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Combination of an e-nose, an e-tongue and an e-eye for the characterisation of olive oils with different degree of bitterness

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ABSTRACT

An electronic panel has been used to characterise the organoleptic characteristics of twenty-five extra virgin olive oils from varieties Hojiblanca, Picual and Arbequina, with different degree of bitterness. The method consists in the combination of three systems: electronic nose, electronic tongue and electronic eye. The Principal Component Analysis (PCA), where PC1, PC2 and PC3 explained 59% of the total variance between the samples, has demonstrated that the capability of discrimination of the combined system is superior to that obtained with the three instruments separately. This improvement is due to the increased information extracted from each sample. Partial Least Squares-Discriminant Analysis (PLS-DA) has allowed separation of the groups in function of olive variety with a root mean square error of prediction (RMSEP) lower than 0.099.

Using PLS1 and PLS2 regression models, good correlations have been found between the signals obtained from the electronic tongue and the polyphenolic content (measured by chromatographic methods) or the bitterness index (scored by a panel of experts) with correlation coefficients higher than 0.9 in calibration and validation.

These preliminary results indicate that the combination of an e-nose, an e-tongue and an e-eye can be a useful tool for the analysis of olive oil bitterness.

1. Introduction

The organoleptic characterisation and the physicochemical analysis of extra virgin olive oils (EVOOs) represent are crucial for their commercial classification in accordance with International Olive Oil Council (IOOC) regulations. At the present time, the sensorial analysis carried out by a panel of trained tasters is the only homologated method for the organoleptic evaluation of virgin olive oils [1–3]. But the cost price of the formation and training of a panel, the impossibility to evaluate large number of samples, the delay of results for several days, and the certain degree of subjectivity have lead to the development of alternative electronic methods to assess the colour, the aroma and the taste of oils. The advantages of electronic systems in comparison with the human senses include higher objectivity and invariable response with time that contribute to the success of routine analysis.

Electronic noses and electronic tongues consist in arrays of non-selective gas or liquid sensors with a broad and partially overlapping selectivity towards compounds present in a sample. The array of sensors is combined with computerised multivariate statistical data processing tools [4–9]. A number of works have been published that have used electronic noses for the characterisation and for the quality control of olive oils [8–11]. In the case of electronic tongues, their capability to analyse and discriminate a variety of beverages such as mineral waters, milks, wines or beers has already been established [5–6,10–12]. However, few works have been focused to the analysis of olive oils using e-tongues. The main reason is the difficulty to carry out electrochemical analysis in a non-conductive liquid with a high viscosity.

An original method based on carbon paste electrodes (CPEs) has been developed to discriminate oils of different origins and qualities using a multisensor system. In this method, the carbon paste is prepared using the olive oil as a binder. The features observed in

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the voltammograms immersed in different electrolytic solutions reflect the electroactive properties of the oils inside the carbon paste [13,14].

Previous works have demonstrated that the simultaneous utilisation of electronic noses and electronic tongues can increase the amount of information extracted from certain samples such as wines [15]. In the case of olive oils, only one work has been published that combines an electronic nose (based on MOX sensors) and an electronic tongue (based on amperometric sensors) to evaluate the oxidation of extra virgin olive oils at different storage periods and conditions [16].

The aim of this work is to evaluate the feasibility of combining an electronic nose, an electronic tongue (based on modified CPE as in Refs. [13,14]) and an electronic eye to the characterisation of virgin olive oils.

For this purpose, twenty-five extra virgin olive oils (EVOOs) from different variety of olives and different degree of bitterness have been analysed using our electronic panel system (EPS).

The establishment of correlations between the official analytical methods and the analysis carried out by multisensor systems is necessary to provide to this new technique a practical utilisation in sensorial analysis. In this work, our interest has been focused to phenolic compounds. These compounds play an important role in the quality of olive oils since they contribute significantly to their stability towards oxidation [17–19]. In addition, polyphenols are the main contributors to olive oil bitterness, astringency and pungency [20–22]. In this work, attempt has been made to correlate the concentration of individual polyphenols or the panel scores with the results of our electronic tongue.

2. Experimental

All the chemicals used were analytical reagent grade and were obtained from Sigma–Aldrich.

2.1. EVOO samples

Twenty-five virgin olive oil samples were obtained using dual phase decanter centrifugation for varieties Picual (4 samples), Arbequina (11 samples), and Hojiblanca (10 samples). The Picual samples were denoted from P1 to P4, Arbequina—from A1 to A11, and Hojiblanca—from H1 to H10.

2.2. Sensory analysis and chromatography

The oil samples were evaluated for bitterness by 12 panel members of the "Instituto de la Grasa" (Institute of Fats) from Sevilla (Spain), following the European regulations [2].

