Evaluation of red wines antioxidant capacity by means of a voltammetric e-tongue with an optimized sensor array

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Abstract

In this work, two sets of voltammetric sensors prepared using different strategies have been combined in an electronic tongue to analyze the complete antioxidant profile of red wines from their direct measurement. To this aim, wine samples were analyzed with the whole set of sensors; afterwards, a feature selection and data compression stage were performed to reduce the large dimensionality of the data set while keeping the relevant information from the samples, using both *kernels* and Discrete Wavelet Transform feature extraction methods. Then, using the obtained coefficients, responses were first evaluated using Principal Component Analysis for visualization of samples dis(similarities), and partial-least squares regression (PLS) and artificial neural networks (ANNs) for building the quantitative prediction models that allowed the quantification of wines antioxidant capacity.

Keywords: Electronic Tongue; voltammetric sensors; wine; polyphenols; antioxidants; feature extraction

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1. Introduction

Wine is an essential component of the Mediterranean diet and might be one of the factors responsible for the low incidence of heart disease in Mediterranean populations [1]. In this sense, the Mediterranean diet has largely demonstrated its health benefits which are related to the intake of foods and beverages rich in antioxidants [2], such as apples and onions, or olive oil and wine. In the case of wine, those effects are mainly related to their content in phenolic compounds [3], which also affect their quality and organoleptic features.

The methods to assess the antioxidant activity are usually based on evaluating the capabilities of an oxidising agent to induce an oxidative damage to a substrate; these, in presence of an antioxidant compound, are inhibited or reduced. The main characteristics of any test for the evaluation of the antioxidant capacity are an appropriate substrate to monitor the inhibition of the oxidation, an initiator of the oxidation (free radical) and an appropriate measure of the endpoint of the oxidation [4].

When approaching the study of the antioxidant activity of wines, it has been recommended to use more than one method. The reason is that each method gives different information: certain antioxidants do not react with certain oxidising species, but they do react with others. As a consequence different reactants provide different results, being able to obtain in this way disparate values among the different methods [5].

On the one hand, the antioxidant activity can be evaluated among other methods by means of the measure of the absorbance capacity of the radical oxygen (ORAC) or the trolox equivalent antioxidant capacity (TEAC) [6]. On the other hand, the measure of phenolic compounds is usually achieved through the Folin-Ciocalteu method [7] or the I_{280} index [8]; the first measures a sample reducing capacity, while the other provides a measure of the sample absorbance at 280nm. Although those indexes are related to total phenolic content, they are also an accepted measure of the antioxidant activity of foods, given the role of phenolic compounds as antioxidants [9].

Moreover, taking into account that the antioxidant activity of wines is mainly related to the phenolic content, Folin index is even preferred by some authors, as it also evaluates the reducing power of wines. However, in some recent works related to the determination of their antioxidant capacity [10], it is stated that a complete antioxidant profile of red wines could be established by coupling (1) evaluation using ABTS (2,2'-

azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) to obtain a measure of total antioxidant capacity, (2) estimation of scavengers activities which give a complimentary information, and (3) use of some type of biomarker methods to provide a measure of the oxidative stress.

Nevertheless, all these techniques have been developed for the analysis of samples at the laboratory level; therefore, it would be desirable to provide methods for on-line analysis because the former also require complex and time-consuming sample pre-treatment procedures.

In this direction, the use of Electronic Tongues (ETs) is growing as a promising approach to analyze liquid samples [11, 12], and can represent a suitable alternative to tackle the determination of antioxidant capacity of wines. Such analytical systems are formed by an array of sensors where several sensing units, which exhibit different responses to various compounds, are coupled with advanced signal processing methods based on pattern recognition or multivariate response models, which allow for the qualitative or quantitative analysis of different sample parameters. To this aim, sensors that might be used are mainly of electrochemical nature, specially of the potentiometric, voltammetric, even of the impedimetric type [12, 13].

The aim of the present work is to examine the potential of an optimized voltammetric electronic tongue to provide a complete antioxidant profile of wine samples at once. To such purposes, two sets of voltammetric sensors prepared using different strategies were evaluated. After samples measurement, a feature selection and data compression stage was performed to reduce the large dimensionality of the data set, while keeping the relevant information from the measurements, employing the Discrete Wavelet Transform (DWT) and *kernel* feature extraction. Finally, obtained responses were analyzed by means of Principal Component Analysis for visualization of samples dis(similarities), and PLS and ANNs to achieve the quantification of wine antioxidant capacity.

2. Experimental

2.1 Reagents and solutions

All reagents used were analytical grade and all solutions were prepared using deionised water from a Milli-Q system (Millipore, Billerica, MA, USA). Copper and platinum nanoparticles (<50nm), polyaniline and polypyrrole, cobalt phthalocyanine

(CoPc), tyrosinase from mushroom (EC 1.14.18.1, 5370U·mg^{-1}), gallic acid, 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium persulfate (di-potassium peroxidisulfate) and 6-hydroxy-2,5,7,8-tetramethychroman-carboxylic acid (Trolox) were purchased from Sigma-Aldrich (St. Louis, MO, USA). KCl was purchased from Merck KGaA (Darmstadt, Germany). Folin-Ciocalteu's reagent and sodium carbonate were purchased from Panreac Química (Barcelona, Spain). HPLC grade ethanol from Scharlau (Barcelona, Spain).

