



## Application of an electronic tongue to study the effect of the use of pieces of wood and micro-oxygenation in the aging of red wine

M. Gay<sup>a</sup>, C. Apetrei<sup>b</sup>, I. Nevares<sup>c</sup>, M. del Alamo<sup>d</sup>, J. Zurro<sup>e</sup>, N. Prieto<sup>f</sup>, J.A. De Saja<sup>f</sup>, M.L. Rodríguez-Méndez<sup>a,\*</sup>

<sup>a</sup> Department of Inorganic Chemistry, E.T.S. Ingenieros Industriales, University of Valladolid, Paseo del Cauce s/n, 47011 Valladolid, Spain

<sup>b</sup> Department of Chemistry, Faculty of Sciences, European Excellence Research Centre for Environmental Problems, "Dunărea de Jos" University of Galati, Romania

<sup>c</sup> Department of Agricultural Engineering, E.T.S.I.A., University of Valladolid, Spain

<sup>d</sup> Department of Analytical Chemistry, E.T.S.I.A., University of Valladolid, Spain

<sup>e</sup> Infirmary School Palencia, University of Valladolid, Spain

<sup>f</sup> Department of Condensed Matter Physics, Faculty of Sciences, University of Valladolid, Spain

### ARTICLE INFO

#### Article history:

Received 24 March 2010

Received in revised form 24 May 2010

Accepted 29 May 2010

Available online 8 June 2010

#### Keywords:

Electronic tongue

Wine

Polyphenol

Chips

Phthalocyanine

### ABSTRACT

The ageing of red wines matured in oak barrels and wines treated soaking pieces of wood of different sizes (chips or staves) in micro-oxygenated stainless steel tanks has been monitored periodically using an electronic tongue, chemical analysis and a panel of experts. The use of micro-oxygenation in stainless steel tanks, lets get wines with characteristics similar to wines aged in oak barrels. However, differences in the phenolic content and in particular in the anthocyanin levels are observed during the first steps of ageing and in the final product.

In the early stages of ageing, Principal Component Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA) calculated from the electronic tongue outputs have permitted the discrimination between wines aged with traditional and alternative methods due to the faster rate of ageing caused by chips or staves. After 5 months of ageing, the use of alternative ageing methods cannot be longer detected. However, when the ageing continues in a reducing atmosphere (bottled wines), the electronic tongue has demonstrated a good capability to discriminate and classify bottled wines previously aged in oak barrels from those previously treated with oak chips and oak staves. The effect of the size of the pieces and of the type of wood can also be detected by the e-tongue. Using Partial Least Squares (PLS-1) good correlations have been found between the electrochemical signals provided by the array of sensors and the polyphenolic content parameters. Good correlations have also been established with the scores given by the panel of experts, in particular with the astringency.

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### 1. Introduction

Recently, alternative ageing methods have been developed that can simplify the traditional maturing of wines in oak barrels [1]. One of these techniques consists in adding small oak wood pieces (chips or staves) to wines kept in stainless steel tanks. A gentle oxidation of tanks is necessary to simulate the micro-oxygenation that occurs in barrels due to the diffusion of oxygen through the barrel pores [2–4]. The oxygen dissolved, promotes the oxidation of certain chemical compounds, resulting in wines with a high degree of similarity with wines aged in oak barrels. For this reason, there is a need to develop methods able to detect the use of such alternative ageing methods.

The phenolic composition of wines is strongly influenced by the experimental conditions of the ageing. It is well known that the final

chemical and organoleptic characteristics of wines depend on the type of wood used (origin, drying and toasting of the wood), contact time with wine, temperature etc. In the case of treatment with pieces of wood additional parameters such as the size or the conditions of the micro-oxygenation have also an important influence in the phenolic spectrum of the final product [5–9]. For this reason, the phenolic composition can help to characterise and detect wine ageing styles.

A promising approach to analyse wines consists in the use of electronic tongues [10–18]. Such systems are formed by an array of sensors where several sensing units, which exhibit different responses to various compounds, are coupled with signal processing methods based on pattern recognition or artificial neural networks.

Our group has developed an electronic tongue dedicated to the analysis of red wines. It consists in an array of voltamperometric sensors chemically modified with electroactive substances (phthalocyanines and perylenes). These materials are sensitive to

\* Corresponding author. Tel.: +34 983 423540; fax: +34 983 423310.

E-mail address: [mluz@eis.uva.es](mailto:mluz@eis.uva.es) (M.L. Rodríguez-Méndez).

several components present in wines including species affecting the pH and species with redox reactivity [19–21]. A data treatment has also been developed to process the voltammetric signals. The e-tongue has been able to discriminate wines of different grape variety or among wines aged in different types of oak barrels [14,16,22]. In addition, in a previous work, our group demonstrated the possibility of using an electronic tongue to discriminate between bottled wines that were aged in barrels or treated with oak chips [23]. In this early work, micro-oxygenation was not used during the treatment with oak chips and wines were analysed only after bottling.

The objective of this work is to establish the capability of our electronic tongue to monitor the ageing of a red wine aged in traditional oak barrels of different origins (French or American) and the same wine aged in stainless steel tanks where pieces of wood of different sizes and origins have been added. In the case of stainless steel tanks, artificial micro-oxygenation has been used in tanks in order to simulate as much as possible the diffusion of oxygen that occurs through the barrel pores. It is important to highlight that in this work the wines have been monitored from the beginning of the ageing in contact with wood (6 months) and continued in wines bottled during 14 months. The high number of samples allowed us enhancing the quality of the mathematical models and looking at the effect of ageing on the intermediate products.

The capability to discriminate between traditional and alternative methods has been evaluated using Principal Component Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA). The wines have also been monitored using standard chemical analysis and by a human panel.

