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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 evaluate the complete antioxidant profile of red wines. To this aim, wine samples were analyzed with the whole set of sensors. In order to reduce the large dimensionality of the data set while keeping the relevant information provided by the sensors, two different methods of feature selection and data compression were used (the *kernels method* and Discrete Wavelet Transform feature extraction method). Then, the coefficients obtained were used as the input variables of Principal Component Analysis (to evaluate the capability of discrimination. Partial-least squares regression (PLS) and artificial neural networks (ANNs) were performer to build the quantitative prediction models that allowed the quantification of the antioxidant capacity of the tested wines.

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 with the intake of foods and beverages rich in antioxidants [2], such as apples, olive oil and wine. A part from the health benefits, the antioxidant capacity is closely related with the quality of foods and beverages because it contributes to their organoleptic characteristics. It also plays a key role in the preservation of foods [3]. In the case of wine, those effects are mainly related with their content in phenolic compounds [4], which also affect their quality and organoleptic features.

The methods to assess the antioxidant activity are usually based on the evaluation of the capability of an oxidizing agent to induce an oxidative damage to a substrate; in presence of an antioxidant compound, these capabilities are inhibited or reduced. The main elements 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 [5].

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 oxidizing species, but they do react with some others. As a consequence, different methods provide complementary information [6].

Among several other methods, the antioxidant activity can be evaluated by means of the measure of the absorbance capacity of the radical oxygen (ORAC) or the trolox equivalent antioxidant capacity (TEAC) [7]. On the other hand, the measure of phenolic compounds is usually achieved through the Folin-Ciocalteu method [8] or the I_{280} index [9]; the Folin method measures the reducing capacity of the sample, while the I_{280} index provides a measure of the sample absorbance at 280 nm. Although these absorbance indexes are related with the total phenolic content, they are also an accepted measure of the antioxidant activity of foods, given the role of phenolic compounds as antioxidants [10].

Taking into account that the antioxidant activity of wines is mainly related with 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 with the determination of their antioxidant capacity [11], 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

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give a complementary information, and (3) use of some type of biomarker methods to provide a measure of the oxidative stress.

In spite of their advantages, all these techniques have been developed for the analysis of samples at the laboratory level and require complex and time-consuming sample pre-treatment procedures.

The use of Electronic Tongues (ETs) is growing as a promising approach to analyze liquid samples [12,13], 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 [14,15]. Voltammetric sensors chemically modified with a variety of sensing materials and biomaterials have demonstrated to be particularly suitable for the analysis of complex samples because they provide sensors with high cross-selectivity [16,17]. In particular, sensors based on phthalocyanines and conducting polymers provide chemical responses related with both the ions and the electroactive molecules (i.e. polyphenols) present in the solution, being particularly sensitive to pH and antioxidants [18,19]. In turn, biosensors based on tyrosinase are highly sensitive to phenols [20]. The use of tyrosinase and phthalocyanines (or conducting polymers) as electron mediators provides sensors with an increased sensitivity and selectivity towards phenols [21].

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. To such purposes, two sets of voltammetric sensors prepared using different strategies were evaluated. After registering the voltammetric responses of the sensors exposed to wines with different oxidation states, a feature selection and data compression stage was performed employing the Discrete Wavelet Transform (DWT) and *kernel* feature extraction. This step is necessary to reduce the large dimensionality of the data set, while keeping the relevant information from the measurements. Finally, the obtained responses were analyzed by means of Principal Component Analysis for visualization of samples dis(similarities), and using PLS and ANNs to achieve the quantification of wine antioxidant capacity.

2. Experimental

2.1. Reagents and solutions

All reagents used were of analytical grade and all solutions were prepared using deionized water from a Milli-Q system (Millipore, MA, USA). Copper and platinum nanoparticles (<50 nm), polyaniline and polypyrrole, cobalt phthalocyanine (CoPc), tyrosinase from mushroom (EC 1.14.18.1, 5370 $U{\cdot}mg^{-1}$), 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 was obtained from Scharlau (Barcelona, Spain).

The lutetium (III) bisphthalocyaninate ($LuPc_2$) was synthesized and purified in neutral radical state following earlier published procedure [22].

2.2. Wine samples under study

A total of 9 red wine samples from Tempranillo grapes, with different oxidation levels were provided by the *Matarromera* group (D.O. Ribera del Duero, Spain) in 2011. Wine oxidation was established according to the results provided by a panel of experts following the established regulations [23,24]; moreover, antioxidant capacity of wine samples was assessed by different standard methods (section 2.3). Table 1 summarizes the information about the wines used. Three replicas of each sample were analyzed.

