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Beer discrimination using a portable electronic tongue based on screen-printed electrodes

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ABSTRACT

There is a wide variety of Lager beer styles that differ, among others characteristics, in their alcohol content and color. Beer contains a high concentration of electrochemical active compounds, some of them related to the color of these beverages. In the present work, the applicability of a portable electronic tongue based on an array of electrochemical screen-printed electrodes was applied to the analysis of *Lager* beers. Multivariate analysis of the obtained signals allowed establishing mathematical models able to predict the European Brewery Convention (EBC) color index and the alcoholic strength with an accuracy of 76% and 86% respectively. Moreover, a discriminant model was established, able to classify beer by styles with 100% degree of accuracy.

1. Introduction

Beer is an alcoholic beverage obtained by fermentation of a starch-rich wort from cereal grain such as malted barley, wheat, maize and rice. Depending on the yeast used, beers can be classified in Lager beer (*Saccharomyces Pastorianus*) or Ale (*Saccharomyces cerevisiae*). Ales are traditionally fermented at warmer temperatures (14/19 °C), while lagers are typically fermented at cooler temperatures (4–9 °C). The cooler fermentation and lower aging temperatures used with lager yeast slow down the yeast activity and require a longer maturation time. The cold environment inhibits the production of fruity aromas and other fermentation byproducts common in ales.

There is a wide variety of Lager beers with pronounced differences among them. The characteristics of each type of beer depend on the raw material used and the parameters employed in the different steps of the production process, including the alcoholic content, which affects the defects of taste in alcohol-free beer (Blanco et al., 2014). Therefore, despite sharing certain steps in their processing, there are some differential aspects in the composition and organoleptic characteristics among the different Lager styles, including color and alcoholic strength. The color of a beer is an important visual cue and part of the overall sensory appeal of evaluating a brew.

Among the substances that contribute to beer characteristics, especially to color, there are a large number of electrochemically active compounds, substances that can be detected by means of electrochemical techniques. These include polyphenolic compounds, natural antioxidants present in plants. Approximately 80% of these compounds come from the malt and 20% from the hop used in the elaboration process. Some of the major phenolic compounds present in beer are phenolic acids (ferulic, cinnamic, chlorogenic, vanillic, gallic, caffeic, and p-coumaric, syringic), derivatives of flavan-3-ol (catechin, epicatechin, procyanidin, prodelphinidin) and flavone glycosides (Leitao et al., 2011). Hodžić et al. showed that there is a relationship between the content of polyphenols and color so that by increasing the content of polyphenols, the intensity of the color of beer increases. A relationship has also been established between polyphenol content and the antioxidant activity (Hodžić et al., 2007).

In addition, several studies have established a relationship between the malting process used and the antioxidant content in beers. Pale malts, subjected to mild heat treatment, exhibit greater antioxidant activity and have a higher content of phenolic compounds than roasted malts, subjected to higher temperatures during drying and roasting (Samaras et al., 2005; Amarowicz, 2009). Recently, it has been shown that antioxidant capacity varies with the type of beer, and this difference could be attributed to different contents of polyphenols, free phenols and phenolic acids (Piazzon et al., 2010). Some beers categorized as Lagers include the following styles: pilsener, which is widely produced industrially and could be considered the most popular type of beer in the world

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(identified by its light yellow to golden color and usually balanced taste); doppelbock, which is a very dark brown version of lager and is exceptionally malty, with very little bitterness; European strong lager, which is a variation of a Malt Liquor, contains more malt for fermentation but it has relatively low hop levels, and lastly alcohol-free beer, which has lower polyphenol and other antioxidant compounds content than other types of beers and whose antioxidant activity is, therefore, also lower.

Although there are traditional methods of analysis for the control of beer production, which include the official analysis methods, recent years have witnessed the increase in the use of cleaner, faster and more economical techniques. In this sense, working methodologies such as electronic tongues (ETs) represent promising chemical systems for the analysis of foods and beverages (Deisingh et al., 2004).

ETs are analytical systems consisting of an array of electrochemical sensors coupled with advanced data processing tools able to interpret the complex electrochemical signals (Cetó et al., 2014a,b, 2012; Gutiérrez et al., 2013; del Valle, 2010; Escuder-Gilabert and Peris, 2010; Winquist, 2008). Their use is becoming more widespread in food analysis, given the advantages offered in tasks such as recognition and classification, quantification of components and prediction of properties. In this sense, there are many reports using ETs for the analysis of beer during its production (Gorjanović et al., 2010). Applications of ETs related to beer analysis are focused on the discrimination of beers and the prediction of some important parameters (Rudnitskaya et al., 2009; Arrieta et al., 2010; Polshin et al., 2010; González et al., 2001), on the monitoring of beer aging and its fermentation process (Ghasemi-Varnamkhasti et al., 2012; Kutyła-Olesiuk et al., 2012) and on the quantification of certain compounds present in beer (Escobar et al., 2013; Ghasemi-Varnamkhasti et al., 2011; Cetó et al., 2013a,b).

