JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Classification and Characterization of Different White Grape Juices by Using a Hybrid Electronic Tongue

Manuel Gutiérrez-Capitán,*[,†] José-Luis Santiago,[‡] Jordi Vila-Planas,[†] Andreu Llobera,[†] Susana Boso,[‡] Pilar Gago,[‡] María-Carmen Martínez,*[,‡] and Cecilia Jiménez-Jorquera[†]

[†]Instituto de Microelectrónica de Barcelona (IMB-CNM), CSIC, Campus UAB, 08193 Bellaterra, Barcelona, Spain

[‡]Misión Biológica de Galicia, CSIC, Carballeira 8, 36143 Salcedo, Pontevedra, Spain

ABSTRACT: A multisensor system combined with multivariate analysis is applied for the characterization and classification of white grape juices. The proposed system, known as hybrid electronic tongue, consists of an array of electrochemical microsensors and a colorimetric optofluidic system. A total of 25 white grape juices representing the large variability of vines grown in the Northwest Iberian Peninsula were studied. The data obtained were treated with Principal Component Analysis (PCA) and Soft Independent Modeling Class Analogy (SIMCA). The first tool was used to train the system with the reference genotypes -*Albariño, Muscat à Petit Grains Blanc* and *Palomino*- and the second to study the feasibility of the hybrid electronic tongue to distinguish between different grape juice varieties. The results show that the three reference genotypes are well differentiated in the PCA model and this can be used to interpolate the rest of varieties and predict their basic characteristics. Besides, using the SIMCA, the system demonstrates high potential for classifying and discriminating grape varieties.

KEYWORDS: *electronic tongue, data fusion, grape juice analysis, grape variety classification, Vitis vinifera*

INTRODUCTION

Grapevine is one of the oldest crops in the world and also one of those of more economic importance. According to Alleweldt and Dettweiler[1] the number of varieties belonging to *Vitis vinifera* L. is estimated to be around 6000, pointing up the great biodiversity that still exists in this crop. However, only a small percentage is used at present for commercial purposes. In the north and northwestern regions of the Iberian Peninsula the diversity of grapevine is still high. The collection of living vines at the Misión Biológica de Galicia (CSIC) research station maintains specimens of 100 accessions recovered in these regions. The characterization and identification of the grape juices obtained from this large number of *Vitis* genotypes around the world is a common and necessary task in viticulture. Besides, this analysis is useful for the producers to decide the procedure of wine elaboration and to control de quality of the must. On the other hand, it is significant to mention the complexity of the grape juice as a sample, given the rapid changes in the chemical composition (sugars, ethanol, pH, amino acids), physical properties (turbidity, density, color) and varietal aromas. For that reason, grape juice requires rapid and reliable measurements in order to act in an efficient way during the elaboration process. Nowadays, the characterization and authenticity of grape juices are based mainly on the analysis of the protein fingerprint[2] or the residual DNA.[3,4] These methods are time-consuming and require complex and expensive equipment.

A promising approach to the analysis of foods and beverages consists of the use of multisensor systems (the so-called electronic tongues).[5] These systems entail the use of an array of chemical sensors with partially selective responses plus a multivariate chemometric tool and permit qualitative (characterization, classification) and/or quantitative (multidetermination) analysis to be obtained in liquid media.[6,7] When the system is based on the data fusion of various measurement techniques (potentiometry, amperometry, conductance, spectrophotometry, etc.), it is called a "hybrid" electronic tongue because it merges variables of different nature.

The electronic tongues have demonstrated their versatility in many types of food samples: beer,[8] meat,[9] olive oils,[10] rice,[11] wine,[12] etc. However, only a few works have attempted to analyze grape juice using multisensor systems due to the complexity and instability of this matrix. Sayago et al.[13] used a tin oxide multisensor as an electronic nose to discriminate grape juices and fermented wine phases from different varieties. Roussel et al.[14] merged variables from an electronic tongue and infrared and ultraviolet spectrometry to classify grape juices in variety categories. Also Moreno-Codinachs et al.[15] achieved the discrimination of varieties from white grape juices by using ion-selective field effect transistor (ISFET) sensors. Finally, Buratti et al.[16] utilized near-infrared and midinfrared spectroscopies together with an electronic nose and an electronic tongue to monitor the alcoholic fermentation of grape juices.