2.3. Spectrophotometric measurements

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

Spectrophotometric measurements were registered in 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 a hydro-alcoholic solution (12%, v/v ethanol) of tartaric acid (3 $g.L^{-1}$) as the blank solution.

2.3.1. TEAC

TEAC measures the antioxidant capacity of a given substance, as compared with the standard, Trolox (a water-soluble vitamin E analogue). 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 734 nm, are generated. Then, antioxidants are added and the scavenging capacity is measured, providing the TEAC value by comparing the previous value to that of Trolox.

The ABTS assay was performed according to a previously reported procedure [25]. 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 2.45 mM). The resulting solution was kept in dark at room temperature for 12 hours prior to its use. $ABTS^{\bullet+}$ solution was diluted with ethanol to an absorbance of 0.70 (±0.02) at 734 nm.

For the assay, 4 mL of $ABTS^{\bullet+}$ solution were added to a 1 cm spectrophotometer cuvette followed by the addition of 10 μl, 20 μl, 30 μl and 40 μl 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 734 nm 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:

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

Prior to wine samples measurement, they were diluted such that, after addition 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).

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

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. This method is based on the reduction of phosphomolybdic-phosphotungstic acid to a blue-colored complex by phenolic compounds in alkaline solution.

As a drawback, this reagent does not measure total phenols uniquely and will react with any reducing substance present in the solution. 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 [23]. First, 200 μl of sample (wines were previously diluted 1:50), 1300 μL of deionized water, 100 μL of Folin-Ciocalteu reagent and 400 μL of a 20% sodium carbonate solution were mixed into an Eppendorf tube. Afterwards, the resulting solution was allowed to react for 30 min in darkness at room temperature (25 °C), and its absorbance was read directly at 760 nm. 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 280 nm. 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 280 nm. One problem with this method is that each class of phenolic substances has a different absorptivity (extinction coefficient, ε) at 280 nm [9]. Thus, the results cannot be related with 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 [23]. For this, wine was first diluted with deionized water (1:50) and then absorbance was measured directly at 280 nm 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 [26,27]. 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, 0.1 g of the mixture was introduced in a plastic syringe (1 mL), 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 CPE biosensors preparation, the three electrodes described in the above paragraph were modified with tyrosinase. The phthalocyanine plays the role of the electron mediator. The enzyme was immobilized using a previously described method [26]. 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 5 μL 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 then dried in air at room temperature. Lastly, the enzyme-immobilized biosensors were washed with phosphate buffer solution twice to remove any unbound enzyme. The biosensors were additionally dried at 10 °C and stored at 4 °C.

In this way, three biosensors were obtained: one containing carbon and tyrosinase (A4), and the others with carbon, cobalt phthalocyanine and tyrosinase CoPc (A5) and carbon, lutetium bisphthalocyanine and tyrosinase (A6).

2.4.2. Graphite-Epoxy Composite (GEC) electrodes

Based on previous experience in our laboratories, an array of 6 voltammetric electrodes were prepared following the conventional graphite-epoxy composite methodology [28]. 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 °C. 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 phthalocyanine (CoPc, B2), conducting polymers (in powder form) such as polypyrrole (Ppy, B3) or polyaniline (PANI, B4), copper nanoparticles (Cu, B5) and platinum nanoparticles (Pt, B6) to the bulk mixture–one component per electrode, plus one unmodified electrode.

2.5. Voltammetric measurements

Cyclic Voltammetry measurements were registered using a 6-channel AUTOLAB PGSTAT20 (Ecochemie, Netherlands), in a multichannel configuration, using GPES Multichannel 4.7 software package. The voltammetric measurement cell was formed by the corresponding working electrodes (the array of voltammetric sensors) 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). For technical reasons, CPE sensors and Graphite-epoxy electrodes were measured separately.

Before performing the measurements in wine samples, both CPE and GEC electrodes were cycled in saline solution in order to get stable voltammetric responses. These voltammograms were considered as a reference signal to evaluate the repeatability and the reproducibility of the sensors. Typically, standard deviations under 5% were considered as acceptable. Sensors with values higher than 5% were discarded.

Once immersed in wines, 2 scans were registered until stabilization of the signal. In Fig. 1 the second scan in shown.