In this work, miniaturized Screen-Printed Electrodes (SPEs) which are cheap, disposable and suitable for working with sample microvolumes (Tymecki et al., 2004; Washe et al., 2013), have been used. Their disposable character avoids cleaning stages, typical of conventional electrodes, and have even demonstrated better properties than other kinds of electrodes in the quantitative determination of some of the compounds present in beer (Matemadombo et al., 2012), including alcoholic strength (Patel et al., 2001; Avramescu et al., 2002) and polyphenol content (Cummings et al., 2001), among others.

The present work has been undertaken to develop a portable electronic tongue formed by Screen-Printed Electrodes (SPE) built commercially with different sensing materials and without any modification. The system has been used to analyze several styles of Lager beers in a wide range of color index and alcoholic strength. The voltammetric signals have been pre-treated using the kernel method and as the input variable of multivariate analysis. This method attempts to be a real tool for classifying beer samples according to their beer style and to establish predictive correlations.

2. Experimental section

2.1. Beer samples

The measurements were made in 25 types of commercial beers (Table 1). Beers were selected attending to their alcoholic strength (from alcohol free to strong beers) and their EBC (European Beer Convention) color value (from golden to dark beers) searching for a wide range in both characteristics. All analyses were carried out from newly opened bottles.

The excess of CO_2 was removed by stirring the samples during 10 min before the measurements.

Table 1
Classification and characteristics of 25 analyzed beers.

Style	Color	%Vol	Number of samples
Free alcohol beer (BS)	Golden	<1.0	8
Pilsener (BP)	Golden	4.2–5.5	9
Doppelbock (BD)	Brown/dark	>7.0	5
European strong *Lager* (*BF*)	Golden/amber	>6.5	3

2.2. Electronic tongue

Electrochemical measurements were carried out in a DropSens stat400 potentiostat (Oviedo, Spain) using four Screen-Printed Electrodes (SPEs) made by DropSens. Their dimensions were $3.4 \times 1.0 \times 0.05$ cm. Voltammograms were registered with DropView 2.0 program. All electrodes employed share a pseudo-reference Ag/AgCl electrode and electrical contacts manufactured in the same material.

- DS-110, working electrode made of carbon.
- DS-250AT, working electrode made of gold.
- DS-410, working electrode made of carbon/Co-Phtahlocyanine.
- DS-550, working electrode made of platinum.

2.3. Voltammetric measurements

Voltammograms were performed between −0.6 V and 1.0 V, with a scan rate of 100 mV s^{-1}. Electrochemical measurements were performed at room temperature (25 °C). The robustness of the system was ensured by measuring the samples seven times with each sensor.

2.4. Data processing

The multivariate data analysis was performed by using the program Matlab V 7.0 and MinitabV 15. Voltammograms were pre-processed by using an adaptation of a data reduction technique based on predefined response "bell-shaped windowing" curves called "kernels" (Parra et al., 2004). The data pre-processing has been done based on a compression method described by Gutiérrez-Osuna and Nagle (1999). The voltammogram curve is multiplied by 10 smooth, bell-shaped windowing functions defined as

$$K_i(V_j) = \frac{1}{1 + \left(\frac{V_j - c_i}{a_i}\right) 2b_i}$$

where a_i, b_i and c_i define the width, shape and center of the different windowing functions K_i. Subsequently, data were integrated with respect to voltage. After compression, each voltammogram has been reduced to a vector of 10 variables. Using this technique, 10 variables were obtained from each voltammogram. A data matrix formed by 175 rows (25 beer samples × 7 repetitions) and 40 columns (10 values for each one of four electrodes employed) was constructed. Principal component analysis (PCA) and linear discriminant analysis (LDS) were carried out using these 10 variables as input data source. Prediction models were performed by partial least squares (PLS). The classification models were subjected to full cross-validation by means of the "leave-one-out" method (Berrueta et al., 2007: Cetó et al., 2013c).

2.5. Color index and alcoholic strength determinations

UV–Vis spectrophotometer Shimadzu UV-1201S was used to determine the color of different beers. Photometric measurements were performed at room temperature on previously degassificated beers. The color of the different samples in units EBC was calculated according to the expression:

$$EBC = 25 \times f \times A_{430}$$

where f is the dilution factor and A_{430} is the absorbance value at 430 nm (EBC, 2006).