In this paper, a hybrid electronic−optic tongue is applied for the characterization and classification of white grape juices. The multiparametric system used is based on an array of electrochemical sensors fabricated with microelectronic technology, and it is constituted by seven ISFET potentiometric sensors sensitive to pH, common ions and generic ones, a conductivity sensor, a redox potential (ORP) sensor and two amperometric electrodes: a gold (Au) microelectrode and a microelectrode for sensing electrochemical oxygen demand (EOD). These microsensors have the advantages of their small

Received: March 24, 2013
Revised: August 28, 2013
Accepted: September 4, 2013
Published: September 4, 2013

size, robustness and rapid response, which in turn provide miniaturized and portable analytical systems for in-field measurements. The optofluidic system consists of a multiple internal reflection (MIR) system fabricated by soft lithography with a high degree of monolithic integration.[17] The main advantage of the MIR configuration is the high sensibility, thanks to a longer optical path. For the data treatment, two different multivariate analysis techniques, principal component analysis (PCA) and soft independent modeling class analogy (SIMCA), are used. The set of samples used consists of 25 different white *Vitis* genotypes maintained at the collection of living vines at the Misión Biológica de Galicia (CSIC). These samples have been grown under the same edaphoclimatic conditions and following the same crop practices, therefore their characteristics are comparable. In this work we have demonstrated that the electronic tongue is able to differentiate the samples according to the three reference varieties of grape juice—*Albariño*, *Muscat à Petit Grains Blanc* and *Palomino*—and also to characterize the different *Vitis* genotypes from these three different varieties.

■ MATERIALS AND METHODS

Reagents and Solutions. All reagents used were of high purity, analytical grade or equivalent. All solutions were prepared with deionized water.

The ion-selective membranes for the ISFETs were prepared from a photocurable polymer (Cytec, USA), using dioctyl sebacate (DOS) and dibutyl phthalate (DBP), both from Fluka (Switzerland), as plasticizers. The recognition elements employed to formulate the potentiometric membranes were valinomycin (potassium ionophore I, Fluka), 4-*tert*-butylcalix[4]arene tetraacetic acid tetraethyl ester (TBCTA, sodium ionophore X, Fluka), *N*,*N*,*N*′,*N*′-tetracyclohexyl-3-oxapentanediamide (ETH 129, calcium ionophore II, Fluka) and tridodecylmethylammonium chloride (TDMACl, chloride ionophore, Fluka). In addition, a recognition element with generic response for anions was used: tetraoctylammonium bromide (TOAB, Fluka). The ionic additive potassium tetrakis(4-chlorophenyl) borate (KpClPB, Fluka) was used when necessary for a correct potentiometric response. All the components of the membrane were dissolved in tetrahydrofuran (THF, Fluka).

For ISFET calibration, solutions with ionic salts with concentrations between 10^{-4} and 10^{-2} mol/L were prepared. In the case of those sensitive to cations (Na^+, K^+ and Ca^{2+}), the corresponding chloride salts were considered. For the Cl^- ion and the generic response to anion ISFETs, solutions of NaCl and KNO_3, respectively, were prepared.

A solution containing 0.1 mol/L KNO_3 was needed to activate the amperometric gold (Au) sensor. In order to calibrate the conductivity sensor, two different standard solutions from Crison (Spain), with nominal values of 1413 μS/cm and 147 μS/cm, were utilized. Two standard redox solutions from Panreac (Spain), with values of 468 mV and 220 mV, were needed to calibrate the ORP sensor. Finally, for the electrochemical oxygen demand electrode, a solution containing 0.1 mol/L NaOH and a glucose stock solution of 25 g/L were used.