A scale of 1–5 was used to determine the intensity of bitterness: 1 indicates imperceptible, 2 indicates slight, 3 indicates moderate, 4 indicates great, and 5 indicates extreme. The scores given by the panellists were averaged and the error was ±0.09.

The phenolic extracts of virgin olive oil were obtained following a previously described procedure [23,24]. The HPLC analysis for analytical separations was performed in a Hewlett-Packard series 1100 liquid chromatographic system equipped with a diode array UV detector, and a Rheodyne injection valve (20 μL loop) A Lichrospher 100RP-18 column (4.0 mm i.d. × 250 mm; particle size 5 μm) (MercK, Darmstat, Germany), maintained at 30 °C. Elution was performed at a flow rate of 1.0 mL min^{-1}, using as the mobile phase a mixture of water/phosphoric acid (99.5:0.5, v/v) (solvent A) and methanol/acetonitrile (50:50, v/v) (solvent B). The solvent gradient changed according to the following conditions: from 95% (A):5% (B) to 70% (A):30% (B) in 25 min, to 62% (A): 38% (B) in 10 min, to 55% (A): 45% (B) in 10 min, and to 47.5% (A):52.5% (B) in 5 min; 100% (B) was maintained for 5 min, and the run was ended. Quantification of phenols was carried out at 280 nm, and the results are expressed in mmol kg^{-1}. Triplicate determinations were made.

2.3. Electronic eye

The transmittance spectra were recorded using a series of LEDs that were selected to cover the range from 780 nm to 380 nm. The tristimulus coordinates were calculated from the reconstructed spectrum, using as reference the illuminant D65 and the CIE 1964 standard observer. The coordinates CIE L^*, a^* and b^* were calculated using the standard procedure [25]. These coordinates represent: L the lightness of the colour ($L^* = 0$ yields black and $L^* = 100$ indicates diffuse white); a^* its position between red/magenta and green (a^*, negative values indicate green while positive values indicate magenta); b^* its position between yellow and blue (b^*, negative values indicate blue and positive values indicate yellow). Hue (H) is associated with the sense of redness, yellowness, blueness, and so forth. Saturation (S) is associated with the strength of hue or the relative admixture with white. The combination of hue and saturation can be described as chromaticity (C).

2.4. Electronic nose

The array of gas sensors was constructed using 13 MOX sensors purchased from FIS and Figaro, selected according to the previous experience of our group [26,27]. Sensors were mounted in a stainless steel test box with a volume of 150 mL. Sensors were polarized using a constant voltage of 5 V provided by a FAC-662B programmable power supply. The scan rate used to measure the changes of the resistance of the sensors was 0.5 s.

2 g of the samples were placed in a 10 mL vials. Then, the vials were thermostated at 40 °C during 15 min in slow agitation, followed by an equilibrium stabilization step of 10 min. A representative sample of the headspace was collected using an automatic system (HP Head Space Sampler) and injected into the sensor chamber using a carrier gas flow (synthetic air at 100 mL min^{-1}). The sensor chamber was maintained at a constant temperature (50 °C) and under a constant flow of synthetic air.

The changes in the resistance were registered using a data acquisition card (PC-LPM-16 from National Instruments) installed in a desktop computer and controlled by software in Visual C++.

2.5. Electronic tongue

For electronic tongue measurements carbon paste electrodes (CPEs) modified with olive oils were prepared as previously described [13,14].

The electrochemistry was carried out in an EG&G PARC 263A potentiostat/galvanostat (Echem M270 Software) using a conventional three-electrode cell. The chemically modified electrodes were used as working electrodes. The reference electrode was an Ag|AgCl/KClsat and the counter electrode was a platinum wire. The electrochemical experiments were performed at a controlled temperature of 25 °C.

Two identical electrodes were prepared for each oil under study. Each replicate was immersed in one electrolytic solution (0.1 mol L^{-1} HCl or 0.1 mol L^{-1} KCl). Electrochemical measurements were carried out by means of SWV (square wave voltammetry). SWV was performed by using a frequency (f) of 15 Hz, an amplitude (E_{sw}) of 0.09 V and a step high (ΔE_s) of 0.005 V. The voltammograms were registered in the potential range from 0.0 V to 0.9 V in the case of HCl and −0.3 V to 0.9 V for KCl.

Table 1
Sensors and variables used for the three sensory systems.

	Electronic nose	Electronic tongue	Electronic eye
Number of sensors	13	8	CIELab coordinates
Signal registered	Change of the resistance in time	Square wave voltammograms	VIS spectra
Parameter after data pre-processing	$\Delta R/R \cdot 100$	10 kernel coefficients	L^*, a, b, C, H, S
Number of parameters included in the data treatment	13	80	6

2.6. Data analysis

All the samples were measured seven times with each electronic system. Pre-processing methods were used individually for each system in order to extract the relevant information from the experimental data. The extracted features were used for statistical analysis. Table 1 collects the sensors, and variables selected for each sensory system.