Prediction models to calculate chemical parameters from data registered with the electronic tongue have been constructed by means of Partial Least Squares (PLS-1). Special attention has been paid to correlations with polyphenolic compounds which are key components of the ageing process.

## 2. Experimental

### 2.1. Wine samples under study

The 13 wine samples prepared are listed in Table 1. Grapes of the variety *Tempranillo* coming from the D.O. Ribera de Duero (Spain) were used in the study. After fermentation, the wine obtained was aged following different methodologies. One part of the wine was introduced in a stainless steel tank of 100 L of capacity with no contact with wood. This wine was used as a control. A second set of samples was prepared by maturing the wine in 100 L stainless steel tanks where pieces of oak wood were added: eight wines were obtained by adding wood samples of different sizes (chips or staves) and of two different origins (American and French) (samples C1–C4 and S1–S4). Finally, a third set of samples was obtained by age-

**Table 1**  
Wine samples under study.

| Sample  | Ageing vessel          | Oak type | Type of wood | Micro-oxygenation |
|---------|------------------------|----------|--------------|-------------------|
| C1      | Stainless steel (100L) | American | Chips        | Yes               |
| C2      | Stainless steel (100L) | American | Chips        | Yes               |
| C3      | Stainless steel (100L) | French   | Chips        | Yes               |
| C4      | Stainless steel (100L) | French   | Chips        | Yes               |
| S1      | Stainless steel (100L) | American | Staves       | Yes               |
| S2      | Stainless steel (100L) | American | Staves       | Yes               |
| S3      | Stainless steel (100L) | French   | Staves       | Yes               |
| S4      | Stainless steel (100L) | French   | Staves       | Yes               |
| B1      | Oak barrel (225 L)     | American | Barrel       | No                |
| B2      | Oak barrel (225 L)     | American | Barrel       | No                |
| B3      | Oak barrel (225 L)     | French   | Barrel       | No                |
| B4      | Oak barrel (225 L)     | French   | Barrel       | No                |
| Control | Stainless steel (100L) | –        | –            | No                |

**Table 2**

Sampling schedule including ageing stage, duration of each step and total elapsed time.

| Sampling | Stage of ageing   | Duration of ageing step, months | Total elapsed time, months |
|----------|-------------------|---------------------------------|----------------------------|
| T1       | Contact with wood | 1                               | 1                          |
| T2       | Contact with wood | 2                               | 3                          |
| T3       | Contact with wood | 2                               | 5                          |
| T4       | Contact with wood | 1                               | 6                          |
| T5       | Bottle            | 5                               | 11                         |
| T6       | Bottle            | 6                               | 17                         |
| T7       | Bottle            | 3                               | 20                         |

ing the wine in oak barrels (225 L of capacity) using American and French oak wood (samples B1–B4).

In order to use a similar surface of wood in contact with wine (in both barrels and tanks where pieces of wood were added), the surface/volume ratio of the 225 L barrels was calculated (surface of the barrels was 2.04 m<sup>2</sup>). Thus, 600 g of chips were added to each tank to obtain a similar surface/volume ratio. Similar calculations were carried out for staves.

In order to monitor the ageing the 13 wines under study were analysed periodically: after 1 month (T1), 3 months (T2), 5 months (T3) and after 6 months (T4) of contact with wood. Then, wines aged by different methods were bottled and analysed periodically after 5 (T5), 11 (T6) and 14 (T7) months of ageing in bottle. The sampling schedule is summarised in Table 2.

### 2.2. Chemical analysis

The chemical analysis included titratable acidity (TA as g L<sup>-1</sup> tartaric acid), volatile acidity (VA as g L<sup>-1</sup> acetic acid), tartaric acid (T as g L<sup>-1</sup> of tartaric acid), dry extract (DE as g L<sup>-1</sup>), tannins (TAN as g L<sup>-1</sup>), alcoholic degree (AD as %), glycerol (G as g L<sup>-1</sup>), reducing sugars (S, as g L<sup>-1</sup>). These parameters were analysed following the international methods [24].

In addition, parameters related with the phenolic content were also analysed: phenolic compounds (TP as g L<sup>-1</sup> gallic acid), low-polymerised phenolics (LPP as g L<sup>-1</sup> gallic acid), high-polymerised phenolics (HPP as g L<sup>-1</sup> gallic acid), analysed following Folin Ciocalteu method [25], anthocyanins (ACY as g L<sup>-1</sup> of malvidin-3-glucoside) analysed according to the Paronetto method [26], catechins (CAT as g L<sup>-1</sup> of (+)-catechin), analysed following Ribereau Gayon and Stonstreet method [27,28].

### 2.3. Electronic tongue

Electrochemical measurements were carried out using a three-electrode cell. The reference electrode was an Ag|AgCl/KCl<sub>sat</sub> and the counter electrode was a platinum wire. Chemically modified

carbon paste electrodes (CPE) sensors were used as the working electrodes. Three phthalocyanines (lutetium and gadolinium bisphthalocyanines and cobalt phthalocyanine) were used as chemical modifiers for the CPEs. In addition, one unmodified carbon paste electrode and one platinum electrode were included in the array. The electrochemical experiments were carried out following a previously published method [19–23]. Cyclic voltammograms (CV) were recorded from  $-1.0$  to  $+1.3$  V at a scan rate of  $0.1 \text{ V s}^{-1}$ . CV were used to analyse the oxidation potentials of the wines under study. For the electronic tongue measurements, Square Wave Voltammetry (SWV) was performed at a potential scan ranging from  $-1.0$  to  $1.3$  V, using  $f = 15 \text{ Hz}$ ;  $E_{\text{sw}} = 90 \text{ mV}$ ;  $\Delta E_s = 7 \text{ mV}$  (except in the case of CoPc,  $\Delta E_s = 5 \text{ mV}$ ). The electrochemical experiments were performed at a controlled temperature of  $25^\circ\text{C}$ .