The alcoholic strength in beer samples was assessed by the EBC method 7.1 (pycnometry) (European Brewery Convention. Analytica EBC, 1975).

3. Results and discussion

3.1. Voltammetric responses

The voltammetric response of the array of electrodes toward beers is illustrated in Fig. 1 for sample BP07.

As observed in the Fig. 1, voltammograms showed redox processes. Due to the surface solution interaction, the position and intensities of the peaks differ from one electrode to the other, confirming that the array selected possess the desirable cross-specificity that arrays of sensors must show.

Since each sensor provides a particular response when immersed in each beer sample, its response could be used to evaluate the ET array capabilities to discriminate between the different group varieties using multivariate data analysis. For this purpose, data was analyzed using two different pattern recognition techniques: principal component analysis (PCA) and linear discriminant analysis (LDA).

3.2. Discrimination of beer using ET

The first classification model was built using PCA in order to reduce the dimensionality of the data and to visualize the different beer groups where possible. This unsupervised technique maximizes the correlation between the original variables forming a small number of new orthogonal and non-correlated variables called *Principal Components* (*PC*) which capture much of the variance in the original variables. This technique classifies the samples according to their electrochemical response, which in turn is a reflection of their chemical composition. In this case, the input variables were the kernels previously obtained by transformation from the voltammetry data. Attending to a minimum PC *eigenvalue*, the number of significant components extracted was four. The accumulated explained variance was 96,924%, a non-large value, distributed in 38.7% (PC1), 28.7% (PC2), 18.2% (PC3) and 11.3% (PC4).

Fig. 2 shows the results of the three-dimensional PCA score plot. Although according to PCA there is no clear discrimination of beers, several trends were observed. On the one hand, similar samples appeared located in the same region of the graph. Moreover, samples with lower alcoholic content and smaller EBC values (free alcoholic beer) were concentrated in the central area of the graph while beers with significantly higher alcoholic content and EBC (Doppelbock) were preferably found in the most remote regions of the graph. This means that the electrochemical response may be related to: (1) the singular characteristics of each beer style, (2) the presence of chemical compounds related to color development and (3) the alcoholic strength of the samples analyzed.

