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Monitoring the aging of beers using a bioelectronic tongue

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ABSTRACT

This paper deals with the implementation and the application of a bioelectronic tongue including three enzymatic biosensors based on tyrosinase and phthalocyanines as electron mediators, to evaluate the changes that occur during the aging of beers. For this purpose, alcoholic and non alcoholic beers, packaged in can and bottle, have been analyzed using cyclic voltammetry. The electrochemical signals showed significant changes during the aging process. The features extracted from the cyclic voltammograms have been used to perform Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA). Data have revealed a clear discrimination among the beer classes in the aging process and the results were confirmed by Probabilistic Neural Networks (PNN) with Radial Basis Functions (RBF) and FeedForward Networks with Backpropagation (BP) learning method. The bioelectronic tongue has demonstrated a good capability to discriminate and classify the beer types satisfactorily in such a way, for all beer treatments, full classification accuracy was found.

1. Introduction

In recent years, the demand for advanced instrumental devices in brewery has increased constantly. Brewing industries try to make beers with characteristic organoleptic properties with small changes from production to consumption stages. The factors determining flavor robustness in beer are complex. Moreover, achieving flavor stability is a major challenge, especially as what happens for the beers between packaging and consumption that is often out of the control of the brewer. Therefore, the quality change after packaging of beer is a main concern because the flavor fingerprint of beer can be influenced by many factors deteriorating gradually the quality of beer. Nonetheless, optimization of the brewing process with respect to flavor stability requires a clear insight of the amount of flavor fingerprint changes during the aging of beer (Vera, Acena, Guasch, Boque, Mestres, & Busto, 2011;Ghasemi-Varnamkhasti, Mohtasebi, Siadat et al., 2011).

Beer flavor is conventionally detected through the combination of common analytical tools (e.g., gas chromatography and FTIR) and human sensory panels (Lachenmeier, 2007; Malfliet et al., 2008; Sohrabvandi, Mousavi, Razavi, & Mortazavian, 2010). The advent of multisensors systems such as electronic and bioelectronic tongues (or array of biosensors) has shown a bright future for the quality enhancement in food industries and many researchers have worked on the application of such systems instead of traditional systems (Apetrei et al., 2011; Ciosek, Kraszewska, & Wroblenski, 2009; Del Valle, 2010; Di Natale et al., 2000; Escuder-Gilabert & Peris, 2010; Ghasemi-Varnamkhasti, Mohtasebi, & Siadat, 2010; Riul, Dantas, Miyazaki, & Oliveira, 2010; Rodriguez-Mendez & de Saja, 2009; Toko, 1998, 2006; Vlasov, Legin, & Rudnitskaya, 2008; Vlasov, Legin, Rudnitskaya, D'Amico, & Di Natale, 2000; Winquist, Holmin, Krantz-Rülcker, Wide, & Lundström, 2000; Winquist, Krantz-Rulcker, & Lundstrom, 2008; Zeravik, Hlavacek, Lacina, & Skladal, 2009). Only few publications dedicated to flavor assessment of beers using electronic tongues have been reported in literature (Arrieta, Rodriguez-Mendez, de Saja, Blanco, & Nimubona, 2010; Ghasemi-Varnamkhasti, Mohtasebi, Rodriguez-Mendez, Siadat et al., 2011; Lvova, Paolesse, Di Natale, & D'Amico, 2006; Polishin et al., 2010; Rudnitskaya et al., 2009; Toko, 1996).

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These works have correlated the attributes obtained by a sensory panel or by chemical parameters obtained by chromatography with the results of electronic tongues. These results were promising for a number of beer samples. The majority of the beer samples studied by these authors were alcoholic beers even though few non alcoholic beer brands were also included in their works. Most of these works have been carried out using potentiometric or amperometric sensors.

Electrochemical sensors (potentiometric, impedimetric and voltammetric) are the most widely used sensing units in electronic tongues. In particular, most of the works in this field involve signal generation from the potentiometric sensors where the potential value created by the diffusion of ions across a membrane is measured (Gutés, Céspedes, Alegret, & del Valle, 2005).

The performance of a voltammetric multisensor system can be improved by using electrodes chemically modified with electroactive materials. Using chemically modified electrodes, peaks associated to the oxidation-reduction of the electrodic material and of the analytes present in the test solution can be observed. The interactions that occur between the electrode and the solution can improve extraordinarily the selectivity of the electrodes (Arrieta et al., 2010; Rodriguez-Mendez et al., 2008).