#### 2.4. Human panel

A human panel was formed by a group of 10 professors in oenology with a large experience in wine taste analysis. Wines were presented in random order to the panellists. The wine tasting took place in an air-conditioned room ( $21^\circ\text{C}$ ) with isolated booths. Judges assessed the taste using a tasting evaluation sheet that included eight sensory descriptors (acidity, tannic intensity, sweet tannin, green tannin, astringency and persistence). The different terms were evaluated in a scale from 1 to 5 (1, null, very weak; 2, weak; 3, medium; 4, strong; 5, very strong). The average of the scores given by the panellists was calculated (RSD 0.4). All the sensory evaluations were carried out under Spanish Standardisation Rules (UNE) [29].

#### 2.5. Data analysis

All samples were measured seven times with each sensor. The analysis was carried out using as input data source pre-processed voltammograms obtained by the adaptation of a data reduction technique based on predefined response “bell-shaped-windowing” curves called “kernels” [22,23]. Using this method, the SWV curve ( $i$  vs.  $E$ ) is multiplied by a number of 10 smooth, bell-shaped-windowing functions, and integrated with respect to potential. The idea behind this pre-processing technique is to capture the information throughout the global response to obtain 10 parameters per curve (Fig. 1).

Principal Component Analysis (a non-supervised technique) and Partial Least Squares Discriminant Analysis (PLS-DA) were used as discrimination and classification tools. PLS-1 regressions were used for estimating the correlations between electrochemical data and the polyphenolic content or the data of the human panel. All computations and chemometric analysis were carried out using the software Matlab v5.3 (The Mathworks Inc., Natick, MA, USA) and The Unscrambler 9.1 (Camo, Norway).

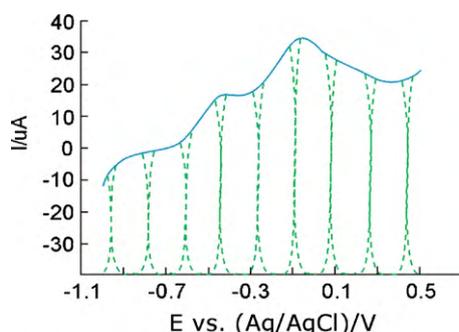


Fig. 1. Square wave voltammogram and kernels which allow obtaining 10 parameters per curve.

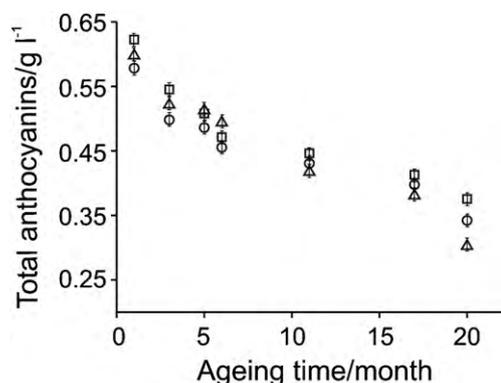


Fig. 2. Variation of anthocyanins content during ageing of wines in (Δ) oak barrel; (○) oak staves; (□) oak chips.

### 3. Results and discussion

#### 3.1. Monitoring the ageing of wines using chemical parameters

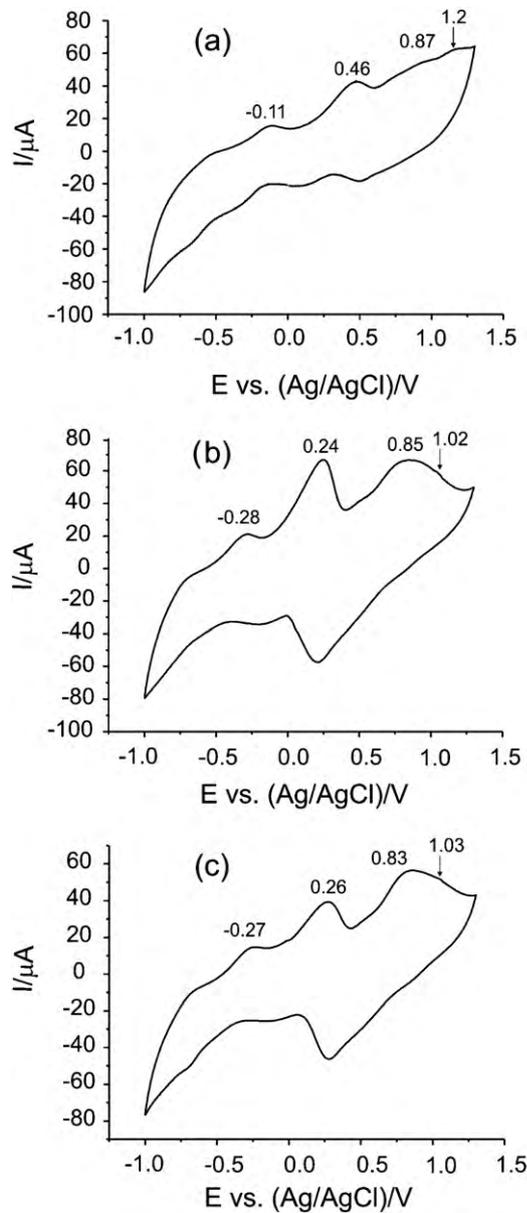
Wines aged in oak barrels and wines treated with oak chips/staves were analysed periodically by chemical methods. The general trends observed in the variations of the chemical parameters were similar for wines aged using both methods. However, several important differences could be observed in the case of polyphenolic compounds especially regarding the anthocyanin levels (Fig. 2). During the contact with wood, the decrease of the anthocyanin concentration was faster than during ageing in bottle [6]. After 1 year in bottle, the anthocyanin levels observed in wines aged using pieces of wood were higher than those observed in wines aged in barrels. Wines treated with staves showed intermediate values between chips and barrels. This result is in good agreement with previously published results [5,30,31] and confirms the importance of the size of the pieces of wood.