- CIELab coordinates were calculated from electronic spectra of olive oil samples and used as input data in statistical data analysis.
- In the case of electronic nose the input variable of multivariate data analysis was the maximum change of the resistance of sensors in the presence of volatile compounds from virgin olive oil samples [26].

In the electronic tongue voltammograms were pre-processed using the adaptation of a data reduction technique based on predefined response "bell shaped-windowing" curves called "kernels" [27]. Using this method, ten parameters per voltammogram were obtained and used the input variable for statistical analysis.

For variable reduction and separation into classes, Principal Component Analysis (PCA) was used. The data were analysed individually for each system, and then a data fusion was carried out in order to have a complete instrumental analysis of olive oils [28].

Partial Least Squares-Discriminant Analysis (PLS-DA) was additionally used as deterministic classification technique. PLS-DA is an extension of PLS; by projecting intercorrelated data from high-dimensional space into low-dimensional orthogonal space; the newly formed variables, which are linear combinations of the original variables, become orthogonal to each other. Through finding the "discriminant plane" to effectively separate data into different classes, PLS-DA is capable of separating "tight" classes of observations on the basis of the X-variables (namely, sensors), according to an Y-vector that encodes the class membership in a set of categorised variables, denoted as positive and negative (1 and 0 values, respectively). PLS2 was used as a prediction technique to correlate the signals obtained by using electronic systems with the values obtained by means of HPLC [28]. PLS1 regression was used to establish the correlations between bitterness degree evaluated by human panel and electrochemical responses [28].

The multivariate data analysis was performed by using the software Matlab v5.3. (The Mathworks Inc., Natick, MA, USA) and The Unscrambler V. 9.1. (CAMO ASA, Trondheim, Norway.)

3. Results and discussion

3.1. Measures with the three electronic systems

The VIS spectra of the EVOO present several peaks, related with the content in pigments such as chlorophyll and carotenoids [29,30]. The twenty-five EVOOs were analysed using the optical system described in Section 2 and the CIELab coordinates were calculated.

The L, a^*, b^*, C, H, and S coordinates were used as the input variable for Principal Component Analysis (PCA). The data matrix was normalised using 1/standard deviation

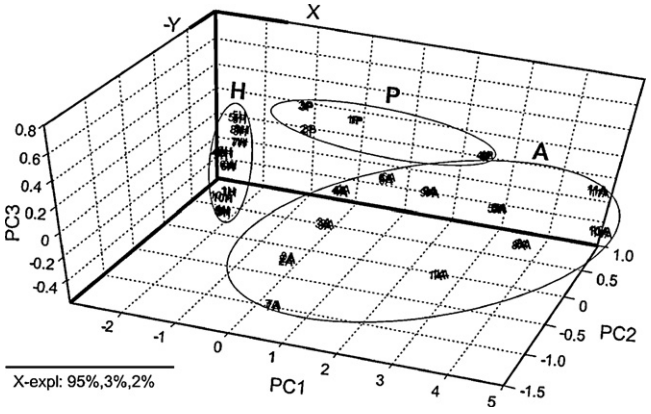

Fig. 1. PCA scores plot resulted form the CIELab coordinates.

As observed in Fig. 1, the PCA results represented as a three-dimensional scores plot of principal components allow obtaining well-defined and separated clusters for each olive variety. The first principal component explains the 95% of the captured information and is mainly responsible for the discrimination of the oils. The samples of the variety Arbequina appear in the right side of the plot (at positive PC1 values), whereas the oils of the variety Hojiblanca appear in the left side of the plot (at negative PC1 values).

In the case of electronic nose, the interaction of the resistive sensors with the headspace of the olive oil produced a rapid variation in the resistance of the sensitive layer [31,32]. As observed in the PCA (Fig. 2), in good accordance with the results obtained with the e-eye, the cluster associated to oils of the variety Arbequina appear in the right side of the diagram and the cluster of the Hojiblanca oils appear on the left side. However, a clear discrimination of the oils could not be achieved.

Measures of the electronic tongue were carried out using the CPE. The square wave voltammetry (SWV) curves showed complex curves where peaks associated to the presence of antioxidants could be observed [13].

