

Flavour characteristics of Spanish and Iranian saffron analysed by electronic tongue

K. Heidarbeigi^{1,2}, S.S. Mohtasebi^{1*}, J. Serrano-Diaz³, C. Medina-Plaza², M. Ghasemi-Varnamkhasti⁴, G.L. Alonso³, M.V. Garcia-Rodriguez³, S. Rafiee¹, K. Rezaei⁵, C. Garcia-Hernandez², J.A. de Saja⁶ and M.L. Rodriguez-Mendez^{2*}

¹Department of Mechanics of Agricultural Machinery, Faculty of Agricultural Engineering and Technology, University of Tehran, Karaj, Iran; ²Department of Inorganic Chemistry, Engineers School, Universidad of Valladolid, Paseo del Cauce, 59, 47011 Valladolid, Spain; ³Cátedra de Química Agrícola, ETSI Agronomos de Albacete, Universidad de Castilla-La Mancha, 02071 Albacete, Spain; ⁴Department of Mechanical Engineering of Biosystems, Shahrekord University, Shahrekord, Iran; ⁵Department of Food Science, Engineering and Technology Faculty of Agricultural and Engineering Technology, University of Tehran, Karaj, Iran; ⁶Department of Condensed Matter Physics, Faculty of Sciences, Universidad de Valladolid, Spain; mohtaseb@ut.ac.ir; mluz@eii.uva.es.

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Abstract

In this study, a portable electronic tongue was developed and successfully used to distinguish and to discriminate quality of saffron samples. The proposed system consisted of an array of voltammetric sensors based on screen-printed electrodes (SPE), where the working electrodes were of different chemical natures (e.g. carbon, gold and modified carbon electrode). Moreover, the carbon electrodes were chemically modified by electrocatalytic copper phthalocyanine derivatives. The electrodes were immersed in saffron infusions to produce complex voltammetric curves that were characterised for each saffron sample. The electrochemical signals provided by the array showed good correlations with the data obtained from traditional chemical analysis, in particular with the kaempferol, a flavonol with antioxidant activity. The calibration model was optimised using more meaningful description of factors. Finally, discriminant analysis showed that the proposed e-tongue based on SPEs modified with phthalocyanine is useful for discrimination of saffron samples with different qualities. All of these results reveal that proposed e-tongue has a high potential for saffron quality control due to its very simple sample preparation and also its fast response.

Keywords: electronic tongue, saffron, screen printed electrode, principal component analysis

1. Introduction

Saffron (*Crocus sativus* L.) is a crop adapted to the climatic conditions of the Middle East, Central Asia and parts of Europe. Iran is the most important producer in the world, distributing low price saffron all over the world. Saffron is a sterile triploid plant that is a member of the Iridaceae family with one stigma formed by three filaments in each flower (Rahaiee *et al.*, 2014; Winterhalter *et al.*, 2000). The stigma, once dehydrated gives the most expensive spice by weight in the world. Saffron spice has a high nutritional value and is used as a food additive and also as an herbal medicine (Caballero-Ortega, *et al.*, 2004; Haghighi *et al.*, 2007). The quality, commercial category and organoleptic properties of saffron mostly depend on the content of certain components such as crocetin esters (colour, golden-

yellow hue, water soluble), picrocrocin (bitter taste), safranal (volatile oil) and flavonoids such as kaempferol (antioxidant character) (Carmona *et al.*, 2007b; Pedroza *et al.*, 2012). In addition, the concentration of crocetin esters, picrocrocin, safranal (Caballero-Ortega *et al.*, 2007), flavonoid fraction (Carmona *et al.*, 2007a), amino acids (Campo *et al.*, 2009) and acetic acid (Carmona *et al.*, 2006a) are different for each sample supplied from different countries and thus could be used to identify quality of samples from different geographic origins (Procida *et al.*, 2009).

The conventional methods used to analyse saffron are liquid chromatography (Verma and Middha. 2010), high-performance liquid chromatography (HPLC) (Campo *et al.*, 2009; Zalacain *et al.*, 2005), near-infrared spectroscopy (Anastasaki *et al.*, 2008; Zalacain *et al.*, 2005), mid-

infrared spectroscopy (Anastasaki *et al.*, 2008) and gas chromatography-mass spectrometry (Carmona *et al.*, 2006a). Two new technologies which have been developed for analysis of foods are called electronic nose (e-nose) and electronic tongue (e-tongue), which are used for analysis of volatile components and liquids, respectively. Moreover, both techniques consisted of an array of electrodes with cross selectivity coupled to pattern recognition software (Baldwin *et al.*, 2011). Although these systems have been widely used to analyse a variety of foods and beverages, such as wines, oils or beers (Gay *et al.*, 2010; Ghasemi-Vamankhasti *et al.*, 2011; Medina-Plaza *et al.*, 2014; Prieto *et al.*, 2011), only two papers have reported the application of an e-nose system for aroma analysis of saffron samples supplied from different geographic origins (Carmona *et al.*, 2006a) and for detecting saffron adulteration (Heidarbeygi *et al.*, 2013).

Up to now, electronic tongues have never been used for analysis of saffron and this research is the first one. The most used methods in e-tongues employ electrochemical techniques, such as potentiometry (Janczyk *et al.*, 2011; Vlasov *et al.*, 2010), amperometry (Scampicchio *et al.*, 2006), cyclic voltammetry (Labrador *et al.*, 2010; Rodriguez-Mendez *et al.*, 2008; Winqvist 2008) or impedance measurements (Pioggia *et al.*, 2007; Riul *et al.*, 2010). Electrodes can be fabricated using a large variety of techniques and can be modified using many different materials. The appropriate selection of the sensitive materials is crucial to construct the adequate array of sensors with the appropriate sensitivity and cross-selectivity for each application.

Screen printed electrodes (SPE) are made by deposition and thermal curing of a thick film of a sensitive material deposited on a ceramic or plastic substrate (Fanjul-Bolado *et al.*, 2008; Wang *et al.*, 1998). The printing inks used to prepare the working electrodes can contain a variety of materials, such as graphite particles, carbon or metal inks (Au, Pt, Pb and Cu) (Kadara *et al.*, 2009; Renedo *et al.*, 2007; Wang *et al.*, 1998) and mixtures of carbon inks with other materials, such as Prussian blue. The SPE devices include three electrodes, working, reference and counter electrode in the same device and they are cheap, disposable, reproducible and simple to use, therefore, they can be used in portable devices (O'Halloran *et al.*, 2001; Wang and Tian, 1992). SPEs have been used to produce e-tongues for analysis of foods and beverages, such as wines or fish freshness (Apetrei *et al.*, 2013b; Matemadombo *et al.*, 2012; Rodriguez-Mendez *et al.*, 2009).