The lutetium (III) bisphthalocyaninate ($LuPc_2$) was synthesized and purified in neutral radical state following earlier published procedures [14, 15].

2.2 Wine samples under study

A total of 9 red wine samples with different oxidation level were provided by the *Matarromera* group (D.O. Ribera del Duero, Spain). Wine oxidation was established according to the results provided by a panel of experts following the established regulations [16, 17]; moreover, antioxidant capacity of wine samples was assessed by different standard methods (section 2.3). Table 1 summarizes detailed information about the wines used.

<TABLE 1>

2.3 Spectrophotometric measurements

For comparison purposes, antioxidant capacity and polyphenolic content of wines were also assessed spectrophotometrycally with three different methods: Trolox Equivalent Antioxidant Capacity (TEAC), Folin-Ciocalteu index (FC) and UV Polyphenol index (I_{280}).

Spectrophotometric measurements were taken using a Schimadzu-UV-1601 spectrophotometer (Kyoto, Japan) and a 1 cm path quartz cell. In all cases, determinations were carried out in triplicate and using as the blank solution an hydro-alcoholic solution (12%, v/v ethanol) of tartaric acid (3g·L^{-1}).

2.3.1 TEAC

TEAC measures the antioxidant capacity of a given substance, as compared to the standard, Trolox. This assay is based on the scavenging of long-lived radical ions (such as $ABTS^{\bullet+}$). Firstly, radicals, which can easily be detected spectrophotometrically at

734nm, are generated. Then, antioxidants are added and its scavenging capacity is measured, providing its TEAC value by comparing the previous value to that of Trolox, a water-soluble vitamin E analogue.

The ABTS assay was performed according to previously reported procedure [18]. First, a ABTS stock solution in water (7 mM) was prepared; followed by the generation of ABTS radical cation ($ABTS^{\bullet+}$) by reacting the stock solution with a potassium persulfate solution (final concentration 2.45mM), which resulting solution was kept in dark at room temperature for 12 hours prior to its use. Lastly, prior to its usage, $ABTS^{\bullet+}$ solution was diluted with ethanol to an absorbance of 0.70(±0.02) at 734 nm.

For the assay, 4mL of $ABTS^{\bullet+}$ solution were added to a 1cm spectrophotometer cuvette followed by the addition of 10µl, 20µl, 30µl and 40µl of previously diluted wine, respectively. The absorbance reading was taken exactly 1 min after initial mixing and up to 10 minutes. The inhibition percentage for the absorbance at 734nm was calculated as the ratio between the decrease of absorbance due to sample addition (A_C-A_S) and the control absorbance (A_C) multiplied by 100, and afterwards, plotted as a function of the added volume:

$$\%I = \frac{A_C - A_S}{A_C} \cdot 100 \qquad (1)$$

Prior to wine samples measurement, those were diluted such that, after introduction of 10-40 µL aliquot of the diluted wine into the assay, they produced between 20%-80% inhibition of the blank absorbance. In our case, the dilution necessary to achieve these inhibition percentages was 1:15 (wine:blank solution).

Analogously, same procedure was followed using Trolox standard (2.5mM prepared in ethanol absolute) instead of diluted wine samples; with a concentration range for the assay in the range from 2.5µM to 15µM (including also the 0). As before, building the absorbance inhibition percentage vs. concentration plot, and calculating its slope.

In this manner, antioxidant capacity, expressed in terms of TEAC, was calculated from the ratio between the slope of the previous plot in the case of wine and the one for Trolox standards.

2.3.2 Folin-Ciocalteu index

FC index is a colorimetric assay measuring the amount of phenol needed to inhibit the oxidation of the Folin-Ciocalteu reagent (a mixture of phosphomolybdate and phosphortungstate, which are reduced to the respective oxides). As a drawback, this

reagent does not measure total phenols uniquely and will react with any reducing substance present. Therefore, it measures the total reducing capacity of a sample, not just the level of phenolic compounds.

The Folin-Ciocalteu test was carried out according to the established procedure for wine analysis [16]. First, 200µl of sample (wines were previously diluted 1:50), 1300µL of deionized water, 100µL of Folin-Ciocalteu reagent and 400µL of a 20% sodium carbonate solution were mixed into an Eppendorf tube. Afterwards, the resulting solution was allowed to react for 30min in darkness at room temperature (25ºC), and finally its absorbance was read directly at 760nm. The total phenolic content (FC Index), expressed in $mg \cdot L^{-1}$ equivalents of gallic acid, was evaluated from the absorbance value by interpolation into the calibration plot obtained with gallic acid standard solutions, multiplying the resulting value by the proper dilution rate.