Fig. 3 shows the scores plot of the three first principal components calculated from the parameters extracted from the curves. The first three principal components explain the 63% of the infor-

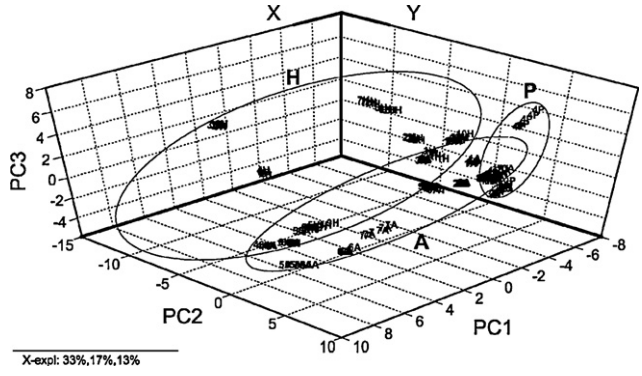

Fig. 2. PCA scores plot for the electronic nose.

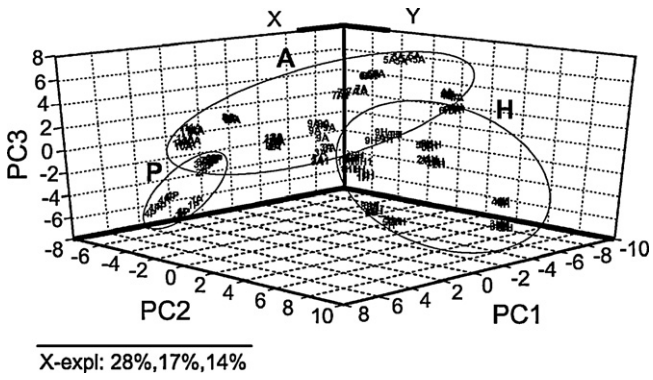

Fig. 3. PCA scores plot using as input kernel coefficients.

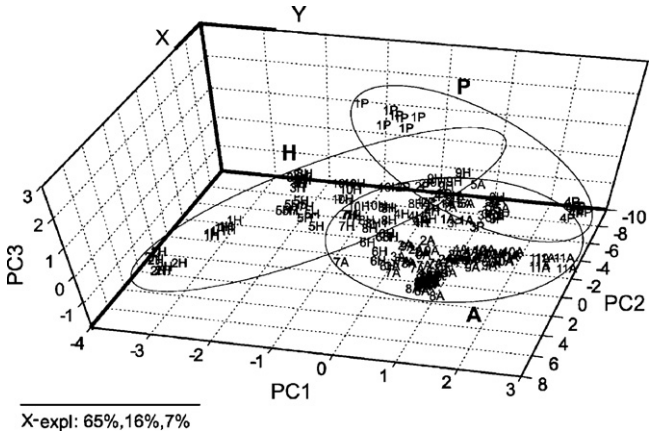

Fig. 4. PCA score plot illustrating the discrimination between the oils by means of EES.

mation (PC1 = 33%; PC2 = 17%; PC3 = 13%). As observed in the figure, EVOO elaborated with the same variety of olive appear in the same region of the diagram, however a net discrimination was not obtained. Using the electronic tongue, the second principal component is mainly responsible for the discrimination of the oils from different variety of olive.

3.2. Results obtained from the electronic panel

After analysing the data with the three electronic systems separately, data from the three systems were merged to obtain a complete characterisation of the samples with the electronic panel. A normalisation step was carried out due to heterogeneity of data magnitudes characteristic for each device. Then PCA was used as exploratory technique and obligatory in the analysis of data structure, followed by application of supervised method such as PLS-DA for EVOO classification.

Fig. 4 shows the PCA scores (PC1 vs. PC2 vs. PC3). The first PC account for the 28% of the variation in the electrochemical signal. PC1, PC2 and PC3 explained 59% of the total variance between the

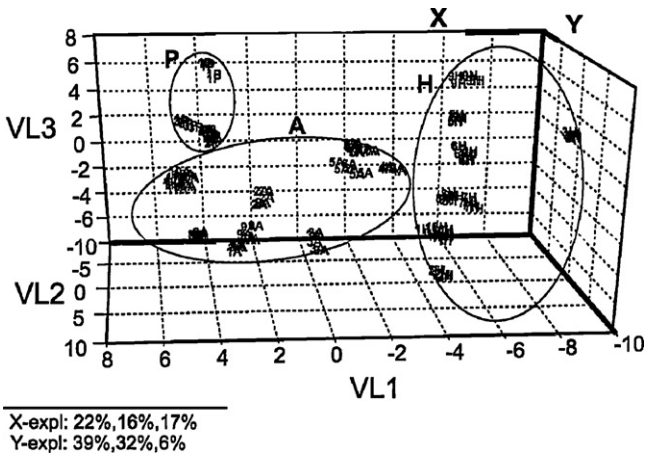

Fig. 5. PLS-DA score plot corresponding to the classification of oil according their olive variety.

samples. As observed in Fig. 4 the separated clusters indicate that the twenty-five olive oils could be clearly discriminated from each other. This is an important issue because the olive oils analysed are of the same quality (extra virgin), being the main difference the level of bitterness assigned by the human panel.