In this work, an array was formed by disposable SPE electrodes prepared from four different working electrodes (carbon, platinum, gold and Prussian blue). In addition, the array was improved by developing novel electrodes where the working electrode was chemically modified with

substituted copper phthalocyanines (CuPc^R), which are well known due to their electrocatalytic properties (Zagal *et al.*, 2012). Moreover, the electrochemical behaviour of the electrodes was investigated and analysed. The capability of the array to discriminate the quality of saffron samples supplied from Spain and Iran was evaluated using principal component analysis (PCA). Moreover, partial least squares analysis (PLS) was used to establish the correlations between the results obtained using traditional chemical analysis and the results obtained from the array of the electrodes.

2. Material and methods

Samples, chemicals and reagent

In total, nine samples of saffron spice were used in this study. The samples were supplied from two different origins: Spain and Iran. Regarding the Spanish saffron spice samples, one sample was selected from the 2013 harvest season and belonged to the commercial category I according to ISO 3632 (ISO, 2011) (sample 1). Other two Spanish samples were selected from category III (ISO, 2011), being one from the 2011 harvest (sample 2) and the other one from the 2010 harvest periods (sample 3). The other two Spanish samples belonged to the protected designation of origin 'Azafrán de La Mancha', being from two different harvest periods: 2013 (sample 4) and 2012 (sample 5), and from two different categories: I and II, respectively (ISO, 2011). Four Iranian samples used in this study were harvested in 2013: one of them was supplied by the Novin saffron company (Mashhad, Iran) (sample 6) another one from a saffron farming area in Qaen County (South Khorasan province, Iran) (sample 7) and the other two samples were chosen from the two commercial categories designated as Selecto (sample 8) and Coupe (sample 9) according to Ministerio de Economía y Hacienda (NCCEA, 1999). Samples 6, 8 and 9 belonged to category I, and sample 7 belonged to category II (ISO, 2011). All reagents and solvents had HPLC purity or analytical grade. Ultra high-purity water was produced using a Millipore Milli-Q System (Bedford, MA, USA). Picrocrocin was isolated using the method described by Serrano-Diaz *et al.* (2011). Standards of kaempferol 3-glucoside (purity >99%) and safranal (88% purity) were supplied by Sigma-Aldrich (Madrid, Spain). Three copper (II) phthalocyanines (Sigma-Aldrich) were used in this study as sensitive materials: copper(II)-octa-butoxy-phthalocyanine (CuPc^{o-but}), copper(II)-tetra-tert-butyl-phthalocyanine (CuPc^{tbut}) and copper(II)-octakis-(octyloxy)-phthalocyanine (CuPc^{o-oxy}).

Moisture and volatile matter content

The moisture and volatile content (M and V) were determined by successive weighing of 1 g of powdered sample introduced into an oven set at 103±2 °C for 16 h

according to ISO 3632 (ISO, 2011). They were calculated with the following ratio (Equation 1):

M and V =

$$100 \times \frac{\text{initial mass} - \text{constant mass}}{\text{initial mass}} \quad (1)$$

UV-Vis determinations

Saffron spice aqueous was extracted with ultra-high purity water with 0.5 g/l concentration to determine their main characteristics of saffron samples according to ISO 3632 (ISO, 2011): colouring strength ($E_{1\text{cm}}^{1\%}$ 440 nm), $E_{1\text{cm}}^{1\%}$ 257 nm, and $E_{1\text{cm}}^{1\%}$ 330 nm. Then, they were magnetically stirred at 1000 rpm for 1 h, at room temperature and in the dark environment. The extracts were filtered through a 0.45 μm polytetrafluoroethylene (PTFE) filters and dilutions 1:10 were performed with ultra-high purity water. All diluted extracts were monitored by scanning from 190 to 700 nm using a Perkin-Elmer Lambda 25 spectrophotometer (Norwalk, CT, USA) with UV WinLab 2.85.04 software (Perkin-Elmer).

Determination of crocetin esters, picrocrocin, kaempferol-3-sophoroside-7-glucoside and safranal

The same extracts were prepared for determination of the main characteristics of crocetin esters, picrocrocin, kaempferol and safranal according to ISO 3632 (ISO, 2011) and then were analysed by a reverse-phase HPLC technique. An Agilent 1200 chromatograph (Palo Alto, CA, USA) was used, operating with a 150 mm $\times$ 4.6 mm i.d., 5 μm Phenomenex Luna C₁₈ chromatographic column (Le Pecq Cedex, France), thermostated at 30 °C. Aliquots of 20 μl of each extract were injected after filtering through a 0.45 μm PTFE filter. Eluents were water (A) and acetonitrile (B) with the following elution gradient: 20% B, 0-5 min; 20-80% B, 5-15 min; 80% B, 15-18 min and 20% B, 18-30 min. The flow rate was 0.8 ml/min. The DAD detector (Hewlett Packard, Waldbronn, Germany) was set at 250 nm for picrocrocin detection, 330 nm for kaempferol-3-sophoroside-7-glucoside and safranal, and 440 nm for crocetin esters.

The identification of eleven crocetin esters and picrocrocin was performed according to Carmona *et al.* (2006b), and that of kaempferol-3-sophoroside-7-glucoside according to Carmona *et al.* (2007b). Safranal was identified by comparing its retention time and also its maximum wavelengths in the UV-Vis absorption spectrum, with the similar parameters of a safranal commercial standard.

Quantification of crocetin esters, picrocrocin, kaempferol-3-sophoroside-7-glucoside and safranal

The quantification of crocetin esters was done based on the following equation (Equation 2) proposed by Sanchez *et al.* (2008):

% of ester I on dry basis =

$$\frac{Mw_i(E_{1\text{cm}}^{1\%} 440 \text{ nm})A_i}{10\epsilon_{t,c}} \quad (2)$$

Where, Mw_i stands for the molecular weight of the crocetin ester i, 440 nm is the colouring strength, A_i is the percentage of peak area of the crocetin ester i at 440 nm and is the molecular coefficient absorbance value (89,000 for *trans*-crocetin esters; and 63,350 for *cis*-crocetin esters) (Speranza *et al.*, 1984).

Picrocrocin was quantified using a six-point calibration curve of picrocrocin isolated from saffron spice (range of 2-315 mg/l; $A = 34.48 C - 17.91$; $R^2=0.9998$). For kaempferol-3-sophoroside-7-glucoside and safranal, six-point calibration curves of kaempferol 3-glucoside (range of 1-200 $\mu\text{g/l}$; $A = 2 \times 10^6 \times C + 156.68$; $R^2=0.9992$) and safranal (range of 0.25-10 mg/l; $A = 32.321 \times C + 1.9479$; $R^2=0.9977$) were used, respectively.