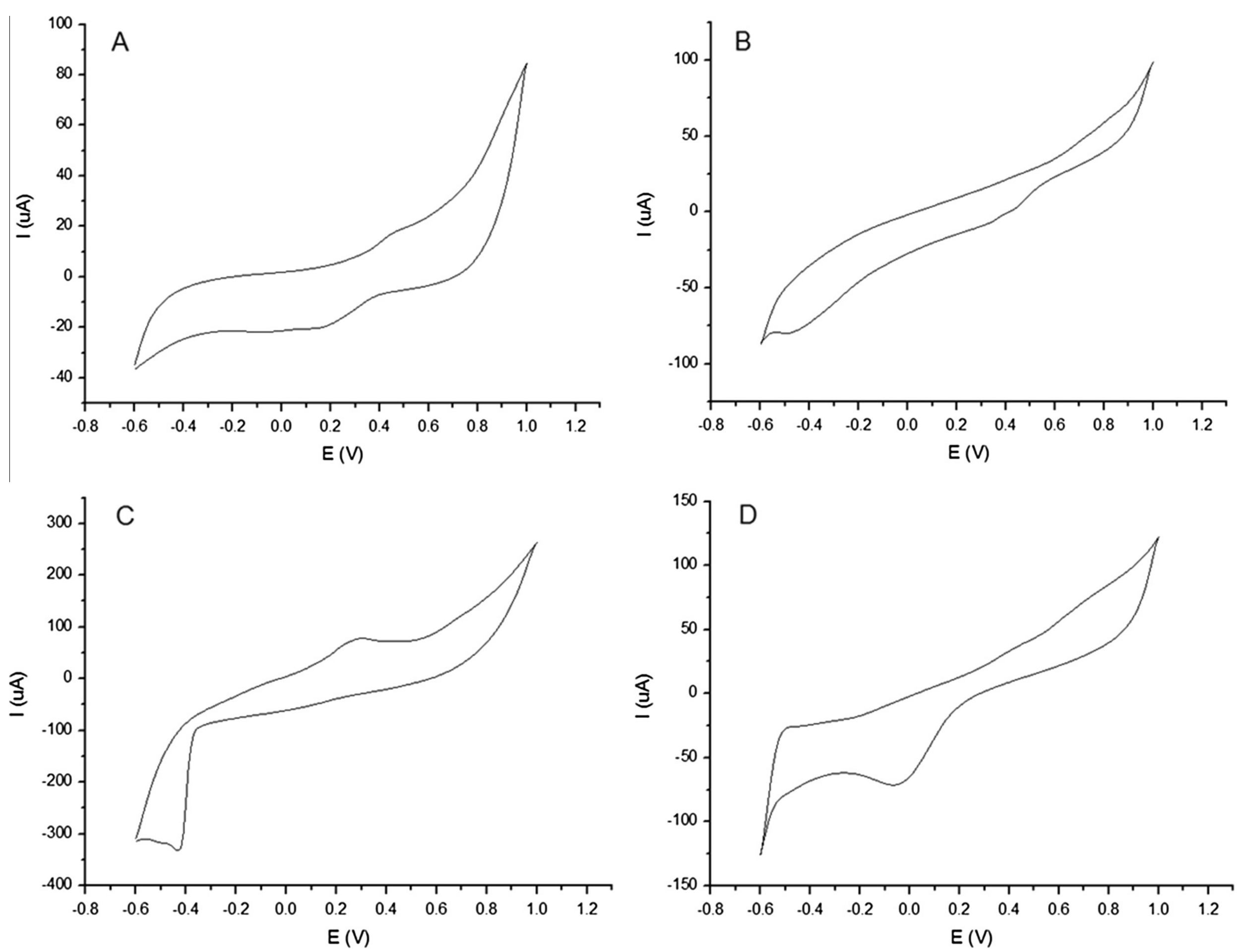

Fig. 1. Cyclic voltammograms registered with the array of sensors exposed to the sample (BP07). (A) DS-110, (B) DS-250AT, (C) DS-410 and (D) DS-550.

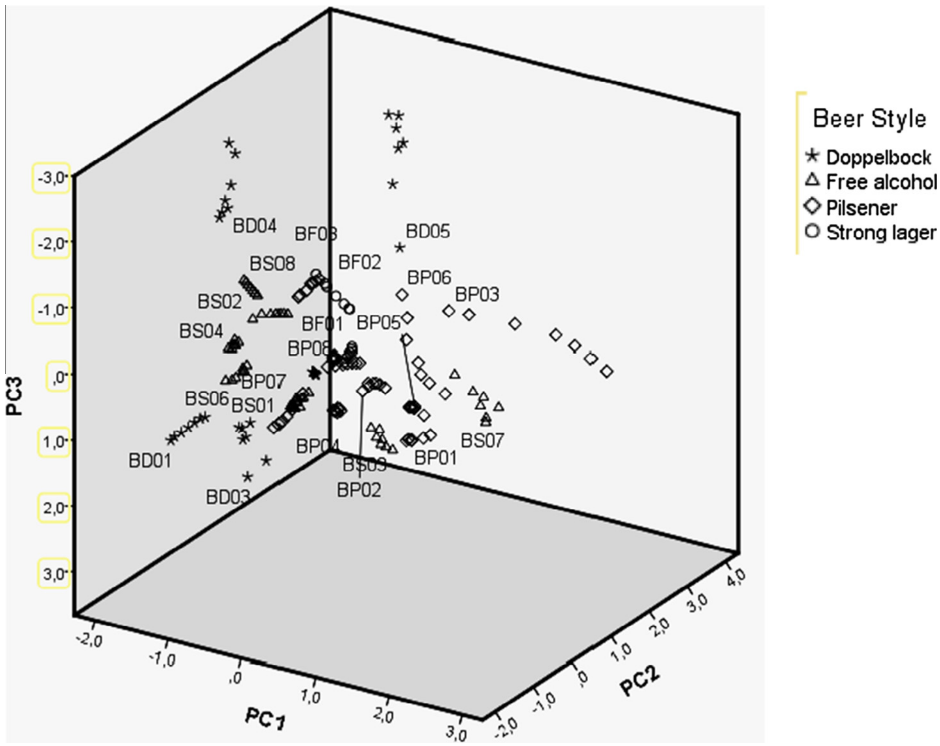

Fig. 2. Results of the three-dimensional PCA score plot.

Data were also treated using LDA analysis, which is a supervised statistical analysis used when groups are known *a priori,* as is the case. It provides *k-1* linear combinations of the considered variables, *k* being the number of groups, called *discriminant functions.* The method measures the distance from each point to each group's centroid and classifies the point to the closest group taking into account the variances and covariances between the variables (Mahalanobis distance).

A linear model was constructed where the input parameters were the ten new variables obtained for each electrode by pretreatment of the data. The beer samples were classified in (1) 8 beers "Free Alcohol", (2) 9 beers "Pilsener", (3) 5 beers "Doppelbock" and (4) 3 beers "European Strong Lager".

