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BioElectronic Tongue for the quantification of total polyphenol content in wine

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ABSTRACT

This work reports the application of a BioElectronic Tongue (BioET) in the estimation of polyphenol content in wine. The approach used an array of enzyme biosensors capable of giving a wide and complete response of the analyzed species, plus a chemometric processing tool able to interpret the chemical signals and extract meaningful data from the complex readings. In our case, the proposed BioET was formed by an array of four voltammetric enzymatic biosensors based on epoxy-graphite composites, one blank electrode and the other three bulk-modified with tyrosinase and laccase on one side, and copper nanoparticles on the other; these modifiers were used in order to incorporate differentiated or catalytic response to different polyphenols present in wine and aimed to the determination of its total polyphenol content value. The obtained voltammetric responses were pre-processed employing the Fast Fourier Transform (FFT); this was used to compress the relevant information whereas the obtained coefficients fed an Artificial Neural Network (ANN) model that accomplished the quantification of total polyphenol content. For comparison purposes, obtained polyphenol content was compared against the one assessed by two different reference methods: Folin–Ciocalteu and UV polyphenol index (I_{280}); good prediction ability was attained with correlation coefficients higher than 0.949 when comparing against reference methods. Qualitative discrimination of individual polyphenols found in wine was also assessed by means of Principal Component Analysis which allowed the discrimination of the individual polyphenols under study.

1. Introduction

Analytical methods with high sensitivity, good selectivity and fast response are needed to meet new challenges in environmental contamination, food safety, and public health. In this sense, winemaking industry is focusing much attention in fast-response and low-cost methods for wine characterization during its elaboration. From the analytical point of view, as the sample, wine is a complex mixture of diverse substances that exhibit biological activity and considerable influence on taste and other properties [1]. Among such compounds, polyphenols are ones of the most important given their antioxidant properties and their effect in wine sensorial features, in particular, colour, body and astringency. The health benefits of phenolic compounds derive from their antioxidant activity acting as free radical scavengers and inhibitors of lipoprotein oxidation [2]; these effects provide a protective effect against aging pathologies like cardiovascular diseases or cancers mutation. Therefore, there is a clear interest to evaluate wine polyphenolic content. Usually, its global content (expressed as equivalents of gallic acid) is around 320 mg L^{-1} for white wine, 820 mg L^{-1} for rosé wine and 2160 mg L^{-1} for red wine [3].

Several methods to quantify polyphenols have been described in the literature [4]. Basically, those methods may be divided into two large groups. On the one hand, there are generic methods such as Folin–Ciocalteu (FC) or polyphenol index (I_{280}), which are widely employed in the wine industry and yield a total polyphenol content value [5,6]. FC method measures the sample reducing capacity and I_{280} index is a measure of the sample absorbance at 280 nm, with different UV contributions that do not necessarily reflect an absolute measurement of the total phenolic content [7]. On the other hand, there are the chromatographic techniques such as High-Performance Liquid Chromatography (HPLC) [8] and Gas Chromatography (GC) [9], which are able to perform the individual determination of polyphenolic compounds. Main disadvantages of these methods are that they need complex and time-consuming sample pre-treatment procedures, and are not suited for on site analysis.

Biosensors provide an interesting alternative to the determination of polyphenols thanks to their low cost and because they can be easily used to carry out on-field analyses [10]. An easy way for biosensor development is the use of bulk-modified composites

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[11], which allows the immobilization of enzymes or other biomolecules at the electrode. The applicability of electrochemical biosensors to the analysis of antioxidant compounds, including polyphenols, is promising and there is a growing interest in the development of such devices. Nevertheless, it is deemed that further work is required to avoid and/or take into account the interference problem [12]. Is for this reason that the combination of biosensors with Artificial Neural Networks (ANNs) modelling may represent an alternative to classical methods, taking benefit of the advantages of both parts. On one hand we have the specificity and selectivity of biosensors [13], and on the other the use of ANNs modelling to derive meaning from complex or imprecise data [14]; for example in multivariate calibration or pattern recognition [15]. This coupling, known as BioElectronic Tongue (BioET) may be postulated as a tool combining ANN to solve interference problems from biosensors, and biosensors as the tool that solves the selectivity problem from the Electronic Tongue (ET). This is the most recent variant of ETs, when biosensors are incorporated in the sensor array. This approach has already been applied towards the analysis of wines [16], and more specifically to the analysis of polyphenols and resolution of their mixtures [17–19]. Apart from BioETs, conventional ET systems based either on the use of voltammetric modified sensors [20] or potentiometric sensors [21,22] have been also used for estimation of polyphenols in wine.