Electrochemical measurements

To start the measurements, the extracts properties (compounds of saffron) were determined according to ISO 3632 (ISO, 2011) and then used in the following experiments.

Electrochemical studies were carried out using SPE purchased from DropSens (Oviedo, Spain). The electrodes contained a working electrode (4 mm diameter), a counter electrode and a silver reference electrode. The electrodes were connected to a potentiostat (μStat 400 BioPotentiostat/Galvanostat; DropSens) adapted to these electrodes fabricated by the same company using a Dropsens boxed connector.

The electrodes included a traditional three electrode configuration printed on the same strip. The initial array consisted of four working electrodes: (1) a carbon disk working electrode (DropSens ref. 110; C-SPE); (2) a platinum disk working electrode (DropSens ref. 550; Pt-SPE); (3) a gold disk working electrode (DropSens ref. 250 AT; Au-SPE) and (4) a Prussian blue/carbon working electrode (DropSens ref. 710; PB-SPE).

Three electrodes, chemically modified by phthalocyanines, were prepared by depositing 10 mM of the corresponding Pc derivative (10 μl in chloroform) on the carbon surface of the working electrode (carbon) surface. The solution was left to be dried at room temperature. Cyclic voltammograms were

registered at a scan rate of 50 mV/s (Apetrei *et al.*, 2013a; Fanjul-Bolado *et al.*, 2007) from -1 to 1 V. Three electrodes were thus obtained: copper(II)-octa-butoxy-phthalocyanine modified electrode ($\text{CuPc}^{\text{o-but}}$ -SPE), copper(II)-tetra-tert-butyl-phthalocyanine ($\text{CuPc}^{\text{tbut}}$ -SPE) and copper(II)-octakis-(octyloxy)-phthalocyanine ($\text{CuPc}^{\text{o-oxy}}$ -SPE).

Statistical analysis

Voltammograms were mathematically pre-processed and then used as a data source for multivariate data analysis according to a previously published method (Apetrei *et al.*, 2007). Pre-processed cyclic voltammograms were obtained by the adaptation of a data reduction technique based on predefined response 'bell-shaped windowing' curves called 'kernels' in order to extract significant information from the voltammetric signals. These coefficients were used as a matrix input in multivariate data analysis. Additionally, the kernel coefficients were used in multivariate calibration method by partial least squares.

A non-supervised multivariate method such as PCA was used for analysis of the voltammetric signals. PCA is a mathematical method used to reduce the amount of data by keeping the important information and finding a correlation in the data set (Esbensen *et al.*, 1994).

Partial least square regression (PLSR) was used to establish mathematical correlations between the signals provided by the array and the results obtained using chemical analysis. Thus, PLS was used to find the fundamental relations between a latent variable (LV) approach in order to model the covariance structures and contained the smallest necessary number of factors (Hoskuldsson *et al.*, 1988). The general idea of PLS is to determine a set of latent variables, corresponding to principal components in PCA but explaining as much as possible of the covariance between the data set and class affiliation matrix. In this research seven latent variables were used for PLS model in some parameters. In PLS modelling, we assume that the investigated system or process actually is influenced by just a few underlying variables, latent variables (LV's). The number of these LV's is usually not known, and one aim of the PLSR analysis is to estimate this number. Then it is essential to determine the correct complexity of the model. Cross-validation is a practical and reliable way to test this predictive significance. After developing a model, differences between actual and predicted Y-values were calculated for the deleted data. The sum of squares of these differences were computed and collected from all the parallel models to form the predictive residual sum of squares, which estimates the predictive ability of the model. This data should be provided in order to justify the number of latent variables employed. Any model needs to be validated before it is used for understanding or for predicting new events. The best validation of a model is

that it precisely predicts the Y-values of observations with new X-values-validation set. Leave-one-out method was used for validation of the data.

All computations and chemometric analysis were carried out using MATLAB v5.3 (The Mathworks Inc., Natick, MA, USA) and the Unscrambler 9.1 (Camo, Norway).

3. Results and discussion

The first step in this work was to analyse the electrochemical behaviour of the SPEs. The electrochemical studies were carried out using four screen-printed electrodes, carbon, gold, platinum and Prussian blue/carbon which were immersed in 0.1 mol/l KCl solutions and in saffron samples. The results showed in Figure 1, demonstrate that as electrodes are immersed in KCl (an inert electrolyte), the shape and position of the peaks depended on the chemical nature of the working electrode. For instance, when using a carbon electrode (C-SPE), no signal was observed. In contrast, when using a Pt-SPE or Au-SPE, the interactions between such metals and water give rise to a variety of peaks. Finally, the electrode modified with Prussian blue (PB-SPE) shows the typical redox response of this compound. The responses were highly reproducible and the standard deviations lower than 5% were found in all cases.

The electrodes were also immersed in saffron solutions with different organoleptic properties. The results are illustrated in Figure 1 right column for saffron (sample 6) and left column for 0.1 mol/l KCl solutions. The cyclic voltammograms showed shifts and changes in the intensity of the peaks associated with the electrode material. These changes are due to the interactions between the components available in the saffron solution and the electrode surface that modify the double layer. In addition, new peaks with one anodic peak at 0.7 V were observed that could be attributed to redox components available in saffron such as kaempferol (Psotova *et al.*, 2004). These peaks are particularly visible in carbon, gold and Prussian blue electrodes.

Further experiments were carried out using carbon electrodes which were chemically modified by copper phthalocyanines derivatives. Copper phthalocyanine has electrocatalytic properties that can be modulated by the central metal ion and also by the substitutes. When using SPE electrodes modified by phthalocyanines, the decomposition of water is facilitated and peaks corresponding to the electrocatalytic oxidation and reduction of water can be observed at high and low voltages respectively (Figure 2). Moreover, the presence of different substitutes modifies the electrocatalytic properties of the electrodes.