Phthalocyanines have also demonstrated to be suitable materials for preparing voltammetric modified sensors because their rich electrochemical behavior. In addition, the electrochemical and sensing properties of phthalocyanines can be modulated by changing the central metal atom or by introducing substituents in the phthalocyanine rings. As reported in the literature (Arrieta, Rodriguez-Mendez, & De Saja, 2003; Gay et al., 2010; Parra et al., 2006), sensors based on phthalocyanines prepared using the carbon paste electrode technique (CPE) have shown good performance and responses towards antioxidants in the experiments of cyclic voltammetry. When a voltage is applied, peaks associated to the oxidation-reduction of the analytes present in the test solution can be observed. In addition, transient responses caused by redox processes associated to the phthalocyanine deposited onto the electrode material are also observed. Interactions between the solution and the phthalocyanine give rise to rich voltammograms with a high degree of selectivity (Rodriguez-Mendez, Gay, & de Saja, 2009). However, the electrochemical behavior of the phthalocyanine electrodes has been found to be strongly dependent on the structure of the electrode, which can be altered by using different electrode preparation techniques (Casill et al., 2005; Rodriguez-Mendez, Gay, Apetrei, & De Saja, 2009).

The concept of biosensor array has been considered in food quality and safety characterization as documented by Gutés et al. (2005). Arrays formed by biosensors show an increased selectivity due to the specificity enzyme-substrate. The performance of biosensors can be improved by introducing electron mediators (ferrocene, conducting polymers, etc) that facilitate the electron transfer from the enzyme to the electrode (Apetrei et al., 2011; Ozsoz, Erdem, Kilinc, & Gokgunnec, 1996; Sergeyeva et al., 1999).

Recently our group has developed biosensors containing tyrosinase (specific for the detection of phenols) that use phthalocyanines as electron mediators (Apetrei et al., 2011). The performance of the arrays of voltammetric sensors formed by chemically modified electrodes can be improved introducing in the array of biosensors. Therefore, the advantages of the selectivity attained by chemically modified electrodes can be enhanced with the help of biosensors. In particular, biosensors containing tyrosinase have been used in multisensor arrays for the detection of phenols and polyphenols. These compounds have an important influence on the organoleptic characteristics of beers (Ghasemi-Varnamkhasti, Mohtasebi, Rodriguez-Mendez, Siadat et al., 2011; Martinez-Perinan et al., 2011).

The objectives of this work were: a) evaluation of the possibility of the use of an innovative multisensory system based on voltammetric sensors chemically modified with phthalocyanines where enzymes have been incorporated. Phthalocyanines have the double role of chemical modifiers and electron mediators, and b) monitoring the aging of beers. For this purpose, an array of three biosensors containing tyrosinase and phthalocyanines was developed. The array was exposed to four selected beers and the aging was monitored periodically. The discrimination capability of the system has been evaluated by use of Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA). The voltammograms recorded using these biosensors were also processed using artificial neural network (ANN).

To date, no research on the aging detection of beer by use of bioelectronic tongue has been published (Ghasemi-Varnamkhasti, Mohtasebi, Rodriguez-Mendez, Lozano et al., 2011; Ghasemi-Varnamkhasti, Mohtasebi, Rodriguez-Mendez, Siadat et al., 2011; Ghasemi-Varnamkhasti, Mohtasebi, Siadat et al., 2011). Therefore, this study was aimed on the development and the application of an array of biosensors coupled with artificial neural network to study the monitoring of the fingerprint changes in beer during the aging process.

2. Materials and methods

2.1. Biosensor implementation

All reagents were obtained from Aldrich and used without further purification, except otherwise indicated. Three electrochemical sensors were fabricated: one unmodified carbon paste electrode (CPE), a CPE based on cobalt phthalocyanine (CoPc) and a CPE based on iron phthalocyanine (FePc). The carbon paste electrodes were prepared by mixing the corresponding phthalocyanine (15% w/w) with carbon powder (Ultracarbon, Ultra F purity) in a mortar. Then a binder (Nujol oil) 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 were introduced in a plastic syringe (1 mL), and compressed. A copper wire was used as a contact. The CPEs were finally smoothed manually by a clean filter paper (Apetrei, Rodriguez-Mendez, Parra, Gutierrez, & De Saja, 2004; Apetrei, Rodriguez-Mendez, & De Saja, 2004; Rodriguez-Mendez, Gay, Apetrei, et al., 2009). Afterward, biosensors were developed as follow: an adequate quantity of tyrosinase enzyme was dissolved in phosphate buffer (0.1 M, pH: 7). Then, the immobilization of tyrosinase was accomplished by addition of 5 μL aliquot of tyrosinase solution and 5 μL of 7% glutaraldehyde solution on the electrode surface (each biosensor had 225 Units/Electrode of tyrosinase). The mixture was then allowed to dry for 1.5 h at room temperature ($23 \pm 2\ ^{\circ}C$) for polymerization. The biosensors were rinsed with deionized water and stored in pH = 7.00, 0.1 M phosphate buffer at room temperature for at least 2 h before the use in the experiments. The biosensors cannot be stored for more than 10 h and they were prepared just before experiments (Dall'Orto, Vago, Carballo, & Rezzano, 2005; Elkaoutit et al., 2008; Elkaoutit, Naranjo-Rodriguez, Temsamani, & de Cisneros, 2007).