#### 3.2. Ageing monitoring by means of the electronic tongue

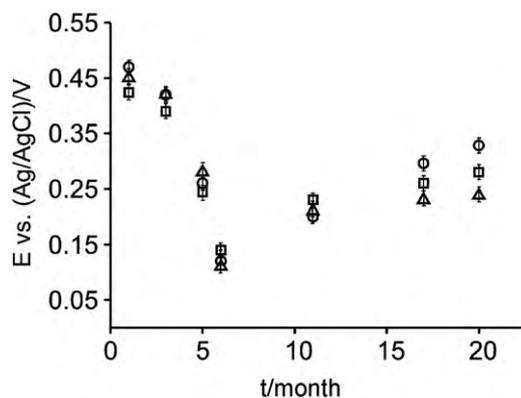
The array of voltammetric electrodes was used to analyse the wines. As it has already been reported, complex voltammograms were obtained [22,23] (Fig. 3). The peak position and their intensities bring information about the chemical composition of the wines [14–22]. Of particular interest are the two peaks associated to polyphenols. The first peak is a reversible peak with an intense anodic wave at ca.  $0.13$ – $0.47$  V. This peak can be associated to phenolic and flavonoid acids which possess easily oxidable ortho-diphenol groups. Such compounds include caffeic acid, gallic acid, tannic acid, catechin, etc. In addition other compounds such as ascorbic acid can also be oxidised in this region [31]. A second broad and also quasi-reversible peak associated to polyphenols can be observed at ca.  $0.9$ – $1.0$  V and corresponds to polyphenols that do not possess ortho-diphenol groups (i.e. *t*-resveratrol, cumaric acid, vanillic acid, etc.).

Due to the variability in the nature and the concentration of polyphenols present in different wines, voltammograms registered from different wines differ one from another. In addition, changes can also be detected during maturing of wines. Ageing in contact with wood, causes an increase of the intensity of the peaks at  $0.13$ – $0.47$  V associated to the presence of polyphenols. The intensity decreases during ageing in bottle.

In addition, during ageing in contact with wood (from T1 to T4), the peak potential of polyphenols at ca.  $0.2$  V shifts to lower values (Fig. 4). This fact can be associated to the modification of the chemical nature of polyphenols that facilitate their oxidation. After bottling, wines change to a reducing atmosphere. Under these



**Fig. 3.** Cyclic voltammetry of a LuPC<sub>2</sub> CPE electrode immersed in (a) C1 (T1); (b) C1 (T3); (c) C1 (T6). Scan rate 0.1 V s<sup>-1</sup>.



**Fig. 4.** Variation of the peak potential associated to polyphenols during the ageing of wines (average value) (Δ) oak barrel; (○) oak staves; (□) oak chips.

**Table 3**

Correlations with chemical parameters. Results of PLS-1 in calibration and validation.

| Parameter                  | Slope | Offset | Correlation | RMSE  |
|----------------------------|-------|--------|-------------|-------|
| <b>Tannins</b>             |       |        |             |       |
| Calibration                | 0.863 | 0.311  | 0.929       | 0.222 |
| Prediction                 | 0.851 | 0.916  | 0.916       | 0.240 |
| <b>Glycerol</b>            |       |        |             |       |
| Calibration                | 0.790 | 1.908  | 0.889       | 0.142 |
| Prediction                 | 0.763 | 2.156  | 0.760       | 0.158 |
| <b>Alcoholic degree</b>    |       |        |             |       |
| Calibration                | 0.824 | 2.429  | 0.908       | 0.166 |
| Prediction                 | 0.814 | 2.715  | 0.886       | 0.184 |
| <b>Dry extract</b>         |       |        |             |       |
| Calibration                | 0.842 | 4.620  | 0.917       | 0.230 |
| Prediction                 | 0.826 | 5.068  | 0.897       | 0.256 |
| <b>Titrateable acidity</b> |       |        |             |       |
| Calibration                | 0.763 | 1.156  | 0.873       | 0.064 |
| Prediction                 | 0.728 | 1.326  | 0.851       | 0.069 |
| <b>Volatile acidity</b>    |       |        |             |       |
| Calibration                | 0.694 | 0.162  | 0.833       | 0.042 |
| Prediction                 | 0.662 | 0.179  | 0.784       | 0.047 |
| <b>Tartaric acid</b>       |       |        |             |       |
| Calibration                | 0.900 | 0.137  | 0.948       | 0.058 |
| Prediction                 | 0.875 | 0.171  | 0.928       | 0.068 |
| <b>Reducing sugars</b>     |       |        |             |       |
| Calibration                | 0.864 | 0.190  | 0.928       | 0.144 |
| Prediction                 | 0.846 | 0.215  | 0.918       | 0.122 |

conditions, the oxidation of polyphenols is more difficult and the oxidation potential increases slowly from T5 to T7. The differences observed between wines aged in oak wood and those treated with oak chips or staves are small and become more important in the last sampling steps (bottled wines). The changes in intensity and the position of the peaks associated to polyphenols can explain the capability of the electronic tongue to monitor the ageing of wines.