2.3.3 I_{280} index

I_{280} index is a direct measurement of the UV absorbance at 280nm. The relation between I_{280} index and phenolic concentration is due to the fact that all phenolic compounds absorb UV light, and even more, all of them have some absorbance at 280nm. One problem with this method is that each class of phenolic substances has a different absorptivity (extinction coefficient, ε) at 280nm [8]. Thus, the results cannot be related to any specific standard and are reported directly in absorbance units or arbitrary units (arb. unit). Despite this method is less sensitive and more unspecific, its usage has grown in the last years given its simplicity and low cost.

Polyphenol index (I_{280}) was determined as previously reported [16]. For this, wine was first diluted with deionized water (1:50) and then absorbance was measured directly at 280nm using a quartz cuvette. The value of I_{280} for each sample was given as the absorbance multiplied by the proper dilution rate.

2.4 Preparation of the sensor array

2.4.1 Modified Carbon Paste Electrodes (CPE) sensors

Based on previous experience in our laboratories, an array of 6 modified CPE voltammetric electrodes were prepared following the conventional carbon-paste methodology [19]. The carbon paste electrodes were prepared by mixing the corresponding phthalocyanine (15% w/w) with carbon powder (Ultracarbon, Ultra F purity) in an agate mortar. Then the Nujol oil (which has the role of binder) was added

and the blend was mixed until a homogenous paste with the appropriate consistence was obtained. Once prepared, 0.1g of the mixture was introduced in a plastic syringe (1mL), and compressed. A copper wire was used as the contact. The CPEs were finally smoothed manually with a clean filter paper.

In this manner, three electrochemical sensors were first fabricated: one unmodified carbon paste electrode (CPE, A1), a CPE based on cobalt phthalocyanine (CoPc, A2) and a CPE based on lutetium bisphthalocyanine ($LuPc_2$, A3).

The other three devices were carbon-paste tyrosinase biosensors. For biosensors preparation, previously described procedure was followed by an immobilization step of the enzyme: tyrosinase in this case. To this aim, a 5 $mg \cdot mL^{-1}$ solution of tyrosinase in phosphate buffer (0.01 M, pH 7.0) was prepared. Then, the immobilization of tyrosinase was accomplished by addition of a 5µL aliquot of tyrosinase solution onto the electrode surface (i.e. 134 tyrosinase U/electrode). After drying, the biosensor was immersed in a glutaraldehyde solution (2%) for 20 minutes and next dried in air at room temperature. Lastly, the enzyme-immobilized biosensors were washed with phosphate buffer solution thrice to remove any unbound enzyme. The biosensor was additionally dried at 10ºC and stored at 4ºC.

In this way, previously prepared sensors were also modified by the immobilization of tyrosinase enzyme, thus resulting in the obtaining of three new biosensors: one only with carbon (A4), and the others with CoPc (A5) and $LuPc_2$ (A6). Thus obtaining a 6 CPE (bio)sensors array. Additionally one platinum electrode (Pt, A7) was also included in the array.

2.4.2 Graphite-Epoxy Composite electrodes

Based on previous experience in our laboratories, an array of 6 voltammetric electrodes were prepared following the conventional graphite-epoxy composite methodology [20]. Resin EpoTek H77 (Epoxy Technology, Billerica, MA, USA) and its corresponding hardener compound were mixed in the ratio 20:3 (w/w); afterwards a 15% of graphite (w/w) and a 2% of the modifier (w/w) were added to the previous mixture before hardening, obtaining the composite. Then, it was manually homogenized for 60 min, and afterwards, the paste was allowed to harden for 3 days at 80 ºC. Finally, the electrode surface was polished with different sandpapers of decreasing grain size, with a final electrode area of 28 mm^2.

In this manner, an array of 6 different graphite-epoxy voltammetric sensors were prepared using bare graphite C (B1) and adding different modifiers such as cobalt phtalocyanine (CoPc, B2), conducting polymers such as polypyrrole (Ppy, B3) and polyaniline (PANI, B4), and nanoparticles of copper (Cu, B5) and platinum (Pt, B6) to the bulk mixture – one component per electrode, plus one unmodified electrode.

2.5 Voltammetric measurements

2.5.1 Carbon Paste Electrodes (CPE) sensors

The voltammetric measurements were taken using an EG&G PARC 263A potentiostat/galvanostat (Echem M270 Software) with a conventional three-electrode cell. The reference electrode was an $Ag|AgCl/KCl_{3M}$ and the counter electrode was a platinum wire; while Pt, CPEs and chemically modified CPEs (bio)sensors were used as the working electrodes.

For the recording of cyclic voltammetric measurements, potential was cycled between -1.0V and +1.0V vs Ag/AgCl (-0.6V and +0.6V for CPE biosensors), with a scan rate of $100mV \cdot s^{-1}$ and a step potential of 4mV (2.4mV for CPE biosensors), and starting at 0V. After each sample measurement, (bio)sensors were cleaned and immersed in a KCl 0.1M solution, running some cyclic voltammograms until the original signal was recovered.