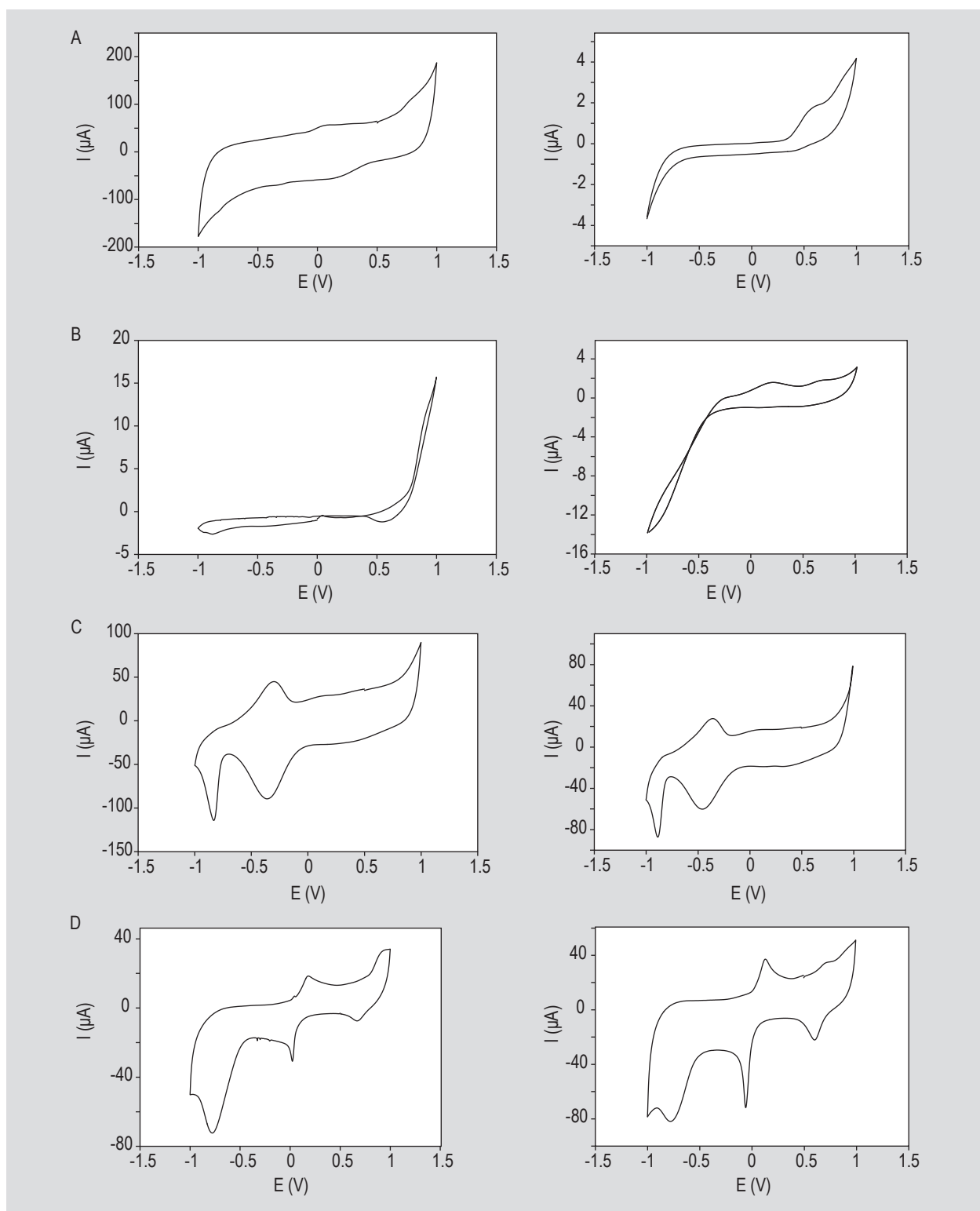


Figure 1. Cyclic voltammograms of sensors based on screen-printed electrodes in 0.1 mol/l KCl (left) and saffron (sample 6) (right) solution with (A) carbon, (B) gold, (C) platinum and (D) Prussian blue/carbon electrodes. Scan rate 0.05 V/s.

Taking into account the previous results, an array was formed to analyse saffron samples with different characteristics. It is important to remark that Pt-SPE and PB-SPE were not included in the array due to problems of

their irreproducibility when immersed in saffron. Cyclic voltammograms obtained by exposing the array of sensors to a saffron solution at a scan rate 50 mV/s are illustrated

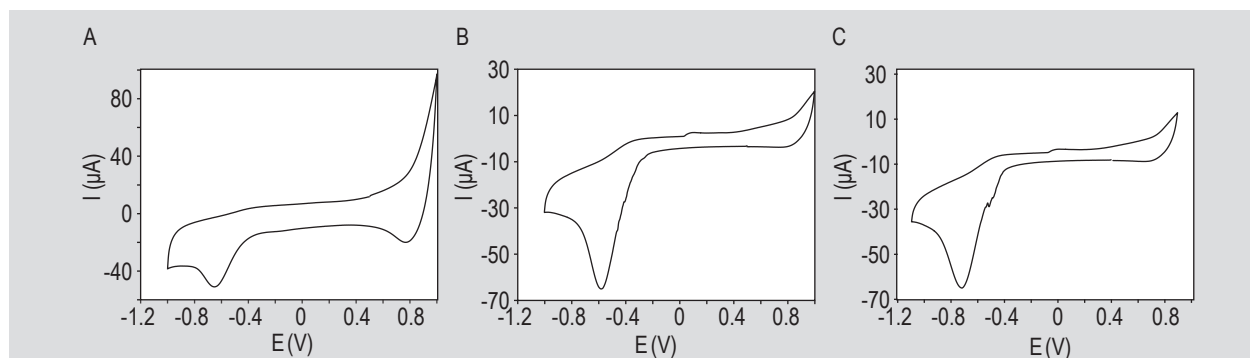


Figure 2. Cyclic voltammograms response of modified carbon electrodes (C-SPE) immersed in 0.1 mol/l KCl: (A) copper(II)-tetra-tert-butyl-phthalocyanine (CuPc^{tbut}-SPE); (B) copper(II)-octa-butoxy-phthalocyanine (CuPc^{o-but}-SPE); and (C) copper(II)-octakis-(octyloxy)-phthalocyanine (CuPc^{o-ooxy}-SPE) modified electrode.

for saffron sample 5 (Figure 3). It should be noted that one emulsion was prepared for each saffron sample.

In cyclic voltammetry, the occurrence of chemical reactions depends on redox processes of many important organic and inorganic compounds and the available surface concentration is affected proportional to electro-active species. Therefore voltammograms shape are changing due to the chemical competition for the electrochemical reactant or product.

The features observed in the voltammograms (Figure 3) reflect the reactions of electroactive compounds (such as crocin and polyphenols: kaempferol) (Dar *et al.*, 2013), which are available in saffron. This means that, the voltammetric responses of the electrodes are specific for each type of saffron. In addition, each electrode immersed in different saffron samples showed a variety of responses. Such pattern of responses can be considered as a characteristic fingerprint of the saffron.