### 3.3. Correlations between electronic tongue and chemical analysis

Partial Least Squares (PLS-1) regressions were performed to model the relationships between the electrochemical signals provided by the array of sensors and the chemical parameters measured in wines. In order to establish a robust model, a test validation was carried out using 84 of the 91 samples (chips, staves and barrels) analysed in the seven the sampling periods (T1–T7) as the training set. In order to validate the model, seven samples (one of each sampling period) were used as the test set. The samples of the validation test selected were all aged using American chips.

In a first approach, the chemicals parameters selected for this study included parameters with a well-known influence in the gustative properties of wines: tannins, glycerol, alcoholic degree, dry extract, total acidity, volatile acidity, tartaric acid and reducing sugars.

Results are shown in Table 3. The best correlations were found for tartaric acid with regression coefficients of 0.948 in calibration and 0.928 in validation. Additionally, low values of RMSE (root mean square error) in calibration (0.058) and prediction (0.068) were accomplished.

Correlations with parameters related with acidity (total acidity and volatile acidity) showed lower correlation coefficients.

Anthocyanins are among the most important phenolic compounds which are responsible of the colour and participate in the

**Table 4**  
Correlations with polyphenols content. Results of PLS-1 in calibration and validation.

| Parameter                           | Slope | Offset | Correlation | RMSE  |
|-------------------------------------|-------|--------|-------------|-------|
| <b>Total Polyphenols</b>            |       |        |             |       |
| Calibration                         | 0.868 | 0.284  | 0.932       | 0.056 |
| Prediction                          | 0.854 | 0.315  | 0.921       | 0.060 |
| <b>Low-polymerised polyphenols</b>  |       |        |             |       |
| Calibration                         | 0.902 | 0.132  | 0.949       | 0.055 |
| Prediction                          | 0.893 | 0.144  | 0.938       | 0.061 |
| <b>High-polymerised polyphenols</b> |       |        |             |       |
| Calibration                         | 0.880 | 0.097  | 0.938       | 0.076 |
| Prediction                          | 0.871 | 0.104  | 0.931       | 0.080 |
| <b>Catechins</b>                    |       |        |             |       |
| Calibration                         | 0.866 | 0.144  | 0.930       | 0.091 |
| Prediction                          | 0.859 | 0.119  | 0.921       | 0.077 |
| <b>Anthocyanins</b>                 |       |        |             |       |
| Calibration                         | 0.914 | 0.042  | 0.956       | 0.024 |
| Prediction                          | 0.908 | 0.044  | 0.950       | 0.022 |

polymerisation and condensation of tannins. These reactions give rise to changes in the phenolic structure that in turn influences the astringency of wines. Due to the important role of polyphenols in the ageing of wines, a PLS-1 model was established in order to find the correlation between the responses of the electronic tongue and total polyphenols (TP), low-polymerised polyphenols (LPP), high-polymerised polyphenols (HPP), catechins (CAT) and anthocyanins (ACY). Also in this case, all the samples (chips, staves and barrels) analysed in the seven of the sampling periods (T1–T7) were included in the study.

As observed in Table 4, excellent correlations were found for all the polyphenols analysed. Root mean square error (RMSE) in calibration and validation are expressed in the original units of the variable (values measured chemically are in the range: PT, 2.1–2.3 g L<sup>-1</sup>; LPP, 1.1–1.5 g L<sup>-1</sup>; HPP, 0.6–1.2 g L<sup>-1</sup>; CAT, 0.7–0.8 g L<sup>-1</sup> and ACY, 0.4–0.5 g L<sup>-1</sup>). RMSEs residual errors obtained for calibration and prediction are lower than 15% for all phenolic compounds under study.

#### 3.4. Correlations between electronic tongue and human panel

Nowadays it is clear that electronic tongues measure the chemical composition of the tested samples instead of human perceptions. However, as the chemical composition is related with tastes and flavours, attempt has been made to find correlations between the results obtained with the electronic tongue and the scores given by a human panel in the gustative phase using PLS-1. Parameters scored included volume, acidity, tannic intensity, sweet tannin, green tannin, astringency and persistency. In good agreement with the results found in the previous paragraphs, the best correlations were found with the tannic intensity and with the green tannin which in turn explain the high correlations also found with the astringency (Table 5).

#### 3.5. Discrimination and classification of wines by means of the electronic tongue

One of the main objectives of this work was to establish the capability of our electronic tongue to monitor the ageing of wines and its capability to detect the particular characteristics induced by traditional and alternative ageing methods.

Wines aged in oak barrels and wines treated with pieces of wood (chips and staves) were analysed periodically using the electronic tongue.

The changes in the chemical composition that occur during maturing of wines, could be detected by the array of sensors faci-