2.2. Measurement procedure

Four beer types from the same brand were provided in this research: alcoholic beer packaged in can (CA), alcoholic beer packaged in bottle (BA), non alcoholic beer packaged in can (CN) and non alcoholic beer packaged in bottle (BN). To mimic the long period storage, the beer samples were stored in an oven (in 40 °C, in dark). This procedure is known as forced aging in the literature (Guido, Fortunato, Rodrigues, & Barros, 2003; Rodrigues et al., 2011;

Fig. 1. Bioelectronic tongue system: a) measuring set up, b) potentiostat c) one sample of biosensors fabricated, d) the biosensors immersed in buffer before experiments.

Walters, Heasman, & Hughes, 1997a,b). The interval time of 5 days was considered among the experiments (non treated beer has been designated as N and F1, F2, F3, and F4 correspond to aged beers measured at 5 days interval).

The measuring system and the biosensor array are illustrated in Fig. 1. Electrochemical experiments were carried out in an EG&G PARSTAT 2273 potentiostat/galvanostat using a conventional three-electrode cell (Fig. 2) containing 50 mL of a mixture of the solution of the phosphate buffer and the beer (for the mixture: pH = 7.00). The beer samples were decarbonated according to the literature (Siebert & Lynn, 2007). The experiments were performed at 20 ± 2 °C and the biosensors were used as the working electrodes and the reference electrode was Ag|AgCl/KCl 3 mol L^{-1} and the counter electrode was a platinum wire as well. For this study, cyclic voltammetry was considered and cyclic voltammograms were recorded from −0.4 to +0.7 V (the scan started at 0.0 V) at a sweep rate of 0.12 Vs^{-1}.

2.3. Feature extraction and data analysis

All beer samples were measured seven times with each biosensor. The voltammograms were pre-processed using the adaptation of a data reduction technique based on predefined response "bell shaped-windowing" curves called "kernels" (Gay et al., 2010; Villanueva et al., 2006). Using this method, the voltammogram curve (i vs. E) is multiplied by a number of 10 smooth, bell-shaped windowing functions, and integrated with respect to potential. Using a program written in Matlab, the area under each voltammogram is divided into 10 parts and the features are extracted from each part. The idea behind this pre-processing technique is to capture the information throughout the global response to obtain 10 parameters per curve. Therefore, the array of three sensors provides 30 variables for each beer. The software of Matlab 7.6 (The Mathworks Inc., Natick, MA, USA) was used to analyze the data collected and to perform artificial neural network as well.

In this work, Principal Components Analysis (PCA) and Linear Discriminant Analysis (LDA) as the data reduction methodologies are used to reduce the number of variables of the dataset and retaining most of the information in the data. Score plots of the data are illustrated and the PCA and LDA results are then confirmed by artificial neural networks.

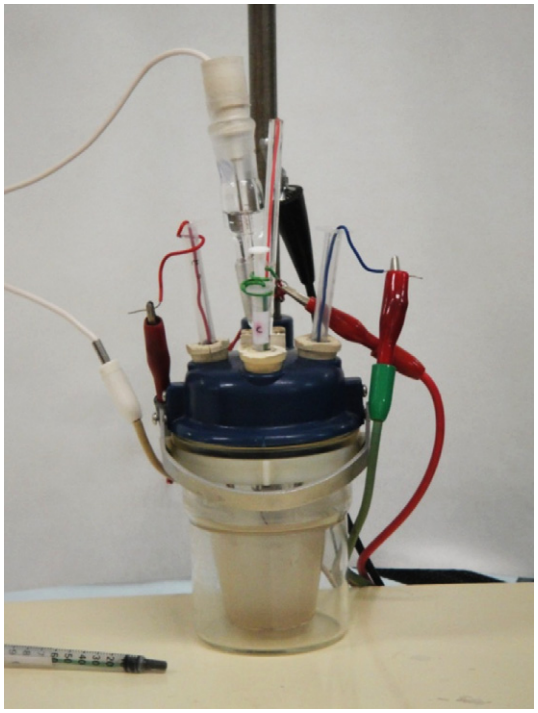

Fig. 2. Electrochemical cell including working electrode (biosensor), reference and counter electrodes.