2.5.2 Graphite-Epoxy Composite electrodes

The voltammetric measurement cell was formed by the 6-sensor voltammetric array and a reference double junction Ag/AgCl electrode (Thermo Orion 900200, Beverly, MA, USA) plus a commercial platinum counter electrode (Model 52–67, Crison Instruments, Barcelona, Spain). Cyclic Voltammetry measurements were taken using a 6-channel AUTOLAB PGSTAT20 (Ecochemie, Netherlands), in a multichannel configuration, using GPES Multichannel 4.7 software package.

Potential was cycled between -1.0V and +1.3V vs Ag/AgCl, with a scan rate of $100mV \cdot s^{-1}$ and a step potential of 9mV. Apart, all experiments were carried out without performing any physical surface regeneration of the working electrodes. In order to prevent the accumulative effect of impurities on the working electrode surfaces, an electrochemical cleaning stage was done between each measurement applying a conditioning potential of +1.5 V for a duration of 40s after each experiment, in a cell containing 25mL of distilled water [21].

2.6 Data processing

Chemometric processing was done by specific routines in MATLAB (MathWorks, Natick, MA) written by the authors, using Neural Network and Wavelet Toolboxes. Partial Least Squares (PLS) regression was done employing The Unscrambler (CAMO Software AS, Oslo, Norway) informatics package. Sigmaplot (Systat Software Inc, California, USA) was used for graphic representations of data and results.

For each sample, one voltammogram was recorded for each sensor from the ET array. In order to reduce the high dimensionality of the recorded signals (samples x sensors x potentials), a preprocessing stage to compress the information from the original signals and extract meaningful data from the readings was required [22]. For this, two different feature extraction tools were used: "bell-shaped-windowing" curves called "*kernels*" [23] and Discrete Wavelet Transform [24].

Then, the obtained coefficients fed the PLS and ANN models which were used for the quantification of wines antioxidant capacity. Similarly, recognition of samples patterns and dis(similarities) was attempted by means of PCA analysis.

3. Results and Discussion

3.1 Voltammetric responses

Different voltammetric responses are observed for each kind of sensor, as shown in Figure 1. Differentiated signals are obtained for each type of sensor used, i.e. showing its distinctive profile; at the same time, it can be seen how currents increase in concordance to antioxidant capacity of wines and with different behaviour for each sensor. Thus, generating very rich data that is very useful as the departure point, where these signals presumably contribute in different manners for model quantification.

<FIGURE 1>

For developing an ET, the first necessary condition is to have analytical signals responding to the phenomena to which the objective is aimed, with variability among them and the different sensors forming the sensor array. However, the high dimensionality and the extreme complexity of the generated signals (the set of

voltammograms) hinders the processing step; thus requiring of a feature extraction stage.

3.2 Feature extraction

The main objective of this step was to reduce the complexity of the input signal while preserving the relevant information; this approach also permits to gain advantages in modelling time, to avoid redundancy in input data and to obtain a model with better generalization ability [25]; i.e. to improve model performance.

In our case, feature extraction process was divided into two steps. First, reduction of the number of sensors that will be used in the modelling stage was attempted by checking autocorrelation between sensor's responses and discarding the ones that presented more similarity or colinearity; afterwards, voltammetric responses of the selected ones were compressed to reduce its large dimensionality.

In order to quantify response similarities, the correlation coefficient (r) and a comparison factor named f_c that considers the area under both signals when superimposed were used [24]. Both factors range from 0 to 1 depending on signals similarity; it values 0 when two signals have nothing in common and increases its value as similarity does. In this sense, f_c computes similarity in a way related to a correlation coefficient "r", but being more sensitive to small differences.

Therefore, the evaluation of sensors autocorrelation will provide a unique numeric value for each sensor, measuring how similar its signal is to another one. Since for both parameters signals comparison is done point by point, response similarities were evaluated separately for the two types of sensors used: CPEs and composites; thus allowing to discard the modified sensors which do not bring new information to the system, i.e. discarding redundant sensors.

In this manner, voltammetric signals were unfolded and normalized between -1 and +1, calculating then proposed parameters, which are summarized in Table 2. As can be seen, "r" values are higher than f_c, presenting little differences in most cases. As expected, the diagonal of the table is "1" in both cases since it corresponds to the correlations of each sensor with itself, and it is also symmetric since it is equivalent to calculate the correlation of "a" and "b" or "b" and "a". As stated, the higher the correlation value is, means higher colinearity in their responses; thus, the ones with lower values will be the ones selected. Specifically, the ones selected were A2 (CoPc),

A5 (CPETyr) and A6 (CoPcTyr) in the case of CPE (bio)sensors, and the composite electrodes modified with B3 (Ppy), B5 (Cu) and B6 (Pt).

<TABLE 2>

After optimizing of the number of sensors that will be considered in the modelling stage, the next step was the compression of their voltammetric signals. In our case this compression stage was achieved by the use of *kernels* and DWT. Hence, each voltammogram was substituted by a number of coefficients (selected from the minimum allowing a good reconstruction of the original data), accomplishing in this way the data reduction, without any loss of relevant information.