**Table 5**  
Correlation with human panel. Results of PLS-1 in calibration and validation.

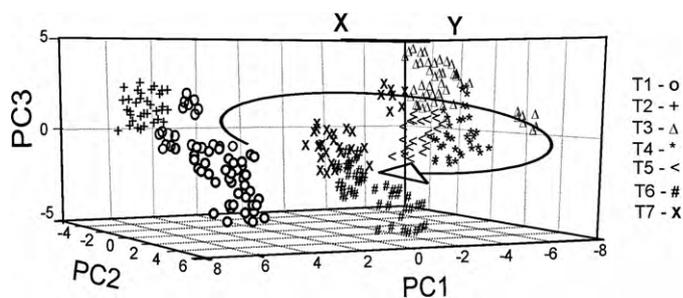
| Parameter               | Slope | Offset | Correlation | RMSE  |
|-------------------------|-------|--------|-------------|-------|
| <b>Volume</b>           |       |        |             |       |
| Calibration             | 0.700 | 1.724  | 0.836       | 0.180 |
| Prediction              | 0.670 | 1.894  | 0.804       | 0.195 |
| <b>Acidity</b>          |       |        |             |       |
| Calibration             | 0.736 | 1.290  | 0.858       | 0.129 |
| Prediction              | 0.710 | 1.418  | 0.819       | 0.145 |
| <b>Tannic intensity</b> |       |        |             |       |
| Calibration             | 0.663 | 2.038  | 0.814       | 0.254 |
| Prediction              | 0.643 | 2.157  | 0.787       | 0.269 |
| <b>Sweet tannin</b>     |       |        |             |       |
| Calibration             | 0.628 | 0.856  | 0.792       | 0.434 |
| Prediction              | 0.604 | 0.910  | 0.763       | 0.460 |
| <b>Green tannin</b>     |       |        |             |       |
| Calibration             | 0.865 | 0.436  | 0.930       | 0.252 |
| Prediction              | 0.850 | 0.488  | 0.916       | 0.276 |
| <b>Astringency</b>      |       |        |             |       |
| Calibration             | 0.816 | 0.811  | 0.903       | 0.345 |
| Prediction              | 0.798 | 0.895  | 0.875       | 0.390 |
| <b>Persistency</b>      |       |        |             |       |
| Calibration             | 0.593 | 2.289  | 0.770       | 0.215 |
| Prediction              | 0.547 | 2.549  | 0.713       | 0.237 |

tating the monitoring of the ageing. Fig. 5 shows the PCA scores plot of the responses of the array of voltammetric sensors immersed in the wines under study. The first three principal components captured the 51%, 11% and 8% of the variance respectively.

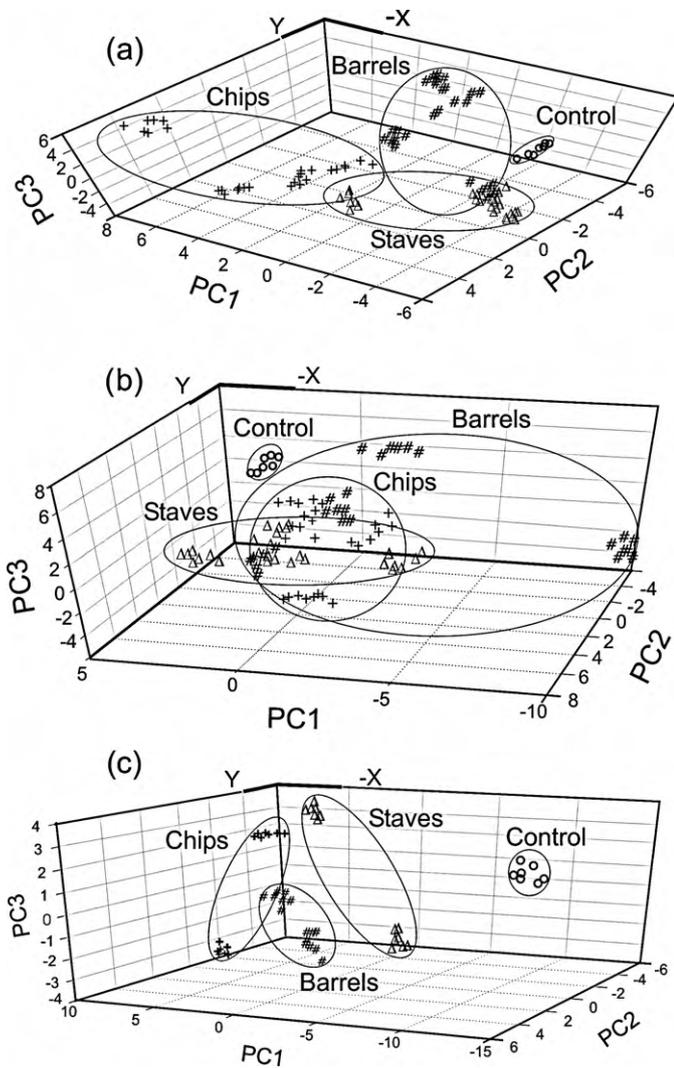
Wines aged during 1 and 2 months (T1 and T2) appear on the left side of the diagram at positive PC1 values, far apart from the other samples (T3–T7) which are located in the right part of the figure at negative PC1 values. Bearing in mind that the array of sensors is sensitive to the antioxidant capability of wines (and the oxygen dissolved plays an important role in this capability), the clear discrimination of wines collected in T1 and T2 can be related with the fast rate of oxygen consumption that takes place during the first months of ageing.

During the oxidative ageing in contact with wood, clusters corresponding to samplings T1–T4 shift from positive to negative first Principal Component (X axis). During maturing in bottle (reductive atmosphere), a displacement of clusters associated to samplings T5–T7 in the opposite sense is observed.

In some cases subclusters inside each sampling period are observed. This can be clearly noticed in samplings T5–T7 and is related to the use of different ageing methods. In order to evaluate the possibility to discriminate between wines aged in oak barrel and wines treated with pieces of wood, the samples collected at each sampling period where analysed separately using PCA.



**Fig. 5.** Score plot of the PCAs of the array of voltammetric sensors exposed to the wines under study. T1 (o), T2 (+), T3 ( $\Delta$ ), T4 (\*), T5 (<), T6 (#) and T7 (x) correspond to samples collected at different elapsed times.