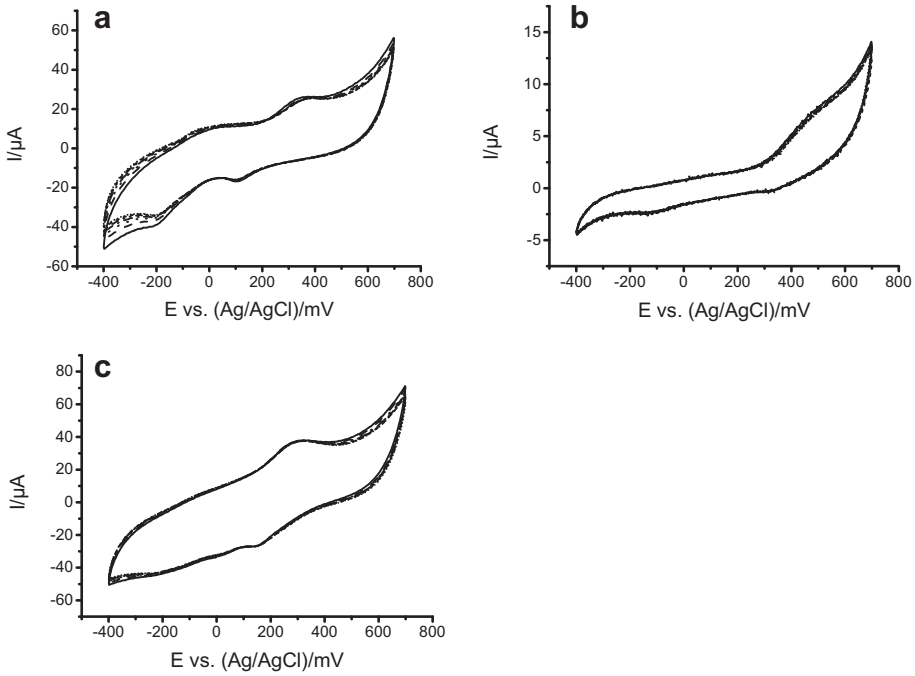

Fig. 3. The seven consecutive voltammograms of the bioarray immersed in alcoholic beer packaged in bottle (BA) with high repeatability and reproduceability: a) CoPc based biosensor, b) CPE based biosensor, c) FePc based biosensor.

A probabilistic neural network (PNN) is used for classification purposes. The PNN is included three layers: the input one has three neurons, corresponding to the three principal components; the hidden layer, with radial basis transfer functions, has the same number of neurons that number of training vectors and a competitive layer in the output. For checking the performance of the network, leave-one-out cross validation method is employed. Leave-one-out consists of training N distinct nets (in this case, N is the number of measurements) by using N – 1 training vectors; while the validation of the trained net is performed by using the remaining vector, excluded from the training set. This procedure is repeated N times until all vectors are validated (Lozano, Arroyo, Santos, Cabellos, & Horrillo, 2008; Lozano, Santos, & Horrillo, 2008). Also, a backpropagation (BP) network topology is formed by three layers: the input layer has three neurons related to the first three components, a variable number in hidden layer, and five neurons in the output layer relevant to the aged beer classes. The network considers the inputs and compares its outputs in opposition to the desired outputs. Errors are then propagated back through the system, causing the system to adjust the weights that control the network. This process takes place over and over as the weights are repeatedly tweaked. During the network training, the same set of data is processed many times as the connection weights are always refined (Ciosek et al., 2005; Lozano, Arroyo et al., 2008; Marini, 2009). In final, the success rates for the classification of each beer type and whole beers are computed.

3. Results and discussion

As mentioned earlier, the aim of this research is to establish if the bioelectronic tongue is able to recognize the beer samples in the aging process. For this purpose, we should consider the voltammograms of the bioarray as the aging fingerprints of the beers under study. The peak position and their intensities give information about the chemical composition and the changes occurring in aging process (Guido et al., 2003).

Performing the experiments, each biosensor showed a particular response when immersed in the beer. This cross selectivity is depicted in Fig. 3 where the voltammograms of the bioarray toward the alcoholic beer packaged in bottle (BA) are illustrated. The voltammograms showed high repeatability and reproduceability in such as way in all cases of use, the coefficient of variation of seven consecutive measurements were lower than 3%.

As seen in Fig. 4, in the voltammogram of non treated beers (N), one peak is seen with an intense anodic wave at ca. 194–467 mV. This peak can be associated to the flavonoid acids which possess easily oxidable orto-diphenol groups (El-Hady & El-Maali, 2008; Korbut, Buckova, Labuda, & Grundler, 2003). Such compounds include caffeic acid, gallic acid, tannic acid, catechin, etc (Bamforth, 2004, 2006; Sohrabvandi, Mousavi, Razavi, Mortazavian, & Rezaei,

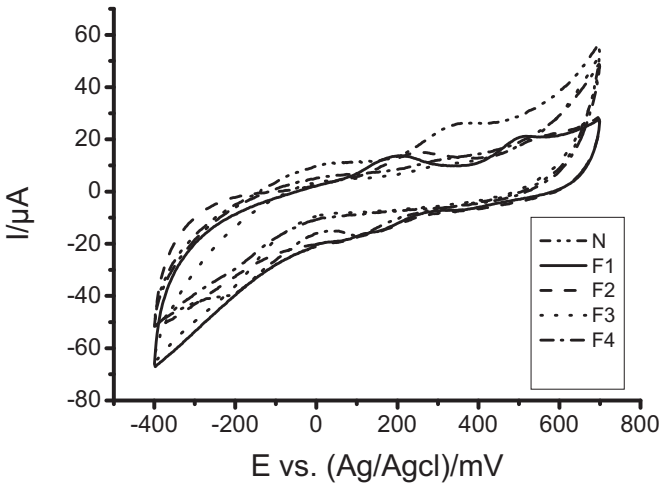

Fig. 4. The voltammograms of CoPc based biosensor for alcoholic beer packaged in bottle (BA), (N is designated as non treated beer and F1, F2, F3, and F4 correspond to aged beers).