2.5.1. Carbon Paste Electrodes (CPE) sensors

For the recording of cyclic voltammetric measurements with CPEs, potential was cycled between -1.0 V and +1.0 V vs Ag/AgCl (-0.6 V and +0.6 V for CPE biosensors), with a scan rate of 100 $mV \cdot s^{-1}$ and a step potential of 4 mV (2.4 mV for CPE biosensors), and starting at 0.0 V. After each sample measurement, (bio)sensors were

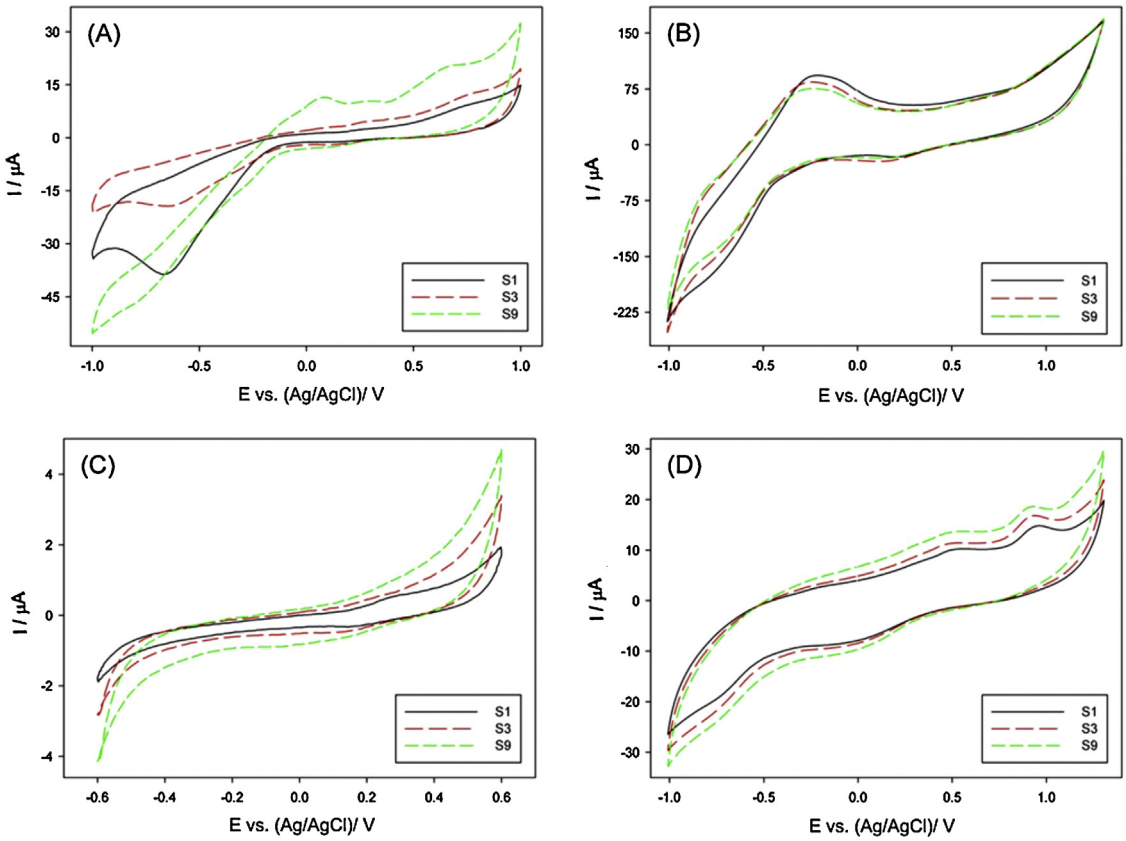

Fig. 1. Example of the different voltammograms obtained with the different selected sensors forming the ET array and for certain arbitrary wine samples. Signals provided correspond to: (A) CoPC modified CPE, (B) GEC composite, (C) CPE-Tyr biosensor and (D) polypyrrol modified composite.

cleaned and immersed in a KCl 0.1 M solution, running some cyclic voltammograms until the original signal was recovered.

2.5.2. Graphite-Epoxy Composite (GEC) electrodes

In the case of GECs, potential was cycled between -1.0 V and +1.3 V vs Ag/AgCl, with a scan rate of 100 $mV \cdot s^{-1}$ and a step potential of 9 mV. 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 40 s after each experiment, in a cell containing 25 mL of distilled water [16].

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 carried out 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.

Each sample was analyzed by registering voltammograms from all the sensors forming the ET array (one voltammogram per sensor). 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 [29]. For this, two different feature extraction tools were used: "bell-shaped-windowing" curves called "*kernels*" [17] and Discrete Wavelet Transform [30].