For statistical analysis, seven replicated cyclic voltammograms were registered in each solution with all sensors. Therefore, 7 cycles were recorded for each saffron sample (5 samples from Spain and 4 samples from Iran) with 5 screen-printed electrodes (C-SPE, Au-SPE, CuPc^{tbut}-SPE, CuPc^{o-but}-SPE and CuPc^{o-ooxy}-SPE). Therefore, 315 curves were used in data analysis. For the reduction of data dimensionality, the kernel method was employed.

The PCA plots are shown in Figure 4. The PCA scores plot shows where the first two components (PC1 and PC2) represent the total variance between the samples measurements (Figure 4A). The scores plot is used to determine whether distinct data clusters exist for pattern recognition. As observed in these figures, all the samples could be clearly discriminated from each other. However, discrimination due to the saffron quality could be detected. Taking into account that this is the first time that an electronic tongue is used to analyse saffron, these results

indicate that this new array of sensors based on screen-printed electrodes, can be a valuable tool for classification of saffron samples in terms of quality.

Sensors are the main components of an electronic tongue system. A key issue is to select the correct sensors able to detect particular components available in saffron while showing a good complementarity. The Loading plot demonstrates that the selected electrodes have a high degree of complementarity and these loadings appear in the four quadrants of the plot (Figure 4B).

Moreover, PLS1 models were established to evaluate whether there is a correlation between the signals provided by the array of sensors and the results of the chemical analysis (Table 1 shows the concentration of each detected compound in the 9 tested samples).

The reduction of the number of LV's leads also to more robust classifier, since the omitted portion of information only caused over fitting of the model. Results of the PLS regression (PLS1) lines for comparison of predicted with calibration values are shown in Table 2 which corresponds to the average of the values obtained for each sample. A cross validation method was used. As observed in Table 2, both the calibration and the validation values show a good quality model performance and there were a good correlations between the voltammetric signals and the classes established. Additionally, low root mean square error of calibration and root mean square error of prediction values were accomplished. The best results obtained for kaempferol, a flavonoid with redox activity available in saffron (with only two latent variables the model could be adequately fitted). These results confirm the good performance of the proposed system.

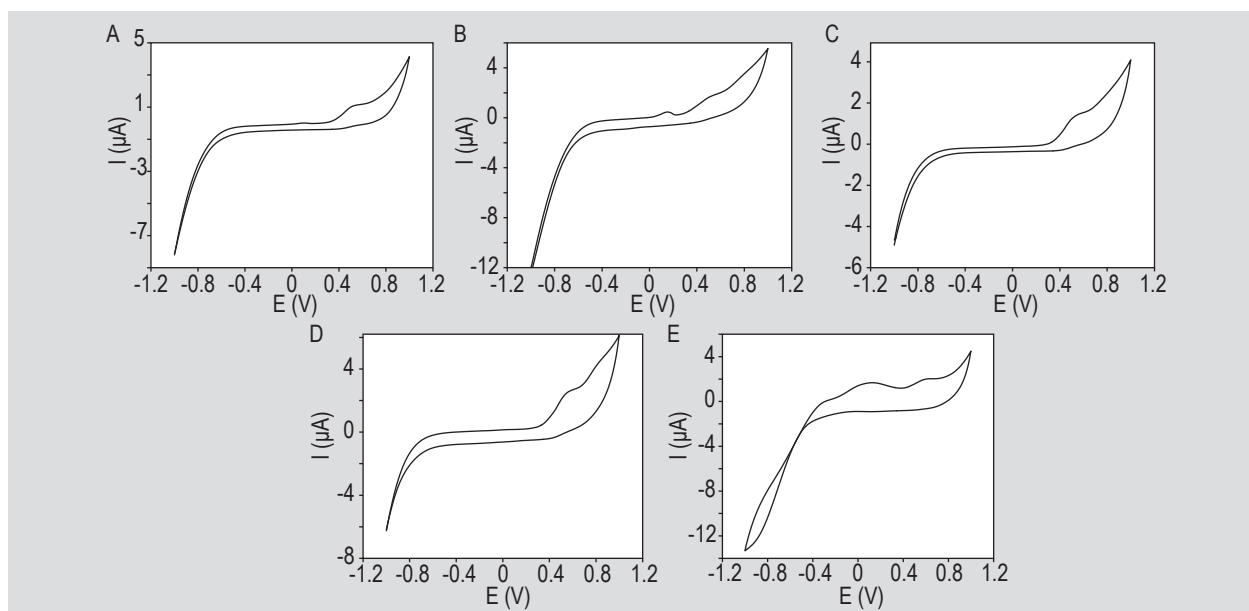


Figure 3. Cyclic voltammograms obtained using the array of sensors based on screen-printed electrodes modified with phthalocyanines in saffron solution (sample 5). Scan rate 0.05 V/s. (A) copper(II)-tetra-tert-butyl-phthalocyanine modified carbon electrode; (B) copper(II)-octa-butoxy-phthalocyanine modified carbon electrode; (C) copper(II)-octakis-(octyloxy)-phthalocyanine modified carbon electrode; (D) carbon electrode; and (E) gold disk working electrode.

Table 1. High-performance liquid chromatography quantitative analysis of 9 saffron samples (mg/100 mg of stigmas) from Iran and Spain (dry basis).^{1,2}

	Wavelength (nm)	Retention time (min)	Spain					Iran			
			1	2	3	4	5	6	7	8	9
Picrocrocin	250	5.96	14.76	7.93	8.97	12.3	11.5	10.97	10.14	12.87	9.5
Kaempferol 3-S-7-G	330	6.50	30.55	6.67	50.95	66.85	63.8	61.4	78.7	138.9	47.6
Safranal	330	17.45	0.32	0.57	0.51	0.30	0.65	0.38	0.37	0.3	0.81
<i>trans</i> -5-tG	440	9.631	0.25	0.16	0.21	0.24	0.22	0.21	0.18	0.22	0.15
<i>trans</i> -5-nG	440	10.13	0.24	n.d.	0.18	0.13	0.14	0.16	0.15	0.154	0.12
<i>trans</i> -4-GG	440	10.244	9.83	5.35	7.21	10.3	7.26	8.12	5.54	8.47	4.93
<i>trans</i> -4-ng	440	10.62	0.25	0.099	0.1	0.57	0.37	0.35	0.26	0.45	0.37
<i>trans</i> -3-Gg	440	10.76	6.63	2.32	3.73	7.38	6.44	8.56	5.88	8.07	6.86
<i>trans</i> -2-gg	440	11.398	1.21	0.29	0.52	1.37	1.42	2.17	1.45	1.66	2.33
<i>cis</i> -4-GG	440	11.846	0.91	1.26	0.98	1.694	0.62	1.15	0.59	0.66	1.03
<i>trans</i> -2-G	440	12.217	0.05	0.12	0.08	0.07	0.07	0.08	0.096	0.04	0.07
<i>cis</i> -3-Gg	440	12.447	0.438	0.424	0.362	1.094	0.379	0.852	0.348	0.443	0.87
<i>cis</i> -2-gg	440	12.864	1.497	0.86	1.22	2.57	2.12	2.65	1.34	3.22	2.26
<i>cis</i> -2-G	440	13.216	0.095	0.05	0.03	0.16	0.088	0.19	0.099	0.11	0.23
Total crocetin esters			21.39	10.91	14.61	25.5	19.1	24.48	15.92	23.48	19.3
Sum of <i>trans</i> crocetin esters			18.46	8.33	12.02	20.01	15.89	19.64	13.54	19.06	14.8
Sum of <i>cis-trans</i> crocetin esters			2.94	2.58	2.59	5.52	3.2	4.84	2.38	4.43	4.39
Ratio <i>cis/trans</i> crocetin esters (%)			15.9	30.98	21.52	27.57	20.16	24.65	17.58	23.23	29.6