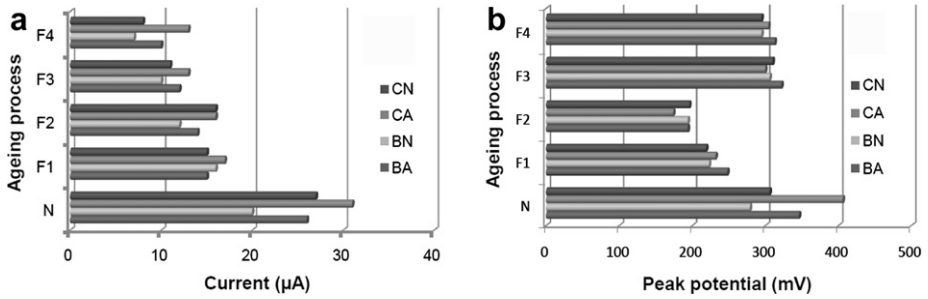

Fig. 5. Fingerprint changes associated with flavonoid during the aging of beer: a) variation of the current relevant to the peak potential, b) variation of the peak potential.

2010). In addition, other compounds such as ascorbic acid can also be oxidized in this region. During the aging of the BA, the intensity of the peak associated to the presence of flavonoid has been decreased (Fig. 5). This means that flavonoid content in beer tends to be decreased in aging process. The latter observation is in close agreement with the findings of Callemien and Collin (2007) who reported that flavanoids significantly change during the storage of beer in such a way (+)-catechin content decrease in the aged beer leading to color instability. Previously, similar reports had been documented in the literature (Vanderhaegen, Neven, Verachtert, & Derdelinckx, 2006; Walters et al., 1997a, b).

Also, as seen in Fig. 5, the peak potential of flavonoids at ca. 346 mV shifts to lower values. This fact can be associated to the modification of the chemical nature of flavonoid that facilitates their oxidation. After packaging, the oxidation of flavonoids is more difficult and the oxidation potential decreases slowly. The changes in intensity and the position of the peaks associated to flavonoid can explain the capability of the bioelectronic tongue to characterize the aging of beers. This ability of bioelectronic tongue is very helpful for the continuous monitoring of such compounds in beer matrix during the production and storage processes because these compounds affect the physical and colloidal stability of beer in these stages and the level of the latter compounds should be controlled in beer (Elkaoutit et al., 2007; McMurrough, Madigan, Kelly, & Smyth, 1996). Therefore, the biosensors employed in the bioelectronic tongue could be considered as an alternative tool instead of colorimetric assay or liquid chromatography (LC) for the analysis of total flavonoids in the range of commercial beers (Cummings et al., 2001; Eggins, Hickey, Toft, & Zhou, 1997).

As follow, the results of individual beers (CA, BA, CN, and BN) as well as whole beer types in five aging treatments are presented and discussed. In this study, principal components analysis (PCA) is used as a data reduction methodology. The objective of PCA is to reduce the dimensionality (number of variables) of the dataset while retaining most of the original variability (information) in the data. This is performed through the construction of a set of principal components which act as a new reduced set of variables. Each principal component is a linear combination of the original variables and they are all orthogonal to each other. For a given data set with N variables, the first principal component has the highest explanatory power (it describes as much of variability of the data as possible), whereas the N^{th} principal component has the least explanatory power. Thus, the *n* first principal components are supposed to contain most of the information implicit in the attributes.

The PCA results of individual beers and whole beers are illustrated in Fig. 6 in which aging groups of beers are clearly discriminated. The first two principal components account the maximum amount of variance in the original dataset. The components accounting for a large variation in the data are known as the new axis to attain the plots of the beer classes called as score plot (Fig. 6). The PCA score plots of PC1-PC2 account for 98%, 96%, 98%, 98%, and 85% of variance for CA, BA, CN, BN, and whole beer types, respectively. A clearly discrimination is seen for individual beer types but the groups shown in Fig. 6e are partially overlapped. This observation shows that in spite of storing the beer samples at the same conditions but the fingerprint changes for each beer type are different in aging process that confirms the profound role of alcohol and packaging container on beer stability as pointed out in literature (Bartolome, Pena-Neira, & Gomez-Cordoves, 2000; Ghasemi-Varnamkhasti, 2011; Hardwick, 1994; Preedy, 2009). Also, as seen in Fig. 6, the speed of fingerprint change in early stages of the aging process is lesser than that of in lately stages indicating the more stability in early stages of aging process; even though in general, the speed of aging is dependent on many factors such as storage conditions and beer composition (Vanderhaegen, Delvaux, Daenen, Verachtert, & Delvaux, 2007).