ETs are a new trend from the sensory field, which can be defined as a multisensor system with marked mix-response, capable of giving a wide and complete response towards the analyzed species; ETs use advanced mathematical procedures for signal processing based on pattern recognition and/or multivariate analysis, able to extract meaningful data from the complex readings [14,15]. A known problem with some ETs is the large dimensionality of the generated data; for example, this occurs when a complete voltammogram is recorded for each sensor forming the array and data needs to be processed with ANN. In these cases, departure information is too complex and needs to be pre-processed. This step is needed to reduce the complexity of the input signal preserving the relevant information. Usually, this has been accomplished by the use of Principal Component Analysis (PCA), "kernel" functions [23], Discrete Wavelet Transform (DWT) [24] or even Fast Fourier Transform (FFT) [25].

The aim of this work is to present a BioET analysis system formed by an array of voltametric enzymatic biosensors, e.g., employing tyrosinase or laccase, incorporated on bulk-modified biocomposites, for the analysis of polyphenols in wine. The combined electrochemical responses obtained from the set of biosensors' voltammograms were pre-processed employing FFT in order to extract the significant information and compress the departure information. Next, the obtained coefficients fed an ANN model specially trained to predict total polyphenol content. Furthermore, distinction of individual polyphenols present in wine was evaluated with PCA and classification was assessed with a PCA-ANN as the pattern recognition algorithm carrying out the identification of the different chemical compounds.

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). Tyrosinase from mushroom (EC 1.14.18.1, 4276 U mg^{-1}), Laccase from *Trametes versicolor* (EC 1.10.3.2, 21 U mg^{-1}), copper nanoparticles (50 nm), gallic acid, (±)-catechin, *p*-coumaric acid, caffeic acid, catechol phenol, *m*-cresol, ferulic acid, chlorogenic acid and quercetin 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).

2.2. Wine samples

2.2.1. Evaluation of total polyphenol content

For this purpose, a total set of 29 wine samples of different varieties and from different regions of Spain where acquired at the local supermarket and analyzed. Samples were chosen in order to obtain a set with sufficiently differentiated total polyphenol content and grape varieties, i.e., including the most typical ones of each region; e.g., *merlot* and *trepat* from *Penedès* region (*Catalonia*), *tempranillo* from *Rioja*, *malbec* from *Castilla* or *petit verdot* from *Andalusia*, all of them from recent harvests on 2008, 2009 and 2010.

2.2.2. Discrimination of individual polyphenols

Nowadays, there is also an increasing demand for highly sensitive and selective analytical methods for the determination of individual polyphenols [4,26]. In this way, despite the great amount of research in the field, the separation and quantification of different polyphenols remain difficult, especially the simultaneous determination of those from different chemical subgroups.

In order to assess the ability of the BioET to discriminate between different polyphenols present in wine, a first qualitative attempt was carried out. For this, some spiked samples were prepared adding different quantities of certain polyphenols to wine samples with low initial content. Polyphenols considered were selected according to their expected presence in wine [27,28]; being gallic acid, (±)-catechin, *p*-coumaric acid, caffeic acid, catechol, phenol, *m*-cresol, ferulic acid, chlorogenic acid and quercetin the compounds selected for the study.

In this manner, 5 separate added samples for each polyphenol tested plus five samples of non-spiked wine were prepared, which means a total of 55 samples divided into 11 classes. To confirm that discrimination between compounds was not due to different amount of polyphenolic compound being added, in all the cases 5 μmol of each compound were added to 25 mL of wine, which represents approximately an increase of 36 mg L^{-1} (200 μM). This small amount was chosen given individual polyphenol concentration is much lower than average total polyphenol content [3]. Apart, solutions were analyzed in random order to be sure their grouping its not a consequence of the order in which samples were analyzed.

2.3. Apparatus

Electrochemical measurements were carried out at room temperature (25 °C) under quiescent condition, in a multichannel electrode configuration using a multipontentiostat AUTOLAB PGSTAT20 (Ecochemie, Netherlands). The voltammetric cell was formed by the array of four composite working electrodes plus a double junction electrode Ag/AgCl Orion 900200 (Thermo Electron Corporation, Beverly, MA, USA) and a platinum-based electrode (Crison 52–67, Barcelona, Spain) used as reference and auxiliary electrodes, respectively.

Spectrophotometric measurements were carried out using a Spectronic Helios Epsilon spectrophotometer from Thermo Electron Scientific Instruments LLC (Madison, WI USA).

2.4. Electrode fabrication

Based on previous studies with polyphenols in our laboratory [18], an array of four working electrodes were prepared following the conventional biocomposite methodology in our laboratories [29]. First, resin EpoTek H77 (Epoxy Technology, Billerica, MA, USA) and its corresponding hardener compound were mixed in the ratio 20:3 (w/w). Then, each electrode was prepared adding a 15% of graphite (w/w) and a 2% of either the enzyme (tyrosinase or laccase) or the modifier (w/w) (copper nanoparticles) into the epoxy resin before hardening—one electrode with each modifier plus a blank electrode without any modifier. Afterwards, the biocomposite was manually homogenised for 60 min. Finally, the biocomposite paste electrode was allowed to harden during 7 day at 40 °C. Electrode surface was then polished with different sandpapers of decreasing grain size, with a final electrode area of 28 mm^2.