The graph could be divided into three regions corresponding to the olive variety of the studied oils: the region corresponding to oils Picual, a second region that includes oils Arbequina that appear close one to the other, finally a third region was observed where oils Hojiblanca were situated. The EVOO discrimination was better when the matrix input contain data provided by all three systems.

For the confirmation of the groups observed in Principal Component Analysis and in order to calculate the errors in calibration and in validation, PLS-DA technique was used. This technique is supervised, therefore the assignment to a certain group is verified and the error in calibration and in prediction is calculated. For validation full cross method was used. Quantitative data of PLS-DA for the three separated systems and for the EPS are collected in Table 2. The results confirm that the EVOO discrimination was better when the matrix input contain data provided by all three systems. Scores plot of the PLS-DA for the electronic panel is presented in Fig. 5. The optimal number of latent variables (LVs) was determined by the lowest value of predicted residual error sum of squares (PRESS).

As observed in the figure, the distribution of the samples was similar as in the case of PCA; a most evident separation of the groups in function of olive variety was observed with a correlation coefficient higher of 0.96 both in calibration and in prediction.

3.3. Correlation between the response of the electronic tongue and the analytical and sensorial data

As stated above, the establishment of correlations between the results obtained by means of electronic systems and the traditional analytical methods (sensorial analysis and chromatography) is of paramount importance in order to evaluate the possible practical

Table 2
Quantitative data of PLS-DA.

Type	EPS		e-nose		e-tongue		e-eye	
	RMSEC	RMSEP	RMSEC	RMSEP	RMSEC	RMSEP	RMSEC	RMSEP
Picual	0.077	0.091	0.137	0.148	0.0917	0.112	0.263	0.269
Arbequina	0.080	0.098	0.152	0.164	0.108	0.137	0.216	0.222
Hojiblanca	0.083	0.099	0.197	0.213	0.113	0.121	0.142	0.145

RMSEC (and RMSEP): root mean square error of calibration (and prediction). A measurement of the average difference between predicted and measured response values (in this case, the measured value corresponds to the positive (1) or negative (0) classification into the variety of olive), at the calibration stage (and at the validation stage).

Table 3
The bitterness panel score of the samples under study.

EVOO	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11
BI	4.05	2.45	2.0	4.25	3.04	1.86	2.31	1.39	4.05	2.05	1.7
EVOO	P1	P2	P3	P4							
BI	2.25	2.5	2.55	1.94							
EVOO	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	
BI	2.75	2.79	3.83	3.50	2.93	3.36	1.94	3.3	3.25	2.95	

applications of such systems. For this purpose, Partial least squares (PLS1 and PLS2) regressions were performed to model the relationships between the electronic signals and the results provided by chromatography or the sensorial analysis.

Due to the complexity of this task and the high number of variables that could be analysed, the study was focused to the search of correlations between the signals provided by the electronic tongue and parameters that could be related with taste sensations. In particular, the study was dedicated to the search of correlations with polyphenols which are the main contributors to olive oil bitterness and the score of bitterness provided by the panel of experts.

3.3.1. Sensorial characterisation

Table 3 shows the result of the scores of the bitterness given by the panel to the twenty-five extra virgin olive oils used in this study. The bitterness degree of the EVOOs ranged from 1.7 to 4.25, therefore samples with a BI almost imperceptible till the value nearer to the maximum were included in the study.

3.3.2. HPLC analysis of phenolic compounds

Tables 4–6 show the mean results (of three replicates) obtained from the analysis of polyphenolic compounds determined by HPLC and expressed in mmol kg^{-1} (where DAOL—dialdehydic form of decarboxymethyl oleuropein aglycone, DALI—dialdehydic form of decarboxymethyl ligstroside aglycone, MAOL—aldehydic form of oeuropein aglycone, MALI—aldehydic form of ligstroside aglycone).

3.3.3. Electronic tongue

Fig. 6 illustrates the square wave voltammetry (SWV) curves obtained using the sensors modified with olive oils immersed in aqueous KCl 0.1 mol L^{-1} and in HCl 0.1 mol L^{-1}. Peaks associated to the polyphenolic content of the oils under study could be observed in the 0.6–0.8 V region. The main differences between curves consist in the position (potential), form and intensity of the peaks observed. Table 7 shows the arithmetical means (seven repetitions) of the peak values associated to this results with a RSD (relative standard deviation) 1%.

Table 4
Quantification of polyphenolic compounds corresponding to samples Picual.