Fig. 3 shows the dispersion of samples in accordance with the three discriminant functions, it shows a clear discrimination between the four styles of beer. The explained variance by each discriminant function (DFs) is 64.2268% (DF1), 29.2273% (DF2) and 6.5459% (DF3).

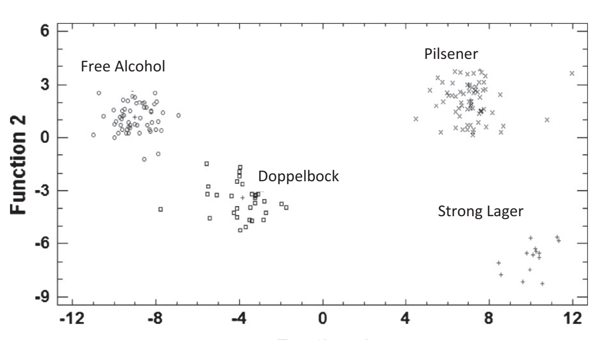

Fig. 3. Score plot of the two first discriminant functions obtained after LDA analysis of the beer samples, according to its type.

The efficiency of the obtained model was estimated attending to the percentage of correctly classified samples according to beer styles; thus the percentage of hits was 100%. Table 2 shows the classification of results of LDA by leave-one-out validation.

The efficiency of the obtained classification 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, 83.7%, and that of specificity was 96.4%.

3.3. Prediction of color and alcohol strength using ET

Prediction of the color and alcohol strength of the beer from the instrumental response is a practically important task since it might potentially reduce the cost of analyzing these attributes. Therefore, an attempt to predict beer attributes from the ET measurements was performed. Calibration models with respect to the attributes were calculated for each attribute separately using Partial Least Squares Analysis (PLS). The obtained model was subjected to cross-validation using the "leave-one-out" method. Although this relationship could be established with the color, according to the properties of the compounds responsible for this parameter, it

Table 2
Confusion matrices built according to beer styles obtained using LDA model for leave-one-out cross validation.

Found				
Expected	Pilsener	Free alcohol	Strong lager	Doppelbock
Pilsener	9	0	0	0
Non-alcoholic	0	8	0	0
Strong lager	0	0	3	0
Doppelbock	0	0	0	5

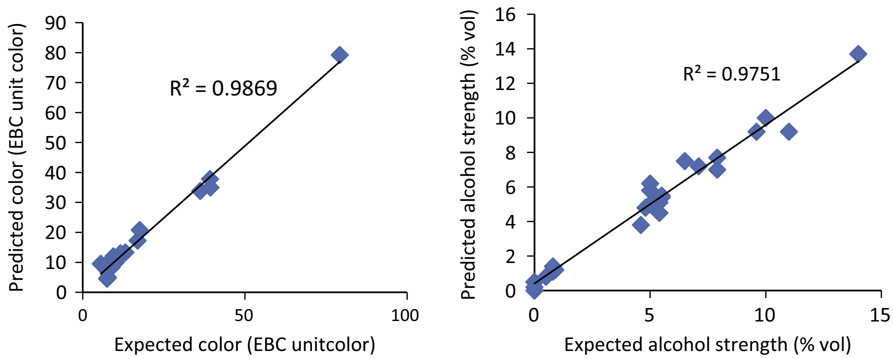

Fig. 4. Plots of PLS calibrations comparing expected and predicted values for EBC color and alcohol strength for analyzed beers.

was also performed in parallel the analysis PLS based on alcohol strength. All the calibration models can be considered satisfactory, as the Root Mean Squared Error (RMSE) values are 1.91 and 0.64 to EBC color and alcohol strength for analyzed beers, respectively. Fig. 4 shows the scatter plots of PLS calibrations comparing measured and predicted values for alcohol strength and EBC color for analyzed beers. According to the results, the success rate of the EBC unit color determined for all beer reaches 76.0% of accuracy and 84.0% for predicted alcoholic content.

4. Conclusions

A simple and portable electronic tongue (ET) system based on disposable SPEs was developed to create a tool capable of distinguishing between different types of beer. Preliminary analyses were done using principal component analysis (PCA), but no good results were obtained. In order to improve the recognition ability of the ET, linear discriminant analysis (LDA) was used and the discrimination and classification of the beer samples was successful, and quite good correlations were obtained.

In the future, the combination of others SPEs may improve de precision of the method and its possibilities. It can be expected that the method could be used to evaluate the antioxidant content in beers (as it has been done in other alcoholic beverages), by analyzing the responses of these electrochemical sensors toward redox compounds and by establishing mathematical models, to find out correlations between actual and predicted values.

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