Hence, the BioET array will be formed by two enzymes, which belong to the class of copper containing oxidases, and that catalyze the oxidation of the phenolic compounds into their quinones, which are directly measurable on an amperometric sensor surface [10]. Also, copper-nanoparticles were chosen given the fact that both tyrosinase and laccase have a copper centre in its active site. Then it was thought that some catalytic effect could be derived; a fact finally proved by sensor's response [18].

2.5. Procedures

2.5.1. Electrochemical measurements

The electrodes were cycled for 3–5 times in buffer solution in order to get stable voltammetric responses before performing the measurements with real samples. Potential was cycled between −0.4 V and +0.8 V vs Ag/AgCl, with a scan rate of 100 mV s^{-1} and a step potential of 9 mV. No pre-treatment or dilution of sample was performed when measuring wine samples; 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 +1.0 V for 40 s after each experiment, in a cell containing 10 mL of distilled water [30]. This step ensures that electrode fouling is minimized, recovering the original signal (baseline) after each measurement.

2.5.2. Spectrophotometric measurements

For comparison purposes, polyphenolic content of wines was also assessed spectrophotometrycally with two different methods: Folin–Ciocalteu index (FC) and UV Polyphenol Index (I_{280}).

The first one 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 other reducing substances present. Therefore, it measures the total reducing capacity of a sample, not just the level of phenolic compounds. The second one is a direct measurement of the 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 [6]. 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 inespecific, its usage has grown in the last years given its simplicity and low cost.

The Folin–Ciocalteu test was carried out according to the established procedure for wine analysis [31]. 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 finally its absorbance was read directly at 760 nm. The total phenolic content (FC index), expressed in mg 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.

Polyphenol index (I_{280}) was also considered [31]. For this, wine was first diluted with deionized water (1:50) and then absorbance was measured directly at 280 nm. The value of I_{280} for each sample was given as the absorbance multiplied by the proper dilution rate.

2.6. Data processing

Chemometric processing was done by specific routines in MATLAB 7.1 (MathWorks, Natick, MA) written by the authors, and using Neural Network Toolbox (v.4 0 6). Sigmaplot 2000 (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 BioET array. In order to reduce the high dimensionality of the recorded signals (samples *x* sensors *x* potentials), a preprocessing stage was required. For this, FFT was used as feature extraction tool to compress the information from the original signals and extract meaningful data from the readings [32]. FFT preprocessing consists in the decomposition of the signal using sine/cosine function pair at different frequencies, calculating a coefficient for each one taking into account its contribution to the original signal [33]. In this manner, as many coefficients as points in the original signal are obtained, where most relevant information is kept by the first coefficients, ergo good signal reconstruction could be achieved just preserving the first coefficients and discarding the rest. Then, the obtained coefficients fed the ANN model which was used for the quantification of total polyphenol content. Similarly, a classification procedure was developed from a PCA pretreatment and an ANN classifier in order to discriminate between the different polyphenols added to wine samples.

The used ANN training algorithm was Bayesian regularization. This algorithm has the particularity that it avoids overfitting without the need to monitor the fitness degree of an internal validation subset [34], then this precaution is not performed. To evaluate the BioET response a jack-knife method was used given the reduced size of the data set [35,36]. This method has the advantage that avoids dependence of predictions from the specific subdivision of data. In this way, train/test data subdivision is repeated randomly *k* times, evaluating model's response for test validation subset each time; afterwards all data is grouped depending if it was intervening in the training process or used in the external test subset, building the response model, which in addition allows to calculate confidence intervals for the predictions.

3. Results and discussion

3.1. Voltammetric responses

Examples of the different obtained signals coming from the BioET array for different wines are shown in Fig. 1. A clear voltammetric response is obtained for all the sensors, with currents