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

3. Results and Discussion

3.1. Voltammetric responses

Different voltammetric responses are observed for each kind of sensor, as shown in Fig. 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 the antioxidant capacity of wines (measured by traditional methods) and with different behavior for each sensor. Thus, generating complex data sets, which are very useful as the departure point, since these signals presumably, contribute in different manners for model quantification.

For developing an ET, the first necessary condition is to have sensors that produce analytical signals responding to the phenomena to which the objective is aimed, with variability among the samples. It is also necessary to have an array of sensors with cross-selectivity. However, the high dimensionality and the extreme complexity of the generated signals (the set of voltammograms) hinder 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 modeling time, to avoid redundancy in input data and to obtain a model with better generalization ability [31]; in summary, feature extraction can improve the model performance.

In our case, feature extraction process was divided into two steps. First, a reduction of the number of sensors to be used in the modeling stage was attempted. This process was carried out by checking the 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 [30]. 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" vs. "b" or "b" vs. "a". As stated, the higher the correlation value is, means higher colinearity in their responses; thus, the sensors 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).

After optimizing the number of sensors that will be considered in the modeling stage, the next step was the compression of their voltammetric signals. In our case this compression stage was achieved by the use of two methods: *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 for data analysis, because the information of the anodic and the cathodic waves is somehow complementary [32]. Thus, multiplying it by a number of 10 smooth, *bell-shaped-windowing* functions, and integrated with respect to the potential [17]. 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 [30]: 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.

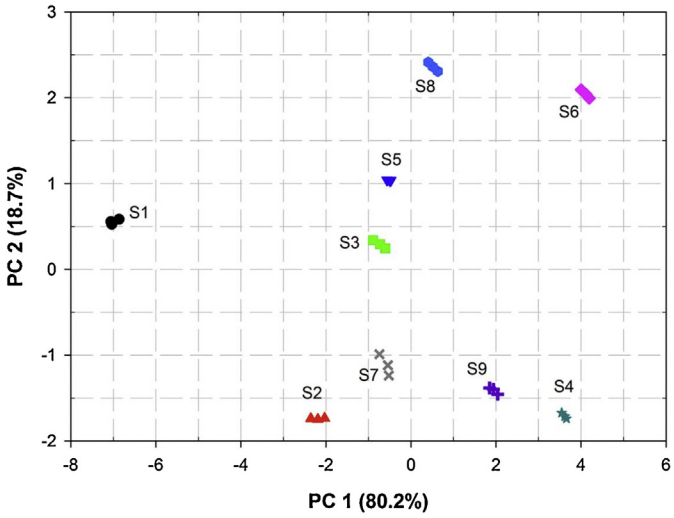

Fig. 2. Scores plot of the first two components obtained after *kernel*-PCA analysis of the considered wine samples (triplicate measurements were carried out).

Therefore, recapitulating, from the initial set of 12 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.

3.3. 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. Thus, a preliminary recognition was attained which allowed the visually identification of samples dis(similarities) and clustering.

Fig. 2 outlines the scores plot obtained in the case of *kernel* preprocessing method. Similar information was obtained from DWT preprocessing. 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. In addition, as it 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. For instance, it could be seen how S1 samples appear far away from the rest at the left part of the plot (negative scores of PC1); S1 samples correspond to a highly oxidized wine (as indicated by the panel of experts and also deducted from its low TEAC value). Opposed to those, S4 and S6 samples, which correspond to reduced wines, appear on the other side of the plot (positive scores for PC1). Wines not oxidized nor reduced (S2, S5 and S7) appear at PC1 values close to zero, while S3 which corresponds to a slightly oxidized wine, appears located at an intermediate position but at positive PC1 values. The case of wine S9 is a particular situation, because it corresponds to a wine that has been micro-oxygenated artificially. S9 appears located in the positive region of PC1, conforming that PC1 discriminates according to the oxidant capacity.

Although PC2 takes account for only the 18.7% of the information, it can provide certain information about the presence of off-flavors. For instance, S8 (which corresponds to a "Brett wine", a wine that shows altered and unpleasant bouquet and palate because it has been contaminated with *Brettanomyces* yest) appear

at the top part of the figure. Also in the upper part and close to S8, it appears S6, a wine with a clearly noticeable reduction of mercaptans, inducing a unpleasant smell of rotten eggs. In contrast, wines free of off-odors appear at negative PC2 values.