In the case of *kernels* functions, only the anodic part of the voltammetric curve was considered, multiplying it by a number of 10 smooth, *bell-shaped-windowing* functions, and integrated with respect to the potential [23]. Using this method, ten parameters per voltammogram were obtained, meaning a compression ratio up to 98.0% for CPE (bio)sensors (997 original data points) and 96.1% for composite electrodes (514 original data points).

Similarly, in the case of DWT, entire voltammetric curve was compressed using Daubechies wavelet, and two different decomposition levels [24]: seventh for CPE (bio)sensors and sixth for composite sensors; obtaining 14 coefficients per voltammogram; i.e. compression ratios of 98.6% and 97.3%, respectively.

Therefore, recapitulating, from the initial set of 13 sensors was optimized into the 6 most significant ones; from those, the corresponding voltammograms were compressed, and the obtained coefficients were the ones that were fed into the chemometric models.

3.2 Qualitative analysis – detection of wine defects

After feature extraction process, which allowed a significant reduction in the dimensionality of recorded signals, the corresponding compressed voltammograms were analyzed by means of PCA. This method allowed to summarize almost all variance contained in the departure information onto a fewer number of directions (the PCs) with new coordinates called scores, obtained after data transformation. Despite PCA cannot be considered as a properly pattern recognition method, as it only provides a visual representation of the relationships between samples and variables, it is a very useful tool because it provides insights into how measured variables cause some samples to be

similar to, or how they differ from each other. Thus, a preliminary recognition was attained which allowed the visually identification of samples dis(similarities) and clustering.

<FIGURE 2>

Figure 2 only outlines the score plot obtained in the case of *kernel* preprocessing method, as similar information was obtained from DWT. Firstly, it should be noticed that with only the first two PCs, the accumulated explained variance was ca. 98.9%; a large value which means that nearly all the variance contained in the original information is now represented by only these two new coordinates. Secondly, as can be seen by simply visually analyzing the plot, some clusters are obtained after this transformation, thus indicating some similarities between those samples.

In this sense, and taking into account results provided by the panel of experts, patterns in the plot were analyzed. Firstly, it could be seen how S1 samples appear far away from the rest at the left part of the plot (negative scores of PC1); this could be explained as those samples correspond to a very oxidized wine (as can also be deducted from its low TEAC value). Opposed to those, S4 samples, which correspond to a reduced wine, appear on the other side of the plot (positive scores for PC1); while S3 which corresponds to a slightly oxidised wine, hence appearing in between both.

S8 corresponds to a "Brett wine", a wine infected by a yeast inducing very unpleasant mouthfeel. Close to this, it appears S6, a wine with a huge reduction of mercaptans, inducing a unpleasant smell of rotten eggs to wine. In this sense, the position of S5 wine samples between those two and S3 (the slightly oxidised wine), can explain why this wine was discarded by the sensory panel.

Lastly, S7 correspond to a correct wine used as control; thus, expecting that samples close to it correspond also to wines without any (or with small) defects. A hypothesis in accordance to the position of S2 and S9 samples. Therefore, the optimized ET array has proved to be able to assess wines quality and distinguish the ones having specific defects.

3.1 Quantitative analysis

From the optimized 6-sensor array, the corresponding voltammograms were compressed, and the obtained coefficients were fed into multivariate calibration models

in order to predict the antioxidant capacity of wines. In this sense, two different methods were evaluated: an ANN as a non-linear data modelling tool and PLS-2 as a linear one. Those were combined with the two different signal compression strategies evaluated, resulting in a total of four different models.

After a systematic evaluation of topologies, the final ANNs models had 60 or 84 input neurons (corresponding to the 10 kernel or the 14 wavelet approximation coefficients obtained from the analysis of each of the 6 sensor signals), 4 neurons and *purelin* transfer function in the hidden layer and 3 output neurons and *purelin* transfer function in the output layer corresponding to TEAC, FC and I_{280} indexes.

For the optimization of PLS models only one consideration was taken into account: the number of latent variables used to build the model. Despite PLS does not need a preprocessing stage, it was found that better models were obtained when this was performed [26]. Thus, the final models were a *kernel*-PLS2 with 3 latent variables, which has a total explained variance ca. XX.X%; and a DWT-PLS2 with 1 latent variable, which has a total explained variance ca. XX.X%.

To evaluate models' performance, and due to the reduced data set, *leave-one-out* cross validation method was used. In this manner, each sample is classified by means of the analysis function derived from the other samples (all cases except the case itself). This process was repeated *k* times (as many as samples) leaving out one different sample each time, the one to be classified, which acts as model validation sample. Thus, with this approach all samples are used once as validation.

Comparison graphs for each compound and model were built grouping the replicas for each individual sample, differentiating when it was intervening in the training process and when used as external test. The predicted indexes were then plotted against the expected ones and fitted with linear least-squares regression. As an example, the obtained results for the DWT-ANN model can be seen on Figure 3, where it may be seen that a satisfactory trend is obtained, with regression lines close to the theoretical ones.