For the ISFET measurements, a reference solution containing an average concentration of the main species present in grape juice was prepared.[18] The composition of this solution is shown in Table 1.

Table 1. Composition of the Reference Solution Prepared for the ISFET Measurements

Component	conc. (mmol/L)
K_2SO_4	3.33
KCl	11.42
Na_3PO_4	2.56
KH_2PO_4	2.18
malic acid	34.68
citric acid	2.68
fructose	541.20
glucose	541.20
tartaric acid	36.58
glycerol	8.20
KOH	8.38

Plant Material. Twenty-five white *Vitis* genotypes were studied (Table 2). All belong to the species *V. vinifera* L., but one (*Catalán Blanco*) is a direct producer hybrid (DPH), obtained from the crossing of a variety of *V. vinifera* L. and an American species. While *Moscatel de Alejandría* is destined for fresh consumption (Table grape), the remaining genotypes are used mainly for winemaking.

Table 2. Set of Genotypes Used for the Study

Studied Varieties		
Albillo Galicia	*Catalán Blanco*	*Moscatel de Alejandría*
Albarín Blanco	*Chasselas Doré*	*Muscat à Petit Grains Blanc*
Albariño	*Chenin Blanc*	*Palomino*
Blanca	*Doña Blanca*	*Ratiño*
Bastardo Blanco	*Godello*	*Savagnin Blanc*
Bastardo Ruzo	*Lado*	*Silveiriña*
Blanca Mantilla	*Loureiro*	*Torrontés*
Brancellao Blanco	*Monstruosa*	*Treixadura*
Caíño Blanco		

Some of these genotypes (*Albarín Blanco*, *Albariño*, *Godello*, *Ratiño* or *Treixadura*) are ancient cultivars traditionally grown in northwestern Spain and northern Portugal, even autochthonous and therefore highly adapted to the edaphoclimatic conditions in these areas. Other genotypes (Palomino, Chasselas, or Savagnin Blanc) were introduced in the Northwest of Spain in the late 19th/early 20th century.[19,20] All genotypes are growing at present at the grapevine collection of the Misión Biológica de Galicia research station (Consejo Superior de Investigaciones Científicas, CSIC), located in Pontevedra (Northwest Spain). All of them, between the 11th and 13th April 1994, were grafted onto rootstocks planted the year before. The plants were therefore 16 years old at the beginning of the study. Ten plants were performed per genotype and were randomly distributed in the same plot.[21] The distance between the plants was 2.5 m and the distance between rows 2 m, rendering a planting density of 2000 plants/ha. All plants were grown as an espalier and pruned using the Sylvoz method. All received identical protection treatments and had been subject to identical cultivation practices. The soil of the plot in which they grew was a sandy loam (70.1% sand, 16.1% silt, 13.8% clay) with 8% organic matter. The vineyard area is a rectangle with an approximate area of 1.4 ha, with a slope of <5% and 35 m above sea level. The plot is equipped with a weather station; the mean annual temperature for the last 50 years is 14.11 °C and the mean annual rainfall 1686.68 mm.

Grape Juice Extraction and Storage. Maturation of all genotypes included in the study was monitored at the end of August 2009. For this purpose, 10 berries from different clusters of each of the 10 plants per genotype were taken twice a week, and the sugar concentration (°Brix) of a 5 mL grape juice sample was determined using a Zuzi 50305150 hand-held refractometer (Auxilab SL, Spain). Between 23th and 30th September 2009 all genotypes were harvested depending on their optimum ripeness (when they stopped concentrating sugar). Berries were selected from the central part of several clusters of each of the 10 plants per genotype. Then, they were crushed in a glass mortar and the juice obtained was static racked at 4 °C for 24 h. The juice was then decanted to fill 50 mL Falcon tubes (two per genotype) and immediately frozen at −40 °C. In the case of *Albariño*, *Muscat à Petit Grains Blanc* and *Palomino*, four 50 mL Falcon tubes were used. These three genotypes were selected as reference genotypes for training the electronic tongue because their grape juices present extreme or intermediate characteristics within the whole set of genotypes included in this work. For example, *Muscat à Petit Grains Blanc* was selected due to its high aromatic intensity, *Palomino* for its