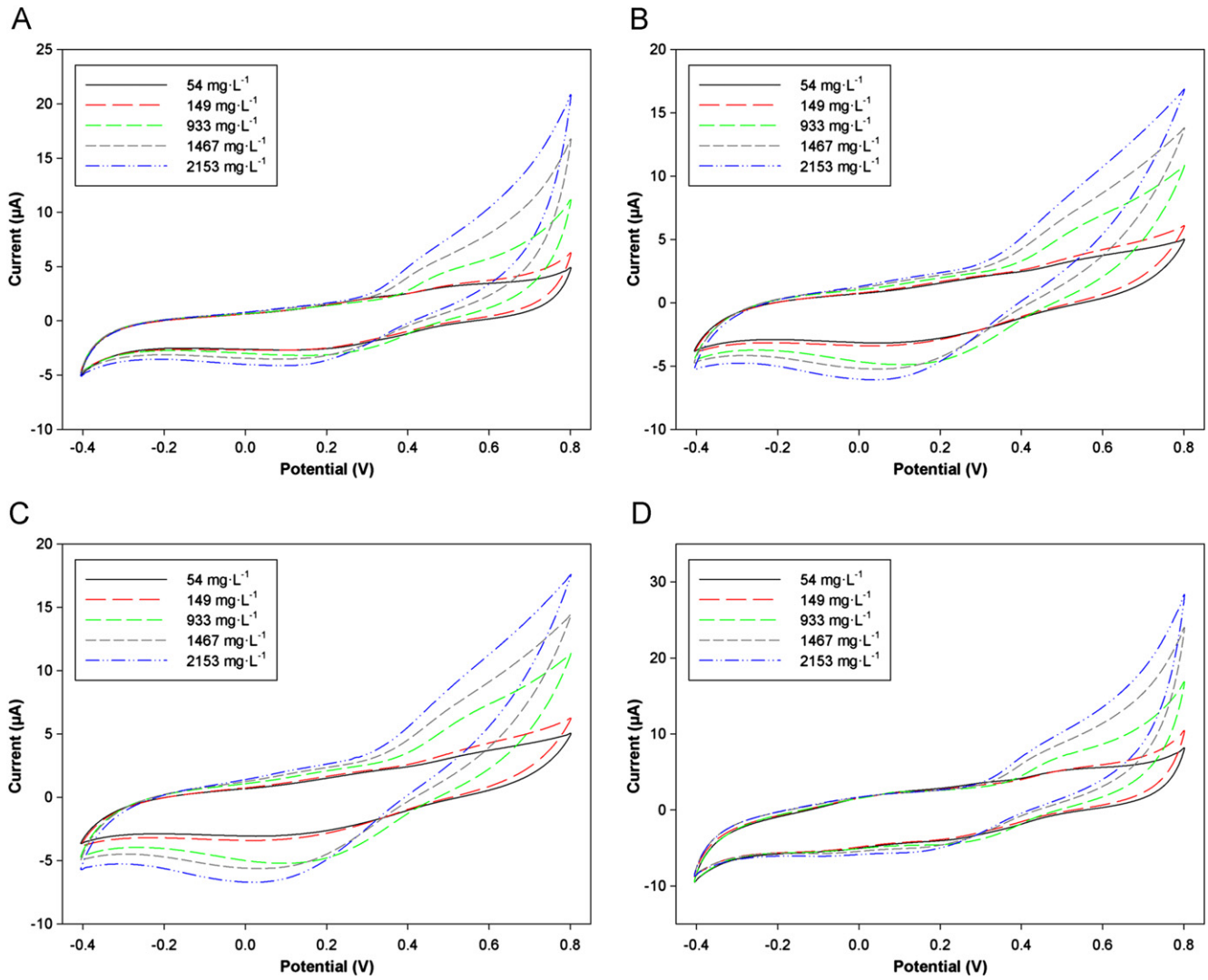

Fig. 1. Example of the different voltammograms obtained for certain arbitrary wine samples. Folin indexes are expressed as equivalents of gallic acid. Also signals provided by different (bio)sensors are shown: (A) graphite-epoxy sensor, (B) tyrosinase biosensor, (C) laccase biosensor and (D) copper nano-particle modified sensor.

monotonously increasing as FC index increases and with differentiated behaviour for each sensor; but as can be seen, higher currents are obtained for the biosensors containing tyrosinase (Fig. 1B) and laccase (Fig. 1C) enzymes, especially in the reduction region close to 0 V where obtained net response is ca. 3 times higher. Also, some degree of catalytic effect is obtained from the copper nanoparticles, a fact somehow explained given both tyrosinase and laccase are copper-containing redox enzymes. These enzymes, both having two copper atoms (three in the case of laccase) within their active site, interact with dioxygen to form a highly reactive chemical intermediate that then oxidizes the substrate; copper nanoparticles may therefore have a similar catalytic effect [18].

3.2. FFT compression

To fully exploit all the information obtained from each voltammogram and not overdosing the associated ANN, a compression step is required given the extreme complexity of the considered departure signals (268 current values × 4 sensors × 29 samples). This step may also help to gain advantages in training time, to avoid redundancy in input data and to obtain an ANN model with better generalization ability [30]. This compression step was achieved in this work using the Fast Fourier Transform (FFT).

In order to quantify the reconstruction degree, the coefficient of determination (R^2) between reconstructed and original signal and a comparison factor named f_c that considers the area under both signals when superimposed were used [24]. The f_c is defined as the ratio of the area intersected by both curves to the total area under both curves. From the set theory, with A and B as the areas under each curve, the f_c can be expressed as $f_c=(A\cap B)/(A\cup B)$. This factor ranges 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.