<FIGURE 3>

<TABLE 2>

In the same way, equivalent plots were built for the other cases (*kernel*-ANN, DWT-PLS2 and *kernel*-PLS2) and regression lines were fitted, which regression

parameters are summarized in Table 2. As expected from the comparison graphs, a good linear trend is attained for all the cases, but as usual in multivariate calibration models, with better performance for the training subset. Regardless of this, the results obtained for both subsets are close to the ideal values, with intercepts close to 0 and slopes and correlation coefficients close to 1; meaning that there are no significant differences between the values predicted by the multivariate calibration methods and the reference ones.

Among the good trend obtained in general, best results were obtained with DWT-ANN model, a fact that can be explained given its superior performance due to its architecture adaptability and the use of both linear and non-linear functions when building the model.

4. Conclusions

Electronic tongues have proved to be useful analytical tools able to provide information for either the organoleptic profile of wines or the simultaneous quantification of three common indexes used to assess wines antioxidant capacity and polyphenolic content. In this sense, good correlations have been found with TEAC, Folin-Ciocalteu and I_{280} indexes, which indicate that the array of sensors presented can be used as an analytical tool to predict the antioxidant capacity of red wines. In this sense, proposed approach represents an alternative to classical methods reducing considerably analysis time (e.g from ca. 30min to ca. 3min in the case of FC index), avoiding the sample pre-treatment (proper dilution factor, etc.) and the use of reagents (Trolox, ABTS, Folin-Ciocalteu reagent, etc.); moreover, providing the quantification of the three indexes (TEAC, FC and I_{280}) from only a single measurement.

Combination of different technologies for constructing voltammetric sensors permitted obtaining an optimized sensor array that yielded best results in both the qualitative and quantitative applications.

Both, *kernels* and DWT methods proved to be very efficient in the compression of voltammetric data, providing similar information as can result from the multivariate data analysis. By using these methods, instead of the entire voltammetric curve, the significant information is reduced to a few coefficients with compression ratio up to XX.X% - XX.X%; with the huge advantage that is the significant reduction of the computational time and the influence of redundant data in the multivariate data analysis

stage. Finally, best results were obtained with the use of ANNs models due to its superior performance.

Acknowledgments

Financial support for this work was provided by *Spanish Ministry of Science and Innovation*, MCINN (Madrid) trough project CTQ2010-17099, and by program ICREA Academia. X. Cetó thanks the support of *Dept. d'Innovació, Universitats i Empresa de la Generalitat de Catalunya* for the predoctoral grant.

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Table 1. Detailed information of the wine samples under study.

Sample	*Wine description*	*Vintage*	*TEAC (mM)*	*Folin index ($mg \cdot L^{-1}$)*	*I_{280} (arb. unit)*
S1	Oxidized wine	1999	4.90	2530	57.7
S2	Correct wine, with some evolution	2006	5.93	2979	61.7
S3	Oxidized wine	2006	5.64	2864	62.5
S4	Red wine, reduced state	2010	5.70	2941	63.1
S5	Wine not accepted by sensory panel	2009	6.20	3166	69.5
S6	Wine with certain reduction degree	2010	5.72	2923	69.2
S7	Correct wine (control specimen)	2010	6.90	3705	69.5
S8	Wine with Brett character	2009	6.47	3672	75.2
S9	Highly elaborated wine, with oak	2010	6.46	3390	75.9

Table 2. Selection and removal of redundant sensors; autocorrelation between its signals.

Composite Paste Electrodes

f_c	A1	A2	A3	A4	A5	A6	7
A1	1	0.697	0.809	0.577	0.086	0.718	0.825
A2	0.697	1	0.564	0.827	0.060	0.501	0.845
A3	0.809	0.564	1	0.467	0.106	0.887	0.667
A4	0.577	0.827	0.467	1	0.050	0.414	0.699
A5	0.086	0.060	0.106	0.050	1	0.120	0.071
A6	0.718	0.501	0.887	0.414	0.120	1	0.592
7	0.825	0.845	0.667	0.699	0.071	0.592	1

r	A1	A2	A3	A4	A5	A6	7
A1	1	0.595	0.758	0.738	0.782	0.815	0.811
A2	0.595	1	0.778	0.600	0.746	0.907	0.734
A3	0.758	0.778	1	0.783	0.800	0.867	0.941
A4	0.738	0.600	0.783	1	0.490	0.664	0.779
A5	0.782	0.746	0.800	0.490	1	0.887	0.841
A6	0.815	0.907	0.867	0.664	0.887	1	0.825
7	0.811	0.734	0.941	0.779	0.841	0.825	1