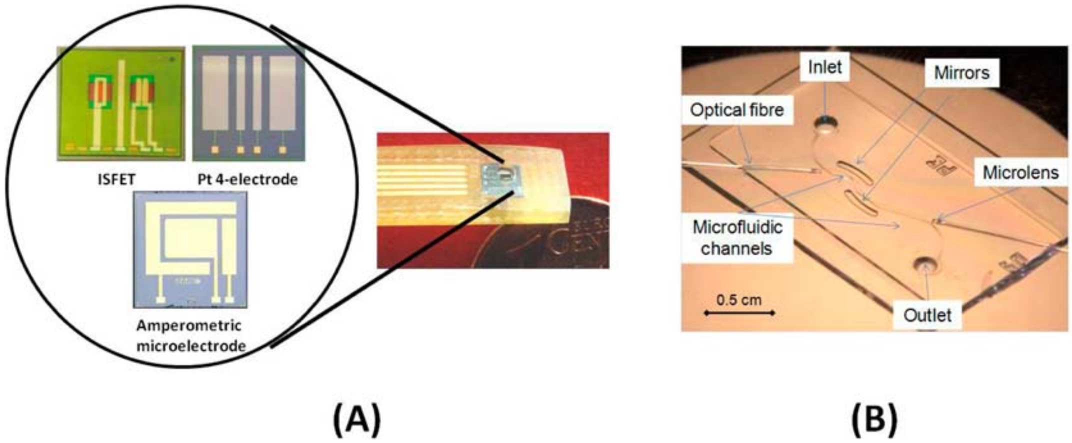

Figure 1. Picture of the microsensors used: (A) chips used for electrochemical sensors and (B) optical MIR system.

neutral juice (under the plot conditions) and low acidity, and *Albariño* for its intermediate juice characteristics and high acidity.

The samples were specially transported from Pontevedra to Barcelona (Spain) where they were defrosted, filtered (0.45 μm) to remove nonsoluble solids and used for the analysis with the electronic tongue.

Hybrid Electronic Tongue. In order to obtain the maximum information about the grape juice, sensors with different nature and with response to different characteristics of the sample were used. Photographs of the chips used for these sensors, the probe and the MIR system are shown in Figure 1.

A set of seven ISFET sensors (Figure 1A) were fabricated using conventional microelectronic technology.[22] One ISFET was used for measuring pH, and the rest were modified with polymeric membranes sensitive to Na^+, K^+, Ca^{2+}, Cl^- and generic response to anions. Therefore, this set of sensors provides information about the concentration of some key ions in the grape juice. Membrane composition and preparation are presented elsewhere.[23−26] The generic ISFET for anions was duplicated in the used array. An Orion 90-02-00 double junction Ag/AgCl reference electrode (Thermo Fisher Scientific, Waltham, MA, USA) with 0.1 mol/L CH_3COOLi solution in its outer chamber was employed for all these potentiometric measurements. For signal conditioning and control data acquisition, a data acquisition card NI USB-6259 M series from National Instruments (USA) was used. This system was connected to the PC through the USB link. The control programs were written by the authors based on LabView graphic language (National Instruments).

Sensors based on Pt 4-electrode configuration (Figure 1A) were employed as conductivity sensor and ORP sensor. The conductivity sensor provides information about the overall ionic charge of the sample while the ORP sensor reports on the global capacity of the grape juice to oxidize or reduce. Their fabrication and characterization are reported elsewhere.[27] For signal conditioning and control data acquisition, a versatile and portable system developed at the IMB was used. This system was connected to the PC through the USB link. The control programs were also written by the authors based on LabView graphic language (National Instruments).