¹ n.d. = not detected.

² The nomenclature adopted by Carmona *et al.* (2006b) is used to abbreviate the names of the crocetin esters: *trans*-5-tG, *trans*-crocetin (β -D-triglucosyl)-(β -D-gentiobiosyl) ester; *trans*-5-nG, *trans*-crocetin (β -D-neapolitanosyl)-(β -D-gentiobiosyl) ester; *trans*-4-GG, *trans*-crocetin di-(β -D-gentiobiosyl) ester; *trans*-3-Gg, *trans*-crocetin (β -D-glucosyl)-(β -D-gentiobiosyl) ester; *trans*-2-gg, *trans*-crocetin di-(β -D-glucosyl) ester; *cis*-4-GG, *cis*-crocetin di-(β -D-gentiobiosyl) ester; *trans*-2-G, *trans*-crocetin (β -D-gentiobiosyl) ester; *cis*-3-Gg, *cis*-crocetin (β -D-glucosyl)-(β -D-gentiobiosyl) ester; *cis*-2-gg, *cis*-crocetin di-(β -D-glucosyl) ester; and *cis*-2-G, *cis*-crocetin (β -D-gentiobiosyl) ester.

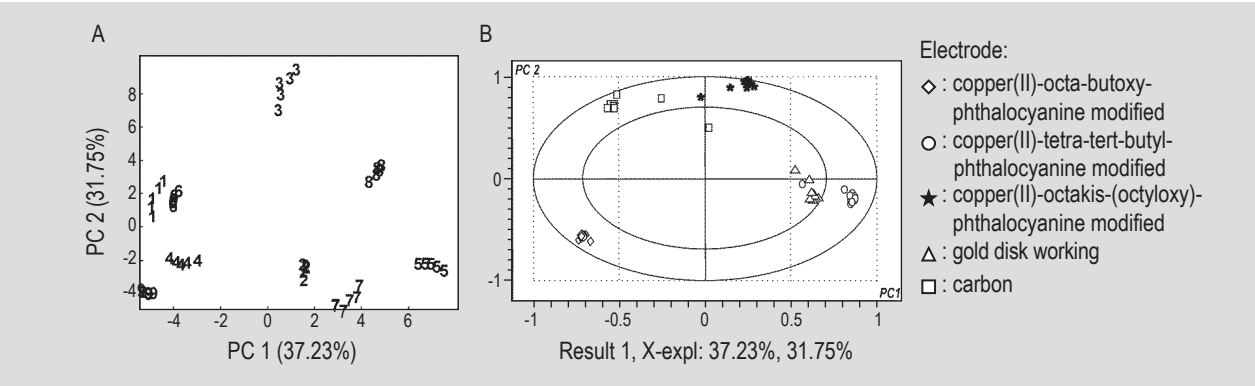


Figure 4. (A) Principal component analysis score plot of the different varieties of saffron. (B) Loading plot of the sensors array in terms of PC1 vs PC2.

Table 2. Partial least squares analysis prediction model using electrochemical data obtained from the sensor array in calibration and prediction.¹

Parameter	RMSEC	RMSEP	R _C	R _P	LV
257 nm	2.534803	3.196066	0.944407	0.910863	7
330 nm	2.300389	2.896448	0.907593	0.851706	6
440 nm	13.491420	15.618980	0.960591	0.946819	5
Picrocrocin	0.598643	0.997948	0.954755	0.873433	7
Kaempferol	14.215310	16.208090	0.914212	0.887377	2
Safranal	0.061975	0.088395	0.931541	0.858255	7
<i>trans</i> -2-G	0.009852	0.012262	0.909491	0.857046	5
<i>trans</i> -2-gg	0.238407	0.324074	0.929070	0.866308	7
<i>trans</i> -3-Gg	0.748915	0.955164	0.919736	0.866778	5
<i>trans</i> -4-GG	0.789489	1.166037	0.903869	0.783901	6
<i>trans</i> -4-ng	0.054632	0.069286	0.932888	0.890706	5
<i>trans</i> -5-nG	0.022652	0.026097	0.926033	0.900771	3
<i>trans</i> -5-tG	0.013882	0.019104	0.906903	0.823037	7
Sum of <i>trans</i> -crocetin esters	0.863964	1.172852	0.973078	0.950434	5
<i>cis</i> -2-G	0.022344	0.030442	0.935945	0.879296	7
<i>cis</i> -2-gg	0.303944	0.362795	0.914108	0.875415	5
<i>cis</i> -3-Gg	0.073466	0.104645	0.961919	0.922616	7
<i>cis</i> -4-GG	0.134001	0.235010	0.919233	0.740742	6
Sum of <i>cis</i> -crocetin esters	0.304497	0.424169	0.960876	0.923865	7
Total sum of crocetin esters	1.168009	1.433916	0.967427	0.950514	5
Relation <i>cis/trans</i> -crocetin esters	1.991465	2.905762	0.918998	0.825535	7

¹ RMSEC = root mean square error of calibration); RMSEP = root mean square error of prediction; R_C = correlation coefficient for calibration; R_P = correlation coefficient for prediction; LV = latent variable.

4. Conclusions

A portable multisensory system based on screen printed electrodes modified with phthalocyanine derivatives was developed and applied to the analysis of saffron of different qualities. The obtained curves using cyclic voltammograms, gave comparable information and provided discrimination performance, in view of the PCA results.

This is the first time that an electronic tongue has been used to analyse saffron quality. Results showed that the e-tongues can be useful for discrimination of different saffron samples in order to control the quality of samples.