Besides the beer groups, the variables could be depicted in the same plot, named loadings, by the values of their coefficients of the eigenvector equations. All of loading plots are not presented here and for example, one loading plot is illustrated in Fig. 7. The plot shows the relative contribution of the variables used in bioelectronic tongue to each principal component; the higher the loading value of a specific biosensor on a principal component, the more variable contribution with this component. It is obvious that having the most important variables could have a significant role in computation stage of the data because sometimes considering many variables to data analysis maybe led to some problems like over fitting in analysis; for instance neural networks are normally simple to implement using a standard program with a user friendly interface. In some cases of ANN, one problem is often that networks based on many input variables need a long computing time. Also, the loadings plot of this data could give us this information that which biosensor(s) could be removed from the array while one wants to reduce the fabrication cost of the biosensor array. The loading plots reveal that CoPc based biosensor has the most contribution in aging fingerprint recognition of beers studied. It is well known that cobalt phthalocyanine complexes can effectively catalyze the electro-oxidation of organic compounds for the function of redox mediators. Electrodes modified with these compounds have shown great promise for the electrocatalytic determination of some phenolic compounds (Gay et al., 2010; Yin, Zhou, & Ai, 2009).

LDA has been also employed in the current study, like PCA, as a feature reduction method determining a smaller dimension hyper plane on which the points will be projected from the higher dimension. However, whereas PCA picks a direction that keep maximal structure among the data in a lower dimension, LDA picks a direction that achieves maximum separation among the groups given. The results obtained by LDA showed a more appropriate

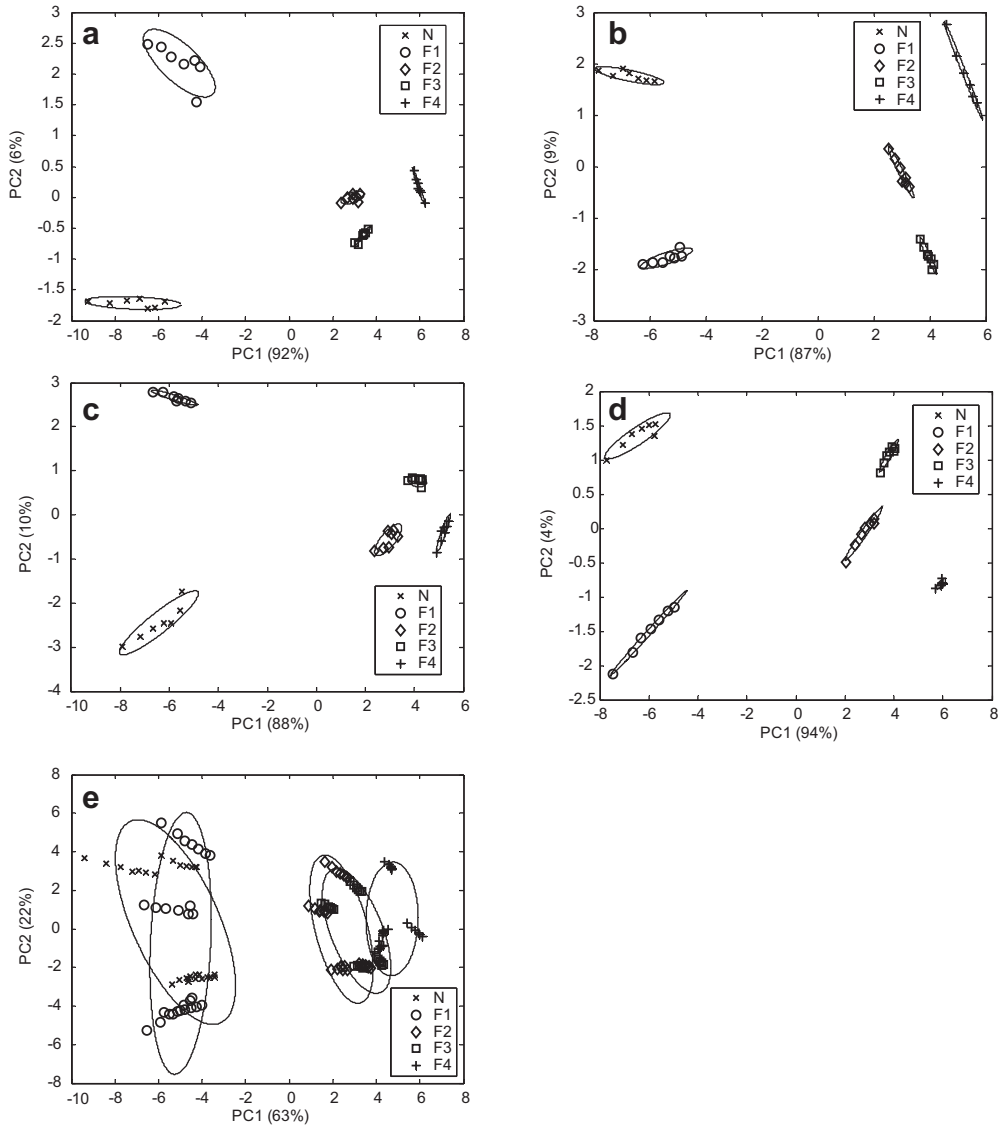

Fig. 6. Score plot of the PCAs of the array of biosensors exposed to the samples of a) CA, b) BA, c) CN, d) BN, e) whole beer types. (N is designated as non treated beer and F1, F2, F3, and F4 correspond to aged beers).