Compound	Sample			
	P1	P2	P3	P4
Hydroxityrosol	0.014	0.024	0.023	0.057
Tyrosol	0.033	0.057	0.028	0.074
Vanillic acid	0.002	0.003	0.000	0.001
Vanillin	0.001	0.001	0.000	0.001
p-Coumaric acid	0.000	0.000	0.000	0.000
Hydroxytyrosol acetate	0.011	0.024	0.020	0.010
DAOL	0.046	0.063	0.094	0.062
Tyrosyl acetate	0.033	0.028	0.033	0.016
DALI	0.112	0.075	0.089	0.079
Pinoresinol	0.011	0.009	0.007	0.006
Cinnamic acid	0.000	0.000	0.003	0.002
ʟ-Acetoxypinoresinol	0.035	0.012	0.019	0.026
MAOL	0.239	0.257	0.287	0.208
MALI	0.104	0.095	0.079	0.074
Ferulic acid	0.000	0.001	0.000	0.000
Luteolin	0.008	0.010	0.009	0.008
Apigenin	0.003	0.003	0.002	0.003
Polyphenols	0.651	0.661	0.693	0.628
Ortodiphenols	0.318	0.379	0.432	0.345
Secoiridoids	0.501	0.490	0.549	0.423

3.3.4. PLS1 regression: EPS vs. bitterness degree (BI)

In order to establish the correlation between the peak potentials obtained with electronic tongue and bitterness degree determined by a human panel test PLS1 regression was used.

As observed in Fig. 7, a very good correlation was found, with a coefficient 0.98 in calibration and 0.97 in validation, using 11 latent variables. For validation of the model full cross-validation method

Table 5
Quantification of polyphenolic compounds corresponding to samples Arbequina.

Compound	Sample										
	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11
Hydroxityrosol	0.009	0.003	0.002	0.007	0.009	0.005	0.005	0.000	0.003	0.009	0.002
Tyrosol	0.011	0.005	0.008	0.005	0.002	0.007	0.004	0.011	0.003	0.018	0.013
Vanillic acid	0.004	0.003	0.003	0.000	0.000	0.001	0.003	0.001	0.000	0.002	0.003
Vanillin	0.001	0.001	0.002	0.002	0.004	0.001	0.001	0.001	0.002	0.003	0.002
p-Coumaric acid	0.000	0.001	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000
Hydroxytyrosol acetate	0.167	0.237	0.134	0.233	0.232	0.232	0.219	0.280	0.253	0.385	0.288
DAOL	0.790	0.524	0.274	1.954	1.401	0.424	0.792	0.105	1.482	0.329	0.271
Tyrosyl acetate	0.012	0.003	0.003	0.000	0.000	0.000	0.006	0.005	0.000	0.000	0.000
DALI	0.183	0.084	0.061	0.000	0.407	0.067	0.135	0.069	0.351	0.197	0.109
Pinoresinol	0.009	0.005	0.006	0.038	0.000	0.006	0.007	0.006	0.000	0.009	0.006
Cinnamic acid	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.001	0.000	0.001	0.000
ʟ-Acetoxypinoresinol	0.080	0.070	0.065	0.032	0.039	0.037	0.066	0.051	0.024	0.070	0.066
MAOL	0.045	0.025	0.019	0.072	0.049	0.025	0.045	0.012	0.068	0.033	0.023
MALI	0.029	0.027	0.025	0.014	0.018	0.050	0.024	0.049	0.013	0.035	0.029
Ferulic acid	0.001	0.000	0.001	0.001	0.001	0.000	0.000	0.000	0.000	0.001	0.001
Luteolin	0.015	0.012	0.014	0.007	0.012	0.012	0.014	0.012	0.007	0.014	0.015
Apigenin	0.005	0.004	0.005	0.001	0.002	0.002	0.004	0.003	0.002	0.002	0.003
Polyphenols	1.363	1.002	0.623	2.365	2.174	0.869	1.326	0.605	2.208	1.107	0.832
Ortodiphenols	1.027	0.800	0.444	2.273	1.702	0.697	1.075	0.409	1.814	0.769	0.599
Secoiridoids	1.047	0.659	0.380	2.041	1.875	0.565	0.997	0.235	1.914	0.594	0.432

Table 6
Quantification of polyphenolic compounds corresponding to samples Hojiblanca.