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References

- Anastasaki, E.G., Kanakis, C.D., Pappas, C.S., Tarantilis, P.A. and Polissiou, M.G., 2008. Geographical origin of saffron spice by mid-infrared spectroscopy (MIR). In: 7th Joint meeting of AFERP, ASP, GA, PSE and SIF, Natural Products with Pharmaceutical, Nutraceutical, Cosmetic and Agrochemical Interest, Athens, Greece. Available at: <http://www.saffic.eu/archivos/POSTER2.pdf>.
- Apetrei, C., Gutierrez, F., Rodriguez-Mendez, M.L. and Saja, J.A., 2007. Novel method based on carbon paste electrodes for the evaluation of the bitterness of virgin olive oils. *Sensors and Actuators (B)* 121: 567-575.
- Apetrei, I.M. and Apetrei, C., 2013a. Voltammetric e-tongue for the quantification of total polyphenol content in olive oils. *Food Research International* 54: 2075-2082.
- Apetrei, M., Rodriguez-Mendez, M.L., Apetrei, C. and Saja, J.A., 2013b. Fish freshness monitoring using an e-tongue based on polypyrrole modified screen-printed electrode. *IEEE, Journal of Sensors* 13: 2548-2554.
- Baldwin, E.A., Bai, J., Plotto, A. and Dea, S., 2011. Electronic noses and tongues: applications for the food and pharmaceutical industries. *Sensors* 11: 4744-4766.
- Caballero-Ortega, H., Pereda-Miranda, R., Riveron-Negrete, L., Hernandez, J.M., Medecigo-Rios, M., Castillo-Villanueva, A. and Abdullaev, F.I., 2004. Chemical composition of saffron (*Crocus sativus* L.) from four countries. *Acta Horticulture* 650: 321-336.
- Caballero-Ortega, H., Pereda-Miranda, R. and Abdullaev, F.I., 2007. HPLC quantification of major active components from 11 different saffron (*Crocus sativus* L.) sources. *Food Chemistry* 100: 1126-1131.
- Campo, C.P., Garde-Cerdan, T., Sanchez, A.M., Maggi, L., Carmona, M. and Alonso, G.L., 2009. Determination of free amino acids and ammonium ion in saffron (*Crocus sativus* L.) from different geographical origins. *Food Chemistry* 114: 1542-1548.
- Carmona, M., Martinez, J., Zalacain, A., Rodriguez-Mendez, M.L., Saja, J.A. and Alonso, G.L., 2006a. Analysis of saffron volatile fraction by TD-GC-MS and e-nose. *European Food Research Technology* 223: 96-101.
- Carmona, M., Sanchez, A.M., Ferreres, F., Zalacain, A., Tomas-Barberan, F. and Alonso, G.L., 2007b. Identification of the flavonoid fraction in saffron spice by LC/DAD/MS/MS: comparative study of samples from different geographical origins. *Food Chemistry* 100: 445-450.
- Carmona, M., Zalacain, A., Salinas, M.R. and Alonso, G.L., 2007a. A new approach to saffron aroma. *Critical Reviews in Food Science and Nutrition* 47: 145-159.
- Carmona, M., Zalacain, A., Sanchez, A.M., Novella, J.L. and Alonso, G.L., 2006b. Crocetin esters, picrocrocin and its related compounds present in *Crocus sativus* stigmas and *Gardenia jasminoides* fruits. Tentative identification of seven new compounds by LC-ESI-MS. *Journal of Agricultural and Food Chemistry* 54: 973-979.
- Dar, R.A., Brahman, P.K., Khurana, N., Wagay, J.A., Lone, Z.A., Ganaie, M.A. and Pitre, K.S., 2013. Evaluation of antioxidant activity of crocin, podophyllotoxin and kaempferol by chemical, biochemical and electrochemical assays. *Arabian Journal of Chemistry*, DOI: <http://dx.doi.org/10.1016/j.arabjc.2013.02.004>.
- Esbensen, K., Schonkopf, S. and Midtgaard, T., 1994. Multi-variate analysis in practice (1st Ed.). CAMO AS, Trondheim, Norway.
- Fanjul-Bolado, P., Hernandez-Santos, D., Lamas-Ardisana, P.J., Martin-Pernia, A. and Costa-Garcia, A., 2008. Electrochemical characterization of screen-printed and conventional carbon paste electrodes. *Electrochimica Acta* 53: 3635-3642.
- Fanjul-Bolado, P., Queipo, P., Lamas-Ardisana, P.J. and Costa-Garcia, A., 2007. Manufacture and evaluation of carbon nanotube modified screen-printed electrodes as electrochemical tools. *Talanta* 74: 427-433.
- Gay, M., Apetrei, C., Nevares, I., Alamo, M., Zurro, J., Prieto, N., Saja, J.A. and Rodriguez-Mendez, M.L., 2010. Application of an electronic tongue to study the effect of the use of pieces of wood and micro-oxygenation in the aging of red wine. *Electrochim Acta* 55: 6782-6788.
- Ghasemi-Vamankhasti, M., Mohtasebi, S.S., Rodriguez-Mendez, M.L., Lozano, J., Razavi, S.H. and Ahmadi, H., 2011. Potential application of electronic nose technology in brewery. *Trends in Food Science and Technology* 22: 165-174.
- Haghighi, B., Feizi, J. and Hemati-Kakhki, A., 2007. LC determination of adulterated saffron prepared by adding styles colored with some natural colorants. *Chromatographia* 66: 325-332.
- Heidarbeigi, K., Mohtasebi, S.S., Foroughirad, A., Ghasemi-Varnamkhasti, M., Rafiee, S. and Rezaei, K., 2015. Detection of adulteration in saffron samples using electronic nose. *International Journal of Food Properties* 18: 1391-1401.
- Hoskuldsson, A., 1988. PLS regression methods. *Journal of Chemometrics* 2: 211-228.
- International Organization for Standardization (ISO), 2011. ISO 3632 saffron (*Crocus sativus* L.). Part 1: specification. Part 2: test methods. ISO, Geneva, Switzerland.
- Janczyk, M., Kutyla, A., Solohub, K., Wosicka, H., Cal, K. and Ciosek, P., 2011. Potentiometric electronic tongues for foodstuff and biosample recognition – an overview. *Sensors* 11: 4688-4701.
- Kadara, R.O., Jenkinson, N. and Banks, C.E., 2009. Characterisation of commercially available electrochemical sensing platforms. *Sensors and Actuators (B)* 138: 556-562.
- Labrador, R.H., Masot, R., Alcaniz, M., Baigts, D., Soto, J., Martinez-Manez, R., Garcia-Breijo, E., Gil, L. and Barat, J.M., 2010. Prediction of NaCl, nitrate and nitrite contents in minced meat by using a voltammetric electronic tongue and an impedimetric sensor. *Food Chemistry* 122: 864-870.
- Matemadombo, F., Apetrei, C., Nyokong, T., Rodriguez-Mendez, M.L. and Saja, J.A., 2012. Comparison of carbon screen printed and disk electrodes in the detection of antioxidants using CoPc derivatives. *Sensors and Actuators (B)* 166-167: 457-466.