classification compared to PCA results as some of them have been illustrated in Fig. 8. In LDA method, the variance between beer groups as well as the variance within beer groups is maximized and looks for a rational rule to recognize between them by use of the formation of the linear functions of the data maximizing the ratio of the between-group sum of squares to the within-group sum of squares. Therefore, the linear functions are limited to be orthogonal. When the linear functions are found, an observation is classified through computation of its Euclidean distance from the class centroids, projected onto the subspace which is defined by a subset of the linear functions. Afterward, the observation is assigned to the closest class. In this study, the performance of this method was assessed in such as way the group centroids were estimated using a leave-one-out cross validation method. The group centroids are computed without reference to the missing data point as well as each observation is removed in turn from the data set. The excluded observation is then classified considering these new class centroids. The data point is then replaced and the next observation removed from the data set accordingly. When all observations have been left out in turn, this process is stopped. Thus, the percentage of the observations correctly classified can be determined through comparison of the true class membership with that estimated by LDA. This presents an appropriate criterion of the reliability of the classification method (Otto, 2007; Zhao, Wang, Lu, & Jiang, 2010).

Then, the PCA and LDA results were confirmed with the ANN studied. In the training of the BP network, different number of neurons in the hidden layer was tried. In addition, several activation functions (pureline, tansig and hard-limit) were tested for the output layer. The optimal number turned out to be 15 neurons by several times tested and better results obtained through pureline activation function. Finally, the classification success was found to be 100% for individual beers and whole beer types under aging process. For instance, Table 1 is presented herein as related to CA beer. The similar results were obtained using PNN as full classification accuracy where the confusion matrix of whole beer types is given in Table 2.

In this research effort, employment of such chemometric methods showed the capability of the bioelectronic tongue to

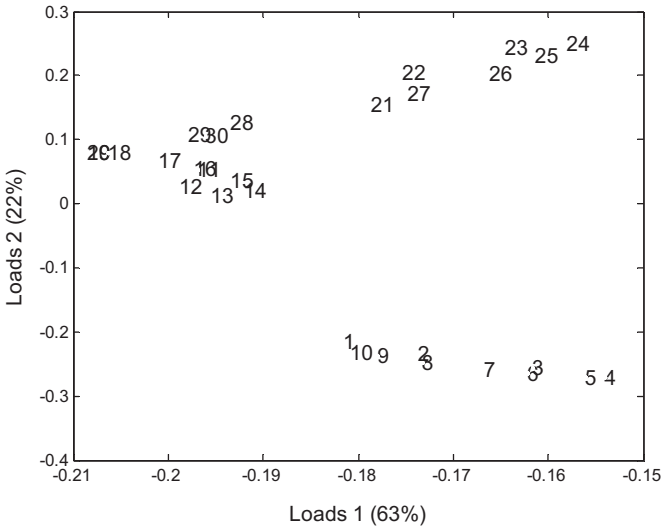

Fig. 7. Loadings plot of the PCAs of the array of biosensors exposed to the whole beer types (variables 1–10, 11–20, and 21–30 are dedicated to CoPc, CPE and FePc based biosensors, respectively).

Table 1
Confusion matrix with 15 neurons in hidden layer for the BP classification of alcoholic beer packaged in can (CA) in aging process.

Real/predicted	N[a]	F1	F2	F3	F4
N	7	0	0	0	0
F1	0	7	0	0	0
F2	0	0	7	0	0
F3	0	0	0	7	0
F4	0	0	0	0	7
Success rate (%): 100					

[a] N : Non treated and F1, F2, F3, F4, F5: Aged beers.