As can be seen in Fig. 2(A), by increasing the number of coefficients we preserve a better representation of the voltammetric signal, but implying a higher complexity for the ANN model. Then, a compromise is needed; we selected the number of coefficients by checking the correlation of the reconstructed vs. original signal representation (Fig. 2B). Both R^2 and f_c had the same trend, but the factor f_c presented better discrimination capability than R; e.g., with only 8 Fourier coefficients, where signal reconstruction is still poor, the obtained value for R^2 was 0.9974, while f_c value was 0.9020. Therefore, the optimal number

of coefficients was selected as the first time f_c surpassed 0.98. This was achieved with the first 32 Fourier coefficients, which allowed a compression of the original information up to 88.1% without any loss of significant information.

Hence, it could be seen the good performance of FFT when applied to voltammetric data, providing similar or even better signal compression/reconstruction ratio than DWT. This is mainly due to the recorded signal, which has some sinusoidal trend, making FFT a very suitable method in these cases. As also happens with Wavelet pre-processing (DWT), both methods are very effective in cases were complex and highly overlapped responses are obtained, given obtained coefficients are a representation of the entire voltammetric signal which in addition is denoised.

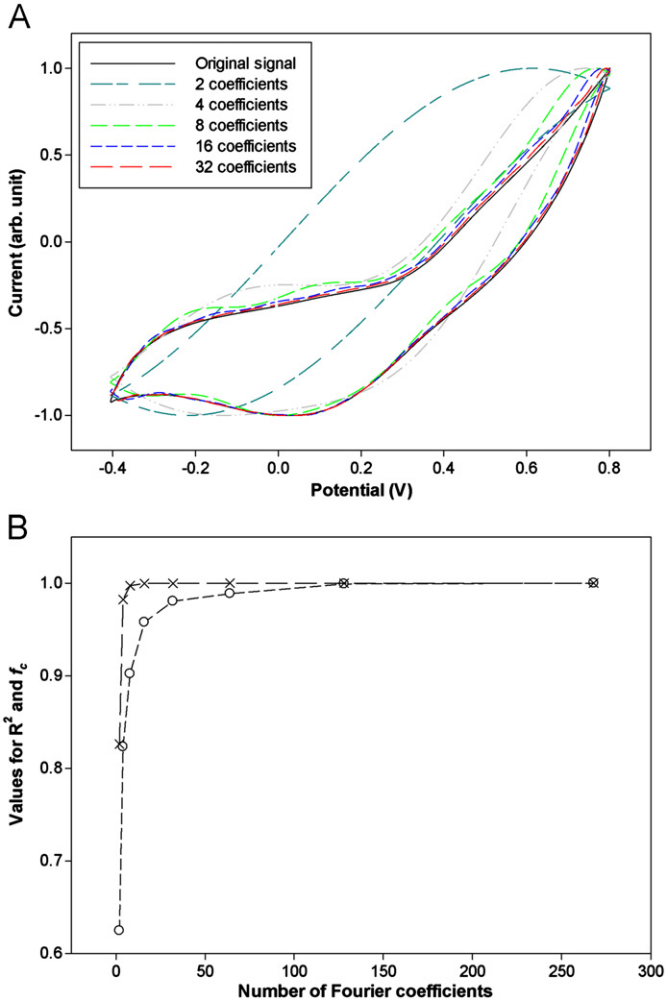

Fig. 2. (A) Reconstruction of the voltammetric signal according to the number of Fourier coefficients employed, tyrosinase biosensor shown as example. (B) Representation of the coefficient of determination (R^2, ×) and f_c (○) against the number of Fourier coefficients used from comparing the raw voltammograms with the reconstructed signals.

3.3. Quantification of total polyphenol indexes

After pre-processing the recorded voltammograms with FFT, the obtained coefficients fed an ANN model in order to predict the total polyphenol index in wines. In order to find the appropriate ANN model, significant effort is needed to optimize the configuration details that determine its operation. Normally, this is a trial-and-error process, where several parameters (training algorithms, number of hidden layers, transfer functions, etc.) are fine-tuned in order to find the best configuration that optimizes the performance of the model. A simplified scheme of the procedure followed for the measurement and data treatment could be seen in Fig. 3.

After some preliminary tests, the final architecture of the ANN model had 128 neurons (4 sensors × 32 coeffs. obtained from the FFT analysis) in the input layer, 6 neurons and *logsig* transfer function in the hidden layer and two neurons and *tansig* transfer function in the output layer, providing the two phenol indexes considered.

Accuracy of the generated model was evaluated employing a jack-knife method to avoid dependence of predictions from the specific subdivision of data, training with 80% of the data (23 samples) and tested with the remaining 20% (6 samples). In this way, train/test data subdivision was repeated randomly 29 times (as many times as samples, similarly to *k*-fold method) in order to ensure that model's accuracy is good enough and BioET performance does not depend on the specific subsets used. Then, once all responses from all the constructed models were obtained, predicted values by each model were grouped depending if they were used in the training process or in the testing subset (again, similarly to *k*-fold method). Finally, average values for each sample were calculated, allowing us to calculate model uncertainties and obtain unbiased data [36].