Two different amperometic microsensors (Figure 1A) were employed: a conventional Au microelectrode fabricated according to standard photolithographic techniques and a composite planar electrode (CPE) for sensing electrochemical oxygen demand (EOD) developed by our group.[28] The first one shows the presence of redox species and their concentration, while the sensor for EOD provides information about simple oxidized organic molecules, like glucose and fructose. In both cases, the amperometric cell contained the working electrode, a Pt commercial electrode as counter electrode (Radiometer, France) and a Ag/AgCl/10% (w/v) KNO_3 reference electrode (Metrohm 0726 100, Switzerland). A μ-Autolab potentiostat/galvanostat (Ecochemie, The Netherlands), using GPES 4.7 software package (General Purpose Electrochemical System), was used for all voltammetric and amperometric measurements. The chips corresponding to these sensors were fixed and encapsulated in a printed circuit board as shown in Figure 1A for connection to the measurement circuit and dipping in solution.

Optical measurements were done using a MIR configuration fabricated in poly(dimethylsiloxane) (PDMS) (Figure 1B). The photonic lab on a chip comprises microlenses, self-alignment structures, microfluidic channels and air mirrors for highly sensitive absorbance measurements.[17] This lab-on-a-chip system can measure only in the UV−vis range due to the limitations of the used spectrometer. For light emission, a deuterium/halogen lamp (Ocean Optics DH-2000) was used. The readout multimode optical fiber was connected to a spectrometer (Ocean optics HR4000) which covers the range between 200 and 1100 nm with a spectral resolution of 2 nm, a signal-to-noise ratio (SNR) > 12 dB and a dynamic range (DR) = 25 dB (or 2.5 au), which ensure the good quality of the registered spectra. Using the Ocean Optics SpectraSuite software (Oracle, USA), the absorption of the sample was recorded.

Methodology. All sensors were characterized before the analysis in order to assess their correct behavior. The analytical response characteristics are reported in a previous work.[29] Sample analysis was carried out under batch conditions using individual sensors. All the different samples were assayed randomly. A complete analysis of a grape juice sample took around 15 min.

For the ISFET set, the output signals corresponded to the relative measurements of each sensor with respect to the reference solution, which was checked periodically. This is a common strategy to correct the possible drift of the sensors.

The 4-electrode sensor was first chemically cleaned successively with ethanol 96%, H_2SO_4 6.0 mol/L and deionized water. Calibration of conductivity sensor was carried out using two standard solutions (1413 and 147 μS/cm). For ORP sensor evaluation, a test with standard redox solutions of 220 and 468 mV (at 25 °C) versus the Ag/AgCl reference electrode was performed. Once the good behavior of the sensors had been confirmed, they were immersed in the sample and the signals were recorded every 30 s during 3 min.

The Au microelectrode was first chemically cleaned as before, followed by an electrochemical activation carried out in 0.1 mol/L KNO_3 where the electrode was cycled from +0.8 to −2.2 V at least 20 times. With this treatment, a constant and stable gold surface is obtained. Then, the electrode was immersed in the sample under study and two cyclic voltammograms (CV) from +1.6 to −0.5 V at a scan rate of 0.1 V/s were run: the first one to stabilize the signal and the second one to obtain the information. During the scans the oxidized gold is then reduced in the same cycle. Therefore, the variation detected in intensity is caused only by the different solution in contact with the electrode. Finally, the electrode was activated after measuring each grape juice sample by running five CV in 0.1 mol/L KNO_3.

Regarding the electrochemical oxygen demand sensor (EOD) based on a CPE, previous studies in our laboratories have demonstrated the response of this electrode toward glucose and some other sugars. These components are present in large concentrations in the grape juice. Using the chronoamperometric mode and setting the potential at +600 mV, the CPE was immersed in NaOH 0.1 mol/L. When the response became stable, 100 μL of the studied grape juice was added, while the intensity was recorded.

In the case of the photonic lab on a chip, measurements were carried out by filling the system with the sample to be analyzed. In order to obtain the spectra in absorbance units, deionized water was used as reference. Measurements were taken prior to and after each grape juice to determine signal drifts due to nonspecific adsorption at the walls. Throughout the experiments, the deionized water reached the same value between the experimental errors, confirming the reversibility of the photonic lab on a chip. Finally, for statistical purposes, for each grape juice type, the average of 10 consecutive scans was considered.