- Medina-Plaza, C., Revilla, G., Munoz, R., Fernandez-Escudero, J.A., Barajas, E., Medrano, G., Saja, J.A. and Rodriguez-Mendez, M.L., 2014. Electronic tongue formed by sensors and biosensors containing phthalocyanines as electron mediators, Application to the analysis of red grapes. *Journal of Porphyrins and Phthalocyanines* 18: 76-86.
- Ministerio de Economía y Hacienda (NCCEA), 1999. Normas de calidad del comercio exterior para el azafran. Ministerio de Economía y Hacienda, Madrid, Spain.
- O'Halloran, M.P., Pravda, M. and Guilbault, G.G., 2001. Prussian blue bulk modified screen-printed electrodes for H₂O₂ detection and for biosensors. *Talanta* 55: 605-611.
- Pedroza, M.A., Carmona, M., Pardo, F., Salinas, M.R. and Zalacain, A., 2012. Waste grape skins thermal dehydration: potential release of colour, phenolic and aroma compounds into wine. *CyTA-Journal of Food* 10: 225-234.
- Pioggia, G., Francesco, F.D., Marchetti, A., Ferro, M. and Ahluwalia, A., 2007. A composite sensor array impedentiometric electronic tongue. Part I. Characterization. *Biosensors and Bioelectronics* 22: 2618-2623.
- Prieto, N., Gay, M., Vidal, S., Aagaard, O., Saja, J.A. and Rodriguez-Mendez, M.L., 2011. Analysis of the influence of the type of closure in the organoleptic characteristics of a red wine by using an electronic panel. *Food Chemistry* 129: 589-594.
- Procida, G., Pagliuca, G. and Cichelli, A., 2009. Geographical differentiation between Italian and Spanish saffron based on volatile fraction composition. A preliminary study. *Agro Food Industry Hi-Tech* 20: 48-53.
- Psotova, J., Chlopikova, S., Miketova, P., Hrbac, J. and Simanek, V., 2004. Chemoprotective effect of plant phenolics against anthracycline-induced toxicity on rat cardiomyocytes. Part III. Apigenin, baicalein, kaempferol, luteolin and quercetin. *Phytother Research* 18: 516-521.
- Rahaiee, S., Hashemi, M., Moini, S., Abbas Shojaosadati, S. and Razavi, S.H., 2015. Comparison of phytochemical constituents and antioxidant activities of aqueous and alcoholic extracts of saffron. *Quality Assurance and Safety of Crops & Foods* 7: 521-529.
- Renedo, O.D., Alonso-Lomillo, M.A. and Arcos Martinez, M.J., 2007. Recent developments in the field of screen-printed electrodes and their related applications. *Talanta* 73: 202-219.
- Riul, J.A., Dantas, C.A.R., Miyazaki, C.M. and Oliveira, J.O.N., 2010. Recent advances in electronic tongues. *Analyst* 135: 2481-2495.
- Rodriguez-Mendez, M.L., Apetrei, C., Gay, M. and Saja, J.A., 2009. Biogenic amines and fish freshness assessment using a multisensor system based on voltammetric electrodes. Comparison between CPE and screen printed electrodes. *Electrochimica Acta* 54: 7033-7041.
- Rodriguez-Mendez, M.L., Parra, V., Apetrei, C., Villanueva, S., Gay, M., Prieto, N., Martinez, J. and Saja, J.A., 2008. E-tongue based on voltammetric electrodes modified with electroactive materials. Applications. *Microchimica Acta* 163: 23-31.
- Sanchez, A.M., Carmona, M., Zalacain, A., Carot, J.M., Jabaloyes, J.M. and Alonso, G.L., 2008. Rapid determination of crocetin esters and picrocrocin from saffron spice (*Crocus sativus* L.) using UV-visible spectrophotometry for quality control. *Journal of Agricultural and Food Chemistry* 56: 3167-3175.
- Scampicchio, M., Benedetti, S., Brunetti, B. and Mannino, S., 2006. Amperometric electronic tongue for the evaluation of the tea astringency. *Electroanalysis* 18: 1643-1648.
- Serrano-Diaz, J., Sanchez, A.M., Maggi, L., Carmona, M. and Alonso, G.L., 2011. Synergic effect of water soluble components on the coloring strength of saffron spice. *Journal of Food Composition and Analysis* 24: 873-879.
- Speranza, G., Dada, G., Manitto, P., Monti, D. and Grammatica, P., 1984. 13-*cis* crocin: a new crocinoid of saffron. *Gazzetta Chimica Italiana* 114: 189-192.
- Verma, R.S., and Middha, D., 2010. Analysis of saffron (*Crocus sativus* L. stigma) components by LC-MS-MS. *Chromatographia* 71: 117-123.
- Vlasov, Y.G., Ermolenko, Y.E., Legin, A.V., Rudnitskaya, A.M. and Kolodnikov, V., 2010. Chemical sensors and their systems. *Journal of Analytical Chemistry* 65: 890-898.
- Wang, J. and Tian, B., 1992. Screen-printed stripping voltammetric/potentiometric electrodes for decentralized testing of trace lead. *Analytical Chemistry* 64: 1706-1709.
- Wang, J., Tian, B., Nascimento, V.B. and Angnes, L., 1998. Performance of screen-printed carbon electrodes fabricated from different carbon inks. *Electrochimica Acta* 43: 3459-3465.
- Winquist, F., 2008. Voltammetric electronic tongues – basic principles and applications. *Microchimica Acta* 163: 3-10.
- Winterhalter, P. and Straubinger, R.M., 2000. Saffron. Renewed interest in an ancient spice. *Food Review International* 16: 39-59.
- Zagal, J.H., Griveau, S., Santander-Nelli, M., Gutierrez-Granados, S. and Bedioui, F., 2012. Carbon nanotubes and metalloporphyrins and metallophthalocynines-based materials for electroanalysis. *Journal of Porphyrins and Phthalocyanines* 16: 713-740.
- Zalacain, A., Ordoudi, S.A., Diaz-Plaza, E.M., Carmona, M., M. Zquez, I.B., Tsimidou, Z. and Alonso, G.L., 2005. Near-infrared spectroscopy in saffron quality control: determination of chemical composition and geographical origin. *Journal of Agriculture and Food Chemistry* 53: 9337-9341.