Compound	Sample									
	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10
Hydroxityrosol	0.108	0.028	0.144	0.018	0.020	0.018	0.064	0.019	0.028	0.056
Tyrosol	0.082	0.066	0.065	0.026	0.039	0.025	0.047	0.033	0.040	0.047
Vanillic acid	0.001	0.003	0.000	0.001	0.000	0.000	0.001	0.000	0.000	0.001
Vanillin	0.001	0.001	0.000	0.000	0.001	0.001	0.001	0.001	0.000	0.000
p-Coumaric acid	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.001	0.001	0.000
Hydroxytyrosol acetate	0.076	0.065	0.052	0.060	0.056	0.049	0.053	0.043	0.025	0.055
DAOL	0.270	0.369	0.360	0.308	0.342	0.410	0.422	0.294	0.302	0.436
Tyrosyl acetate	0.015	0.014	0.023	0.019	0.017	0.020	0.024	0.029	0.028	0.016
DALI	0.228	0.268	0.250	0.228	0.292	0.307	0.267	0.238	0.331	0.301
Pinoresinol	0.005	0.004	0.002	0.005	0.002	0.003	0.001	0.002	0.003	0.003
Cinnamic acid	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
ʟ-Acetoxypinoresinol	0.051	0.051	0.037	0.045	0.061	0.047	0.047	0.058	0.038	0.049
MAOL	0.338	0.367	0.394	0.327	0.422	0.350	0.362	0.338	0.456	0.379
MALI	0.089	0.143	0.174	0.135	0.163	0.093	0.119	0.106	0.208	0.139
Ferulic acid	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001
Luteolin	0.019	0.019	0.016	0.018	0.020	0.018	0.019	0.019	0.014	0.018
Apigenin	0.010	0.010	0.008	0.009	0.010	0.009	0.009	0.009	0.007	0.009
Polyphenols	1.293	1.411	1.525	1.199	1.445	1.350	1.436	1.190	1.481	1.510
Ortodiphenols	0.811	0.848	0.966	0.731	0.860	0.844	0.920	0.713	0.825	0.944
Secoiridoids	0.926	1.147	1.178	0.997	1.218	1.159	1.170	0.976	1.297	1.255

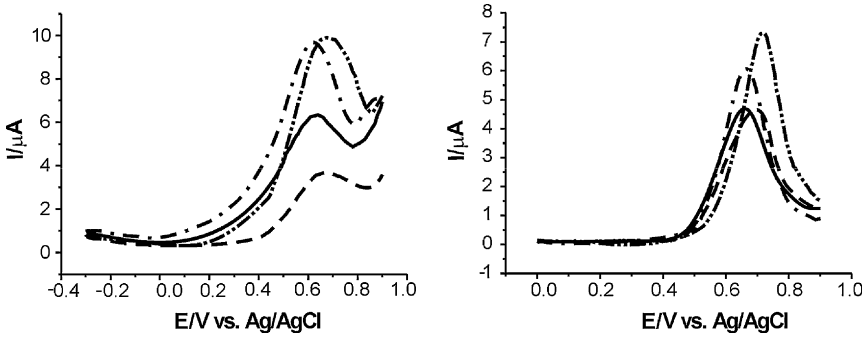

Fig. 6. SWV curves (direct scan) corresponding to EVOO–CPEs modified with Picual simples P1 (–· –· –·), P2 (–·· –·· –··), P3 (—) and P4 (- - -) immersed in (a) 0.1 mol L^{-1} KCl and (b) 0.1 mol L^{-1} HCl.

Table 7
The peak potential registered by SWV in anodic and cathodic scan.

EVOO	HCl		KCl	
	Anodic/V	Cathodic/V	Anodic/V	Cathodic/V
P1	0.637	0.574	0.630	0.325
P2	0.687	0.469	0.696	0.293
P3	0.657	0.499	0.705	0.323
P4	0.665	0.594	0.639	0.197
A1	0.708	0.546	0.746	0.128
A2	0.663	0.518	0.669	0.190
A3	0.665	0.557	0.742	0.098
A4	0.554	0.274	0.556	0.122
A5	0.538	0.308	0.535	0.179
A6	0.519	0.432	0.536	0.183
A7	0.529	0.448	0.483	0.219
A8	0.706	0.583	0.650	0.214
A9	0.694	0.405	0.730	0.147
A10	0.680	0.564	0.602	0.318
A11	0.684	0.549	0.605	0.321
H1	0.662	0.538	0.633	0.132
H2	0.708	0.427	0.617	0.044
H3	0.596	0.205	0.521	0.022
H4	0.631	0.191	0.545	0.016
H5	0.527	0.302	0.557	0.144
H6	0.556	0.286	0.545	0.176
H7	0.660	0.538	0.525	-0.010
H8	0.644	0.565	0.543	0.036
H9	0.499	0.339	0.500	0.203
H10	0.669	0.552	0.597	0.138

was used. This result indicated that the organoleptic characteristics of the oils, in particular the bitterness, are strongly related to the responses provided by the electronic tongue.

3.3.5. *PLS2 regression: EPS–polyphenolic compounds*

Finally, the correlations between signals obtained with EPS and the concentration of polyphenolic compounds determined by HPLC were established. The *X* matrix was obtained using data registered by EES and the *Y* matrix was built using data obtained by HPLC.