Comparison graphs of predicted vs. expected concentration for FC (Fig. 4) and I_{280} (Fig. 5) indexes were built, both for train and test subsets, to check the prediction ability of the obtained ANN model. It may be seen that a satisfactory trend is obtained for both indexes, with regression lines almost indistinguishable from the theoretical ones. Also, as usual in ANN models, lower dispersion and uncertainties are obtained for the training subsets.

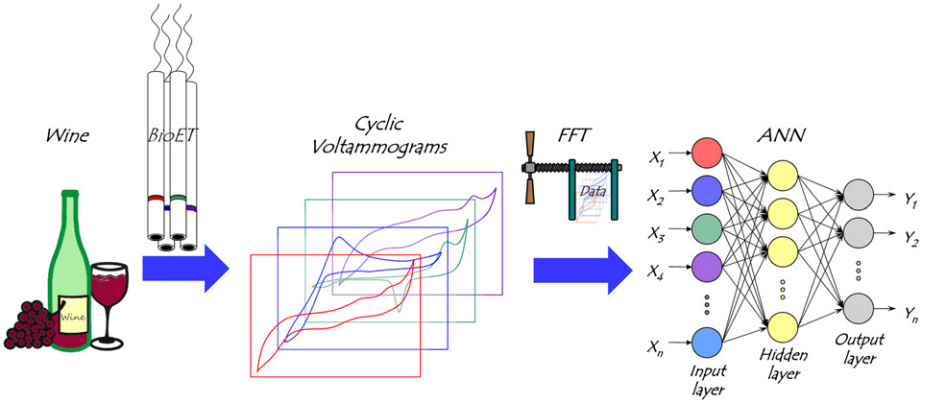

Fig. 3. Voltammetric BioET approach. Voltammetric signals are compressed employing FFT and the obtained coefficients are taken as input in the ANN. Appropriate weights and biases are applied by the learning algorithm until the targets are reached within the established error.

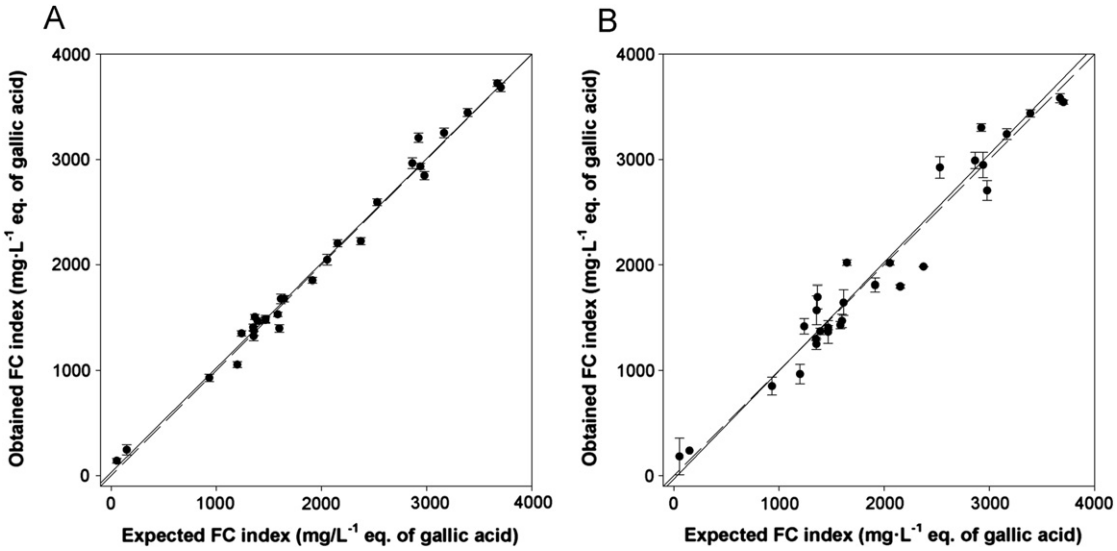

Fig. 4. Modelling ability of the optimized FFT–ANN. Sets adjustments of expected vs. obtained concentrations for Folin–Ciocalteau index, both for (A) training and (B) testing subsets. Dashed line corresponds to theoretical diagonal line. Results provided correspond to the average of the values obtained for each sample after 29 repeated calculations, done with random division of samples for train/test subsets each time. Uncertainties calculated at the 95% confidence level.