Graphite-Epoxy Composites

f_c	B1	B2	B3	B4	B5	B6
B1	1	0.825	0.831	0.865	0.381	0.558
B2	0.825	1	0.992	0.713	0.462	0.677
B3	0.831	0.992	1	0.719	0.458	0.672
B4	0.865	0.713	0.719	1	0.329	0.483
B5	0.381	0.462	0.458	0.329	1	0.682
B6	0.558	0.677	0.672	0.483	0.682	1

r	B1	B2	B3	B4	B5	B6
B1	1	0.993	0.993	0.878	0.91	0.919
B2	0.993	1	0.996	0.916	0.929	0.925
B3	0.993	0.996	1	0.901	0.909	0.909
B4	0.878	0.916	0.901	1	0.894	0.82
B5	0.91	0.929	0.909	0.894	1	0.909
B6	0.919	0.925	0.909	0.82	0.909	1

Table 3. Results of the fitted regression lines for the comparison between obtained vs. expected values, both for the training and testing subsets of samples and the three considered indexes.

	Training subset									
	DWT-ANN					***DWT-PLS2***				
	Correlation	Slope	Intercept	*RMSE*	*Total NRMSE*	Correlation	Slope	Intercept	*RMSE*	*Total NRMSE*
TEAC (mM)	0.951	0.861	0.825	0.174		0.775	0.601	2.39	0.375	
Folin index (mg·L^{-1})	0.985	0.922	242	66.5	0.070	0.754	0.568	1352	242	0.171
I_{280} index (arb. unit)	0.981	0.940	4.03	1.15		0.951	0.904	6.47	1.83	
	Testing subset									
	DWT-ANN					***DWT-PLS2***				
	Correlation	*Slope*	*Intercept*	*RMSE*	*Total NRMSE*	*Correlation*	*Slope*	*Intercept*	*RMSE*	*Total NRMSE*
TEAC (mM)	0.774	0.747	1.57	0.372		0.368	0.295	4.25	0.500	
Folin index (mg·L^{-1})	0.920	0.868	429	146	0.147	0.401	0.311	2171	321	0.230
I_{280} index (arb. unit)	0.926	0.890	7.12	2.25		0.836	0.658	23.0	2.69	

RMSE: Root Mean Square Error; NRMSE: Normalized Root Mean Square Error

	Training subset									
	kernel-ANN					***kernel-PLS2***				
	Correlation	Slope	Intercept	*RMSE*	*Total NRMSE*	Correlation	Slope	Intercept	*RMSE*	*Total NRMSE*
TEAC (mM)	0.935	0.756	1.45	0.210		0.944	0.972	0.333	0.132	
Folin index (mg·L^{-1})	0.951	0.773	708	125	0.103	0.991	0.990	60.5	51.3	0.049
I_{280} index (arb. unit)	0.960	0.821	12.0	1.77		0.989	0.995	0.722	0.613	
	Testing subset									
	kernel-ANN					***kernel-PLS2***				
	Correlation	*Slope*	*Intercept*	*RMSE*	*Total NRMSE*	*Correlation*	*Slope*	*Intercept*	*RMSE*	*Total NRMSE*
TEAC (mM)	0.775	0.761	1.51	0.380		0.667	0.470	3.16	0.417	
Folin index (mg·L^{-1})	0.910	0.871	437	158	0.165	0.861	0.591	1238	203	0.202
I_{280} index (arb. unit)	0.860	0.760	15.8	3.03		0.667	0.490	32.6	4.08	