recognize the beers in terms of the fingerprint changes during the aging process. As found in this study, the fingerprint of the beer samples changes in aging process mimicking the long period storage (Walters et al., 1997b). The level of stability of such beers could be revealed or characterized by bioelectronic tongue as done in this study. According to the fingerprints detected by bioelectronic tongue, brewers could assess several strategies which could improve the stability of beer. However, many factors contribute to the aging of beer and can be basically divided into intrinsic factors (i.e. compositional ones) and extrinsic factors (i.e. events and conditions out with the beer but to which the beer is exposed). Although the formula makes no attempt to weigh the various parameters, it is clear that a change in any one of them will impact flavor stability. Also, it is worth mentioning that the composition of beer is established on the basis of the raw materials used in its manufacture and on the processing procedures employed. From grain and hops growing in the field right way through to closing the final container, there are forces at play that can impact the quality and the ensuing shelf life of the product (Bamforth, 2004, 2006). Therefore, using bioelectronic tongue to aging fingerprint characterization of beer in aging process, a brewer could obtain valuable information on the level of stability of beer to manage or control the factors contributing to flavor stability of beer (Ghasemi-Varnamkhasti, Mohtasebi, Rodriguez-Mendez, Lozano et al., 2011; Ghasemi-Varnamkhasti, Mohtasebi, Rodriguez-Mendez, Siadat et al., 2011; Ghasemi-Varnamkhasti, Mohtasebi, Siadat et al., 2011). In addition, using variable selection methods, it is feasible to obtain more exact information on the compounds possibly responsible for the differences among the beer samples under aging process.

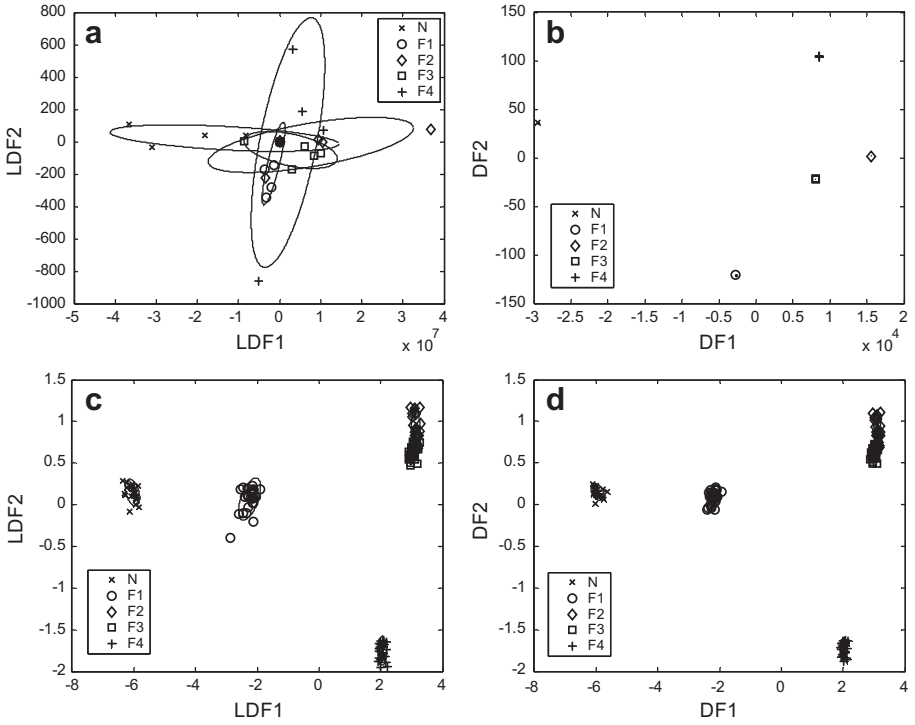

Fig. 8. Score plot of the array of biosensors exposed to the samples: a) CA by LDA, b) CA by LDA without validation, c) whole beer types by LDA, d) whole beer types by LDA without validation. (N is designated as non treated beer and F1, F2, F3, and F4 are designated as aged beer treatments).

Table 2
Confusion matrix for the PNN classification of whole beer types in aging process.

Real/predicted	N[a]	F1	F2	F3	F4
N	28	0	0	0	0
F1	0	28	0	0	0
F2	0	0	28	0	0
F3	0	0	0	28	0
F4	0	0	0	0	28
Success rate (%): 100					

[a] N : Non treated and F1, F2, F3, F4, F5 : Aged beers.

4. Conclusion

The capability of a bioarray based on phthalocyanine derivative electrodes coupled to ANN for the discrimination of beer samples during the aging has been evaluated. The aging process of beer and the changes arisen during this phenomenon can be detected and characterized using a bioelectronic tongue. This capability is related to the changes in the phenolic composition and in particular the changes of flavonoid levels that occur during aging. These changes are detected from the changes in peak position and current intensity values of the voltammograms attained. Application of this bioarray is of industrial interest because it could be used to address the level of beer stability to achieve high quality of beer. Since the bioarray developed in the current study can detect the antioxidant in beer, it can be used in brewery line to monitor the changes of these compounds as affected by production circumstances. For instance, monitoring of some phenolic compounds like flavonoid is very helpful in fermentation stage even though for each application, technical problems have to be solved.

More recently, integrated or combined systems have been reported to food evaluation (Apetrei et al., 2010; Casale, Oliveri, Armanino, Lanteri, & Fornia, 2010). This idea could be considered where combination of bioelectronic tongue, electronic nose and eye are used to get the comprehensive fingerprints related to odor, color and taste of the beer under aging.

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