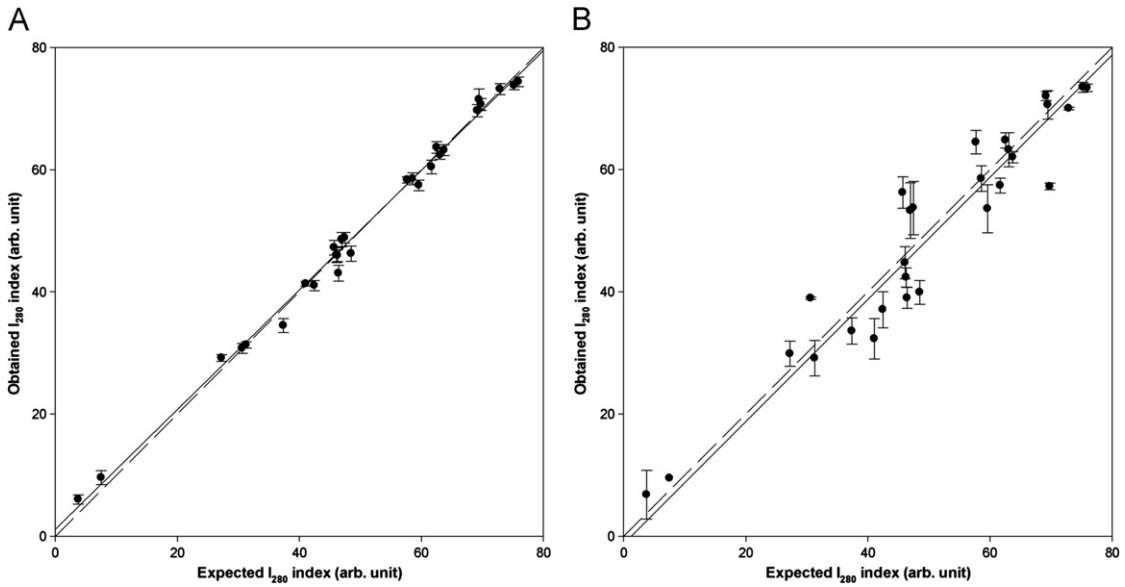

Fig. 5. Modelling ability of the optimized FFT–ANN. Sets adjustments of expected vs. obtained values for I_{280} index, both for (A) training and (B) testing subsets. Dashed line corresponds to theoretical diagonal line. Results provided correspond to the average of the values obtained for each sample after 29 repeated calculations, done with random division of samples for train/test subsets each time. Uncertainties calculated at the 95% confidence level.

Table 1 summarizes the obtained regression parameters. As expected from the comparison graphs, a good linear trend is attained for all the cases, but with better correlation coefficients in the training subsets due to the lower dispersion. Despite this, the results obtained for both subsets are close to the ideal values, with intercepts close to 0 and slopes and correlation coefficients practically 1.

As an additional verification of the proposed approach, a Student's paired samples *t*-test was performed between both reference methods and the BioET methodology. Obtained experimental *t* values were 0.025 and 0.127 for the comparison against FC and I_{280}, respectively, while the critical tabulated *t* value with 95% confidence level and 28 degrees of freedom was 2.048. Therefore, from the comparison graphs and these *t*-test results it could be concluded that there are no significant differences

Table 1
Results of the fitted regression lines for the comparison between obtained vs. reference values for the BioET, both for Folin–Ciocalteu and I_{280} indexes. Results provided correspond to the average of the values obtained for each sample after 29 repeated calculations, done with random division of samples for train/test subsets each time. Uncertainties calculated at the 95% confidence level.

	Correlation	**Slope**	**Intercept**	**RMSE** (mg L^{-1})
Folin–Ciocalteu index (54 to 3705 mg L^{-1} gallic acid)				
Train subset	0.994	0.993 ± 0.008	29.5 ± 17.4	102
Test subset	0.978	1.027 ± 0.033	-31.6 ± 74.0	205
	Correlation	**Slope**	**Intercept**	**RMSE** (arb. unit)
I_{280} **index** (3.78 to 75.93 arbitrary units)				
Train subset	0.995	0.979 ± 0.007	1.15 ± 0.39	1.85
Test subset	0.949	0.999 ± 0.046	-1.23 ± 2.57	5.17

between the BioET predicted values and the ones obtained with the reference methods.

3.4. Discrimination and classification of individual polypehnols

In this qualitative approach, spiked wine samples with reference polyphenols were analyzed in random order with the BioET array, following equivalent measuring procedure as before. Then, the obtained responses were processed employing PCA analysis and grouped using cluster analysis tools, which 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. Thus, a preliminary recognition was attained which allowed the visually distinction of the formed groups.

As can be seen in Fig. 6, with the two first PCs, the explained variance accumulated was ca. 98% and a clear distinction between clusters is obtained; this large value means that nearly all the variance contained in the original information is represented by the two new coordinates. Patterns in the figure evidence that samples are grouped based on which polyphenol was added. These well established clusters clearly separate the main classes of samples corresponding to: (I) wine, (II) gallic acid, (III) (±)-catechin, (IV) *p*-coumaric acid, (V) caffeic acid, (VI) catechol, (VII) phenol, (VIII) *m*-cresol, (IX) ferulic acid, (X) chlorogenic acid and (XI) quercetin. This satisfactory separation of classes validates on its respect, the proper selection of (bio)sensors to form the BioET.