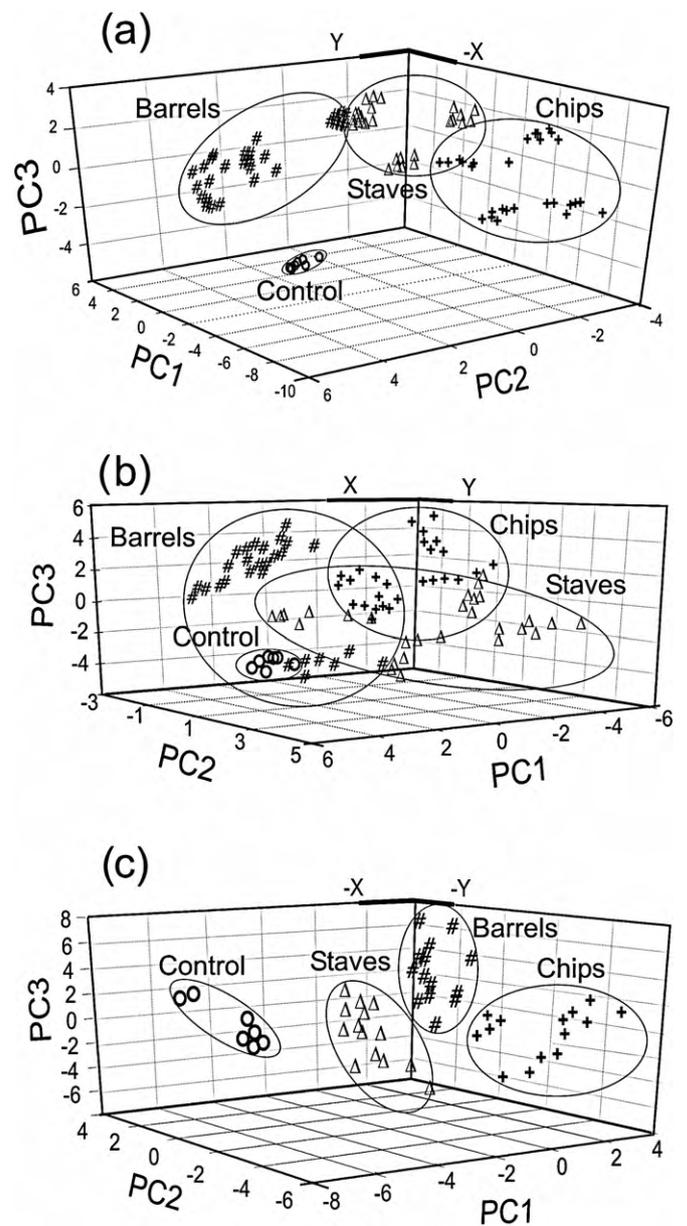
**Fig. 6.** PCA scores plot of wines sampled at (a) T1, (b) T4 and (c) T6. Symbols refer to: (o) control wine; (+) wines treated with chips; ( $\Delta$ ) wines treated with staves and (#) wines aged in barrels.

Fig. 6a shows the result of the PCA using samples collected at T1. The wine control (non contact with wood) appears far apart from the wines aged in contact with wood. Clusters corresponding to wines aged in barrels, and using chips and staves appear partially discriminated, probably because pieces of wood induce an accelerated ageing. When the ageing continued, the differences between wines decreased progressively (Fig. 6b), and after 3 months of ageing (sampling T4) wines could not be longer distinguished.

Then when wines were bottled (T5), differences increased progressively and wines elaborated following different methods could be distinguished using the array of sensors (Fig. 6c). Here again, sub-clusters are observed that correspond to the use of different types of wood (American or French) during the ageing.

The results shown in Figs. 5 and 6 obtained using the electronic tongue are in good agreement with the results found by chemical analysis because PCA detect patterns in the data measured in wines aged by different methods only at the beginning of the ageing and in the final stages.

In order to evaluate the capability of the system to predict the type of method used for ageing, prediction models based on PLS-DA were constructed. Fig. 7 shows the result of the PLS-DA calculated



**Fig. 7.** PLS-DA scores plot of wines sampled at (a) T1, (b) T2 and (c) T5. Symbols refer to: (o) control wine; (+) wines treated with chips; ( $\Delta$ ) wines treated with staves and (#) wines aged in barrels.

for wines bottled at T1, T2 and T7. The relative location of the samples retains the general structure of the PCA score plots shown in Fig. 5, confirming the previous observations.

One of the main interests of the discrimination between wines aged in oak barrel and wines treated with pieces of wood is to detect the use of such alternative practices in the final product. When a wine is consumed it may have suffered variable periods of bottling. For this reason, the capability of the system to predict the ageing method was evaluated constructing a model formed by samples T5–T7 where different bottling periods were contemplated. Table 6 collects the quantitative data derived from the PLS-DA classification model. As observed, both the calibration and validation values involved a good-quality modelling performance (slope near 1, offset near 0 and large correlation between sensors and categorised variables) In addition, almost negligible RMSEC and RMSEP values were accomplished. Sensitivity and specificity were found >0.880 in all cases.

**Table 6**  
PLS-DA prediction models using T5–T7.

|                | Slope    | Offset   | Correlation | Residual error |
|----------------|----------|----------|-------------|----------------|
| <b>Barrels</b> |          |          |             |                |
| Calibration    | 0.669011 | 0.110330 | 0.817931    | 0.271207       |
| Prediction     | 0.533707 | 0.667900 | 0.667900    | 0.356975       |
| <b>Chips</b>   |          |          |             |                |
| Calibration    | 0.598239 | 0.133920 | 0.773459    | 0.298798       |
| Prediction     | 0.503298 | 0.169557 | 0.678737    | 0.347477       |
| <b>Staves</b>  |          |          |             |                |
| Calibration    | 0.814750 | 0.061750 | 0.902635    | 0.202896       |
| Prediction     | 0.665368 | 0.084832 | 0.821902    | 0.269914       |

#### 4. Conclusions

The use of alternative ageing practices such as the use of pieces of wood and micro-oxygenation can be detected using an electronic tongue during the first steps of ageing and in the final product. This capability is related to the changes in the phenolic composition and in particular the anthocyanin levels that occur during ageing. The system has been able to detect the use of pieces of wood of different sizes and is sensitive to the origin of the wood (American or French). Prediction models have been particularly useful to detect the use of such alternative practices in the bottled wines. Good correlations have been found with chemical parameters, especially with polyphenols and with the astringency scored by a panel of experts. The good correlations obtained indicate that the array of sensors presented can be used as an analytical tool to predict the polyphenolic content of wines. The electronic tongue can be a complementary method to the traditional ones to detect the use of alternative ageing methods.