Data Treatment and Analysis. The obtained data were treated using two different multivariate methods. The principal component analysis (PCA) was used at first to evaluate the system's ability to characterize the samples. For this purpose, a response model was generated with the reference samples of *Albariño*, *Muscat à Petit Grains Blanc* and *Palomino* (four samples of each variety). Then, the rest of the grape juices were interpolated in this PCA to analyze their relative position in the new space. Second, the Soft Independent Modeling Class Analogy (SIMCA) classification method was applied to prove the feasibility of the electronic tongue to distinguish between the grape juice samples. For both methods, the original values were previously autoscaled (all the variables were centered and set to a standard deviation equal to 1) to avoid that the variables have more influence on the model only by having different nature and units. Besides, in all the analyses the cross-validation method was used and the generated models were also centered. To control all these parameters and to perform the analysis, the Unscrambler v. 10.1 (CAMO, Norway) informatics package was used.

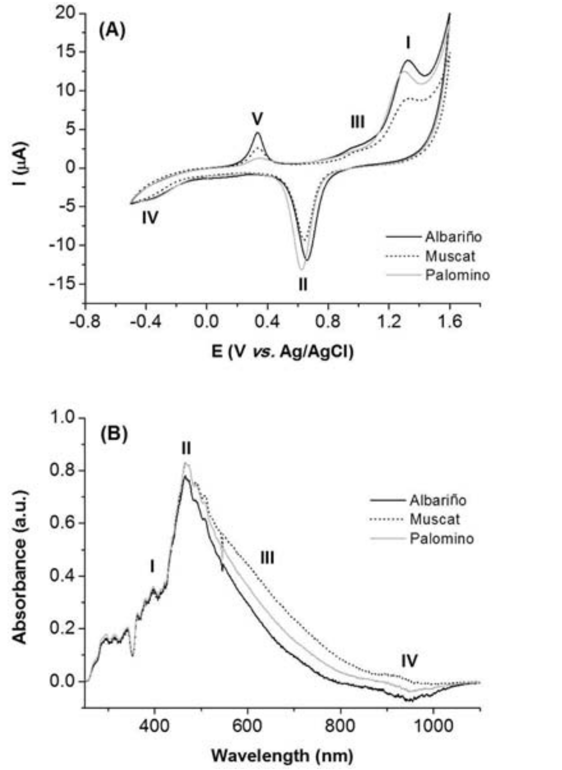

Figure 2. (A) Cyclic voltammograms obtained with the gold microelectrode and (B) absorbance spectra obtained using deionized water as reference for the three grape juice used as reference. Selected values of either current intensity or absorbance were used as variables for the models.

RESULTS AND DISCUSSION

Identification of Input Variables. One of the most important steps in a multivariable analysis is the selection of the variables that will form the model. The idea is to obtain the maximum information of the sample but with the minimum number of variables in order to simplify the experimental set up and the model management. In the case of the seven ISFETs, the input data corresponded to the relative signal of each ISFET with respect to the synthetic reference solution.

For the conductivity and ORP sensors, the absolute signal after three minutes recording was included in the model. This time was set to obtain a stable signal for the ORP sensor. In the case of the EOD sensor, the intensity at 120 s after the grape juice addition was used as variable.

For the Au amperometric microelectrode, cyclic voltammograms (CV) were carried out to obtain the input data for the model. The CV curves obtained for *Albariño*, *Muscat à Petit Grains Blanc* and *Palomino*, the three genotypes used as reference in this study are shown in Figure 2A. As can be observed, at +1.6 V the oxidation of water has not reached the maximum (the higher current measured is around 15 μA). Besides, taking into account that the grape juices have a low pH (around 3), the oxidation of water is shifted to more positive potentials. The CVs show two redox peaks corresponding to the oxidation (peak I) and reduction of the electrode Au (peak II) at +1.31 V and +0.65 V, respectively. As can be seen, the current intensity of these peaks is different for each variety, especially for the oxidation one, which is less intense in the case of the *Muscat à Petit Grains Blanc* sample. Besides, two smaller shoulders are observed at +1.01 V and at −0.38 V (peaks III and IV) with different intensities depending on the grape variety. It is possible that these peaks correspond to the polyphenol content since their antioxidant capacity is well-known.[30] Finally, a well-defined peak appears at +0.35 V (peak V), which may be also related with the antioxidant properties of grape juices. This peak is more intense in the case of the *Albariño* sample than in the other two varieties. Therefore, the current intensities of these five peaks were used as variables.