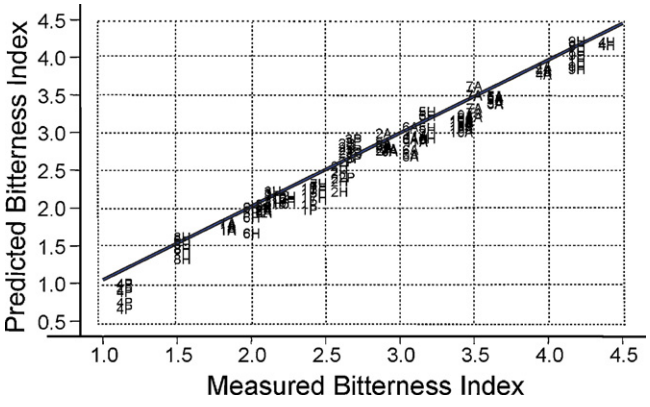

Fig. 7. Plots of predicted bitterness intensity obtained with electronic tongue vs. the values of bitterness intensity obtained by the panel of experts.

Table 8
Quantitative data of PLS2 regression.

Compound	Calibration				Validation			
	Slope	Offset	Rc	RMSEC	Slope	Offset	Rp	RMSEP
Hydroxityrosol	0.899	0.002	0.948	0.010	0.870	0.003	0.925	0.013
Tyrosol	0.962	0.001	0.980	0.004	0.938	0.002	0.971	0.005
Vanillic acid	0.898	0.0001	0.948	0.0004	0.875	0.0001	0.925	0.0005
Vanillin	0.965	4E-5	0.982	0.0002	0.953	5E-5	0.975	0.0002
p-Coumaric acid	0.867	3E-5	0.931	0.0002	0.846	4E-5	0.909	0.0002
Hydroxytirosol acetate	0.959	0.005	0.979	0.021	0.943	0.007	0.971	0.025
DAOL	0.952	0.021	0.976	0.094	0.939	0.027	0.964	0.114
Tyrosyl acetate	0.932	0.0009	0.965	0.003	0.920	0.001	0.952	0.004
DALI	0.965	0.006	0.982	0.020	0.950	0.009	0.975	0.024
Pinoresinol	0.969	0.0001	0.984	0.001	0.955	0.0002	0.978	0.001
Cinnamic acid	0.958	1E-5	0.979	0.0001	0.938	1E-5	0.969	0.0001
ι-Acetoxypinoresinol	0.954	0.002	0.976	0.004	0.934	0.003	0.967	0.005
MAOL	0.965	0.007	0.982	0.030	0.956	0.009	0.975	0.035
MALI	0.901	0.008	0.949	0.017	0.887	0.009	0.933	0.019
Ferulic acid	0.834	7E-5	0.913	0.0002	0.789	8E-5	0.879	0.0002
Luteolin	0.955	0.0006	0.977	0.0009	0.947	0.0007	0.969	0.001
Apigenin	0.957	0.0002	0.978	0.0006	0.948	0.0003	0.970	0.0007
Polyphenols	0.967	0.039	0.983	0.091	0.952	0.057	0.975	0.111
Ortodiphenols	0.967	0.027	0.983	0.081	0.954	0.037	0.974	0.099
Secoiridoids	0.956	0.040	0.978	0.100	0.940	0.055	0.968	0.121

The polyphenolic concentrations predicted by the PLS2 model were compared with measured data (real) obtained by HPLC.

Good correlations were obtained both in calibration and validation, using 19 latent variables. The optimal number of latent variables (LVs) was determined by the lowest value of the predicted residual error sum of squares (PRESS). In the development of PLS2 model, full cross-validation was used to evaluate the quality and to prevent overfitting of calibration model. Also in this case, regressions with correlation coefficient higher than 0.9 were accomplished with all polyphenolic compounds analysed by HPLC (Table 8).

4. Conclusions

The combination of electronic systems coupled to multivariate data analysis can represent a useful device for the characterisation of extra virgin olive oils. PCA has demonstrated that the capability of discrimination of the electronic panel. The use of PLS-DA as supervised method for classification has evidenced the compatibility of data fusion from all three systems, showing a good predictive capacity according to the olive variety. PCA and PLS-DA have demonstrated that the capability of discrimination and prediction can be improved by merging the signal coming from the three instruments.

Regression models PLS1 have evidenced good correlations (correlation coefficients higher than 0.9) between electronic tongue data and bitterness scores determined by a panel of experts. PLS2 models have permitted to predict accurately the polyphenolic content of EVOO that were determined by HPLC.

According to these results, the electronic panel appears as a complementary tool in the characterisation of olive oil samples in the routine analysis.

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