RMSE: Root Mean Square Error; NRMSE: Normalized Root Mean Square Error

FIGURE CAPTIONS

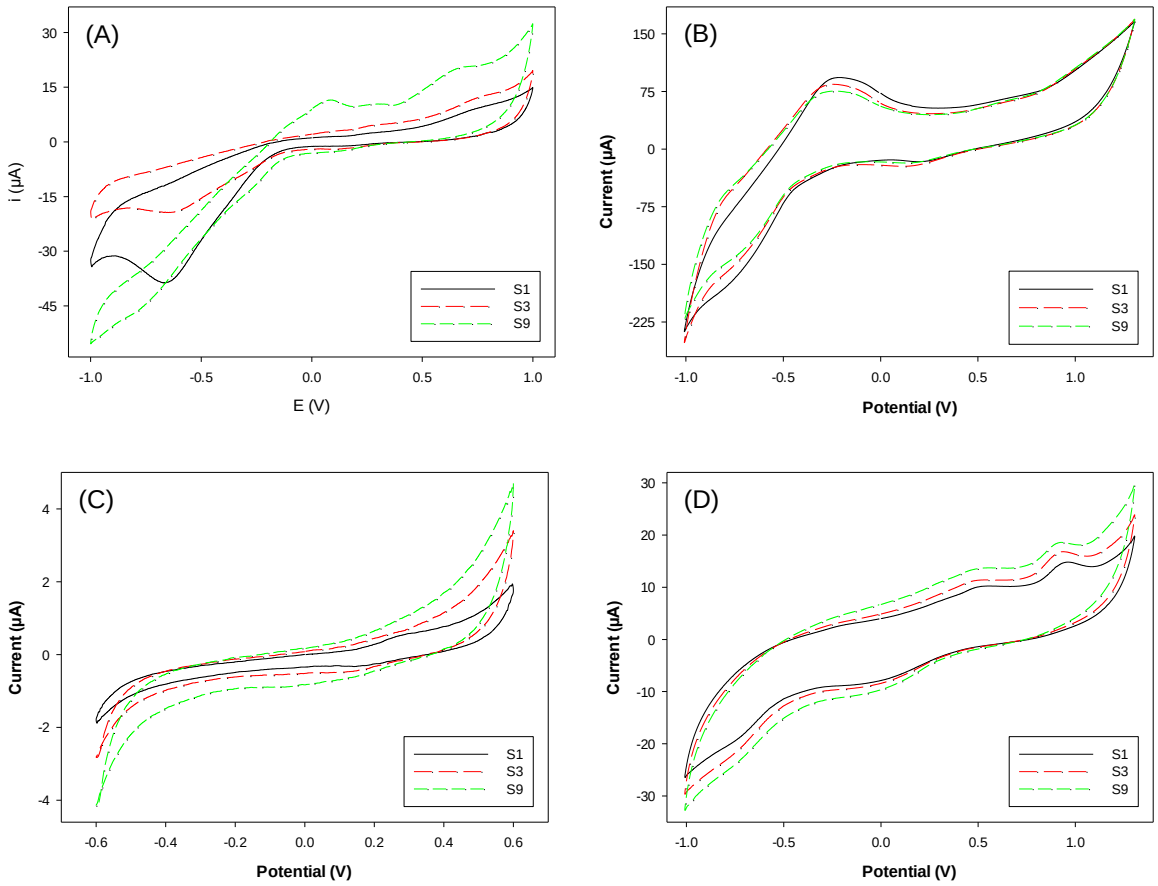

Figure 1. Example of the different voltammograms obtained with the different selected sensors forming the ET array and for certain arbitrary wine samples. Signals provided correspond to: (A) CoPC modified CPE, (B) GEC composite, (C) CPE-Tyr biosensor and (D) polypyrrol modified composite.

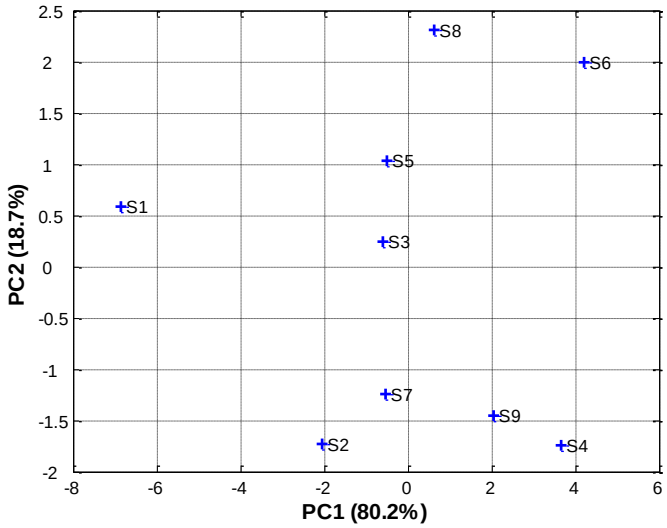

Figure 2. Score plot of the first two components obtained after *kernel*-PCA analysis of the considered wine samples.

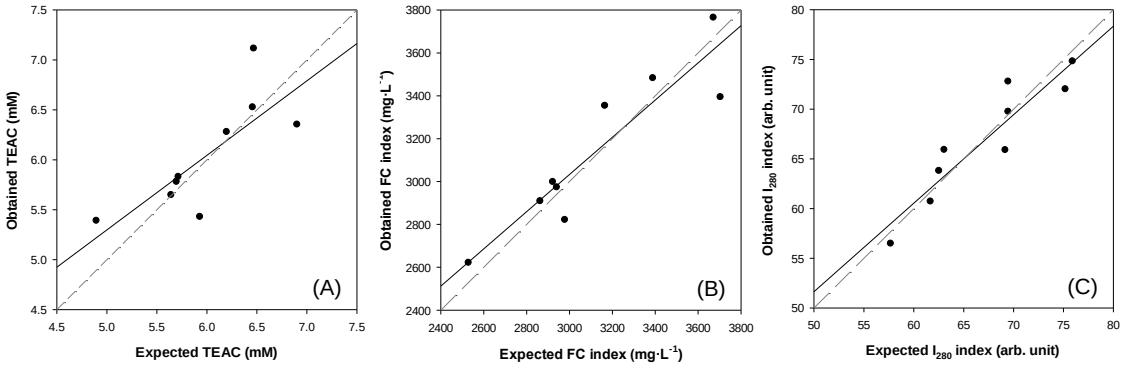

Figure 3. Example of the modelling ability of the optimized DWT-ANN showing the set adjustments of obtained vs. expected indexes for (A) TEAC, (B) Folin-Ciocalteu and (C) I_{280}. Dashed line corresponds to theoretical diagonal line, while plotted data correspond to testing subsets (●, solid line).