#### Acknowledgements

Financial support from CICYT (Grant no. AGL2006-05501/ALI) is gratefully acknowledged. One of us M.G. wants to thank MICINN for grant FPI (AGL2006-05501, BES-2007-14435).

#### References

- [1] P. Arapitsas, A. Antonopoulos, E. Stefanou, V.G. Dourtoglou, *Food Chem.* 86 (2004) 563.
- [2] V.L.G. Afonso, *J. Food Sci.* 67 (2002) 2415.
- [3] I. Cutzach, P. Chatonnet, D. Dubourdiou, *Food Chem.* 48 (2000) 2340.
- [4] J.I. Campbell, M. Sykes, M.A. Sefton, A.P. Pollnitz, *Aust. J. Grape Wine Res.* 11 (2005) 348.
- [5] M. del Alamo, I. Nevares, *Anal. Chim. Acta* 563 (2006) 255.
- [6] M. del Alamo, I. Nevares, L.M. Cárcel, L. Navas, *Anal. Chim. Acta* 513 (2004) 229.
- [7] E. Cadahia, L. Munoz, B. Fernandez de Simon, M.C. Garcia-Vallejo, *Agric. J. Food Chem.* 49 (2001) 1790.
- [8] M. Laszlavik, L.G. I, S. Misik, L. Erdei, *Am. J. Enol. Viticult.* 46 (1995) 67.
- [9] G. De Coninck, A.M. Jordao, J.M. Ricardo-Da-Silva, O. Laureano, *J. Int. Sci. Vigne et Vin* 40 (2006) 25.
- [10] K. Toko, *Mater. Sci. Eng. C* 4 (1996) 69.
- [11] P. Ciosek, W. Wroblewski, *Analyst* 132 (2007) 963.
- [12] Y. Vlasov, A. Legin, A. Rudnitskaya, C. Di Natale, A.A. D'Amico, *Pure Appl. Chem.* 77 (2005) 1965.
- [13] R.H. Labrador, J. Olsson, F. Winquist, R. Martinez-Manez, J. Sotoa, *Electroanalysis* 21 (2009) 612.
- [14] V. Parra, T. Hernando, M.L. Rodríguez-Méndez, J.A. de Saja, *Electrochim. Acta* 49 (2004) 5177.
- [15] L.M.I. Codinachs, J.P. Klock, M.J. Schoning, *Analyst* 133 (2008) 1440.
- [16] V. Parra, A.A. Arrieta, J.A. Fernández-Escudero, H. García, C. Apetrei, M.L. Rodríguez-Méndez, J.A. de Saja, *Sens. Actuators B* 115 (2006) 54.
- [17] G. Verrelli, L. Lvova, R. Paolesse, *Sensors* 7 (2007) 2750.
- [18] M. del Valle, S. Alegret, A. Merkoçi, A. Baldi, A. Ipatov, A. Bratov, C. Jiménez-Jorquera, *Compr. Anal. Chem.* 49 (2007) 721.
- [19] A. Arrieta, M.L. Rodríguez-Méndez, J.A. de Saja, *Sens. Actuators B* 95 (2003) 357.
- [20] A.A. Arrieta, C. Apetrei, M.L. Rodríguez-Méndez, J.A. de Saja, *Electrochim. Acta* 49 (2004) 4543.
- [21] S. Casilli, M. De Luca, C. Apetrei, V. Parra, Á.A. Arrieta, L. Valli, J. Jiang, M.L. Rodríguez-Méndez, J.A. De Saja, *Appl. Surf. Sci.* 246 (2005) 304.
- [22] V. Parra, Á.A. Arrieta, J.A. Fernández-Escudero, M. Íñiguez, J.A. de Saja, M.L. Rodríguez-Méndez, *Anal. Chim. Acta* 563 (2006) 229.
- [23] C. Apetrei, I.M. Apetrei, I. Nevares, M. del Alamo, V. Parra, M.L. Rodríguez-Méndez, J.A. De Saja, *Electrochim. Acta* 52 (2007) 2588.
- [24] EEC, Community methods for the analysis of wines, Commission Regulation, 1990.
- [25] O. Folin, V. Ciocalteu, *J. Biol. Chem.* 73 (1927) 627.
- [26] L. Paronetto, *Polifenoli e tecnica enologica*, Edagricole, Bologna, 1977.
- [27] J. Ribereau-Gayon, E. Stonstreet, *Bull. Soc. Chim.* 9 (1965) 2649.
- [28] Y. Glories, *Conn. Vigne Vin* 18 (1984) 195.
- [29] ISO Norms, N.A. AENOR 71 (1997) 970.
- [30] M. del Alamo, I. Nevares, L. Gallego, C. Martin, S. Merino, *Anal. Chim. Acta* 621 (2008) 86.
- [31] P.A. Kilmartin, H. Zou, A.L. Waterhouse, *J. Agric. Food Chem.* 49 (2001) 1957.