This is the reason why, S7 which corresponds to a control wine (absence of oxidation or defects) appears in the inferior part of the graph at negative PC2 values and PC1 values close to 0; thus, it can be expected 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.

3.4. 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 modeling 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 [33]. Thus, the final models were a *kernel*-PLS2 with 3 latent variables, which has a total explained variance ca. 95%; and a DWT-PLS2 with 3 latent variables, which has a total explained variance ca. 98%.

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 Fig. 3, where it may be seen that a satisfactory trend is obtained, with regression lines close to the theoretical ones.

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 3. 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 from both subsets are of good quality (taking into account that wine is an extremely complex matrix), 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.

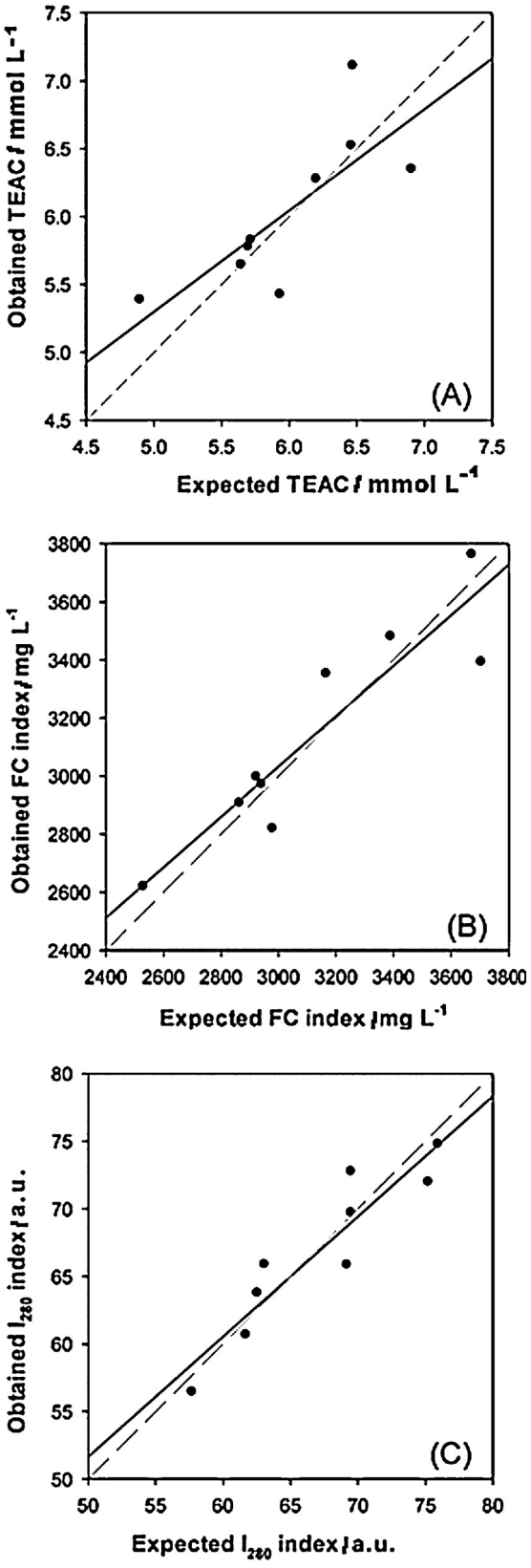

Fig. 3. Example of the modelling ability of the optimized DWT-ANN showing the set adjustments of obtained vs. expected indexes for (A) TEAC, (B) Folin-Ciocalteu and (C) I_{280}. Dashed line corresponds to theoretical diagonal line, while plotted data correspond to testing subsets (●, solid line).

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

The optimized electronic tongue presented here has proved to be a useful analytical tool able to provide information of the antioxidant capacity of wines. In this sense, good correlations have been found with TEAC, Folin-Ciocalteu and I_{280} indexes. The proposed approach represents an alternative to classical methods reducing considerably analysis time (e.g from ca. 30 min to ca. 3 min 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 a single measurement.

The combination of different technologies to obtain voltammetric sensors with high degree of cross-selectivity has demonstrated to be a good strategy that yielded improved results in both qualitative and quantitative applications.

Both, *kernels* and DWT methods proved to be efficient approaches for the compression of voltammetric data, and showed similar performance. By using the data provided by these methods instead of the entire voltammetric curve, the significant information is reduced to a few coefficients with compression ratio up to 96%-98%; with a 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 the superior performance.

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