Again, an ANN was trained to act as a classifier of the above stated groups. Unlike the previous case, where the responses obtained from the model were quantitative variables, in this case the output of the ANN model was formed by binary predictors (1/0) for each of the classes. As in the previous case, the ANN configuration needs to be optimized. Then after some preliminary tests, the final ANN architecture model had 3 neurons (corresponding to the first three components of the PCA, with explained variance accumulated ca. 99%) in the input layer, 7 neurons in the hidden layer and 11 binary (1/0) neurons in the output layer (one for each class) with *logsig* transfer function in the three layers. The backpropagation algorithm was used to train the network and the expected output error was programmed to reach a value of 0.01.

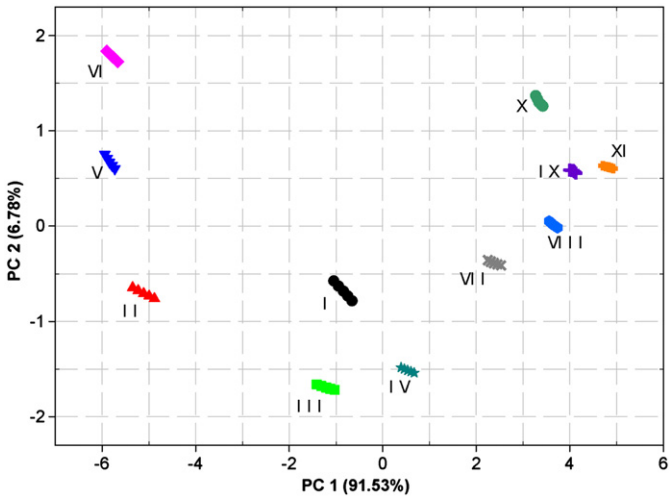

Fig. 6. Score plot of the first two components obtained after PCA analysis of the spiked wine samples. A total of 55 spiked wine samples were analysed. As can be seen, clear discrimination is obtained for the different polyphenols considered: (I) wine, (II) gallic acid, (III) (±)-catechin, (IV) *p*-coumaric acid, (V) caffeic acid, (VI) catechol, (VII) phenol, (VIII) *m*-cresol, (IX) ferulic acid, (X) chlorogenic acid and (XI) quercetin.

PCA-ANN model was trained with 60% of the data (33 samples) and confusion matrix was performed using the information of the testing set (remaining 40% of the data; 22 samples) in order to characterize the accuracy of the identification model and obtain unbiased data. Unlike the previous case, a k-fold strategy was used instead of jack-knife data division, given in this case it was dealing with a qualitative approach, which means an easier modelling situation and with less statistical parameters; besides that, five replicates for each sample were measured.

From the classification results, the corresponding confusion matrix was built. Correct classification for all the classes was obtained (i.e., a classification rate of 100% for each of the groups), as could be explained from the direct visualization of the PCA analysis. The percentage of correct classifications was estimated, from individual sample calculation in the test subset, as 100%. The efficiency of the classification obtained was also evaluated according to its sensitivity, i.e., the percentage of objects of each class identified by the classifier model, and to its specificity, the percentage of objects from different classes correctly rejected by the classifier model. The value of sensitivity, averaged for the classes considered, was 100%, and that of specificity was 100%.

4. Conclusions

In summary, a BioElectronic Tongue based on voltammetric enzyme-modified biosensors with different modifiers (tyrosinase and laccase on one side and copper nanoparticles on the other) has been applied in wine analysis in order to create a tool capable of quantifying total polyphenol content and discriminate individual polyphenolic compounds. Pre-processing tools such as FFT (an approach rarely used in literature with voltammetric data) could provide reasonable compression of data preserving relevant information, while the use of ANN allowed us to predict phenolic content index obtained with two different reference methods (Folin–Ciocalteu and I_{280} indexes). Finally, PCA allowed the discrimination of individual polyphenolic compounds.

With this and the previous results, proposed BioET has demonstrated its powerful applications in winemaking industry in the analysis of polyphenols found in wine, both in the quantitative analysis of total polyphenol content and in the resolution and quantification of phenolic mixtures, and also in the qualitative discrimination and classification of polyphenolic compounds. Its performance characteristics may satisfy food industry requirements of precision, rapidity, sensitivity, simplicity and low cost required to be considered as a useful analytical tool.

Furthermore, it represents an alternative to Folin–Ciocalteu method reducing considerably analysis time (from 30 min to ca. 3 min), avoiding the sample pretreatment (proper dilution factor) and the use of reagents (Folin–Ciocalteu and sodium carbonate). At the same time and after proper training the BioET, it is possible to perform a discrimination of individual phenolic compounds in an application more comparable to HPLC.

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