The absorbance spectra of the grape juices were analyzed in order to choose the optical variables. In Figure 2B, the absorbance using deionized water as background for the three reference juices is depicted. These spectra are the average of 10 consecutive scans, using an integration time of 500 ms. The values measured for the grape juices are higher than 1/3 of the whole dynamic range, therefore these data are feasible for grape juice differentiation. As can be seen, the spectra obtained are quite similar within the three grape varieties. Generally speaking, *Muscat à Petit Grains Blanc* presents a higher absorbance in comparison with the other two, especially in the range between 500 and 1000 nm. Although the color defined for wine and grape juice covers the absorbance at 420, 520, and 620 nm,[31] in our case these values do not coincide with peaks of maximum absorbance. In order to not lose information about the samples we decided to consider the maximum variation of signal between samples; then absorbance values at 395, 465, 650, and 945 nm were chosen. Therefore, the final models were composed of 19 variables depicted in Table 3.

Table 3. Variables Considered To Construct the Response Models

Sensor	Variables
ISFETs	pH, Na^+, K^+, Ca^{2+}, Cl^- and two generic to anions
Pt 4-electrode	conductivity and ORP
gold microelectrode	current intensity at +1.31, +1.01, +0.65, +0.35 and −0.38 V
EOD sensor	current intensity at 120 s after the addition of grape juice
optical MIR system	absorbance values at 395, 465, 650, and 945 nm

Generation of the PCA Response Model. The first study performed with this multisensor system was the analysis of the *Albariño, Muscat à Petit Grains Blanc* and *Palomino* juices in order to build the response model. This calibration (training) set is composed of four samples of each grape variety, a total of 12 samples. With the data obtained from the selected variables, a principal components analysis (PCA) which explains 59% of the total variance was performed (see Figure 3). In the scores plot (Figure 3A), samples formed well differentiated groups in the two component space showing a clear clustering for each genotype. As can be observed, the PC 1 separated the *Albariño* grape juices while the PC 2 distinguished between the *Muscat à Petit Grains Blanc* and *Palomino* groups. At this point, it is interesting to know which variables are responsible of this distinction. The loadings plot (Figure 3B) of the PCA analyzes the weight of each variable in the constructed model, directly related with its distance to the origin: the more away, the more significance in the model. As can be seen, the PC 1, which distinguished the *Albariño*, is constituted basically by the pH ISFET and a set of variables related with the oxido-reduction proprieties, higher than the other two reference genotypes: EOD (glucose, fructose), ORP (global oxido-reduction capacity) and the intensities of the gold microelectrode at +1.01 V (polyphenols) and at +0.35 V. This result agrees with the fact that grape juices or wines from *Albariño* are known by their aromatic quality, with high total acidity values and moderate alcohol content.[32,33] On the other hand, the absorbance at 650 nm and some ionic variables, concretely ISFETs with generic response to anions and conductivity have more importance in the PC 2, which separates *Muscat à Petit Grains Blanc* from *Palomino*. This means that *Muscat à Petit Grains Blanc* presents a higher color intensity and more concentrated species, which can explain a higher aromaticity. On the contrary, there are a set of variables close to the origin and inside the low significance zone which do not supply any useful information for the characterization. As can be observed, these variables are related to the concentrations of cations (Na^+, K^+ and Ca^{2+} ISFETs), the absorbance at 395 nm and the intensity of current at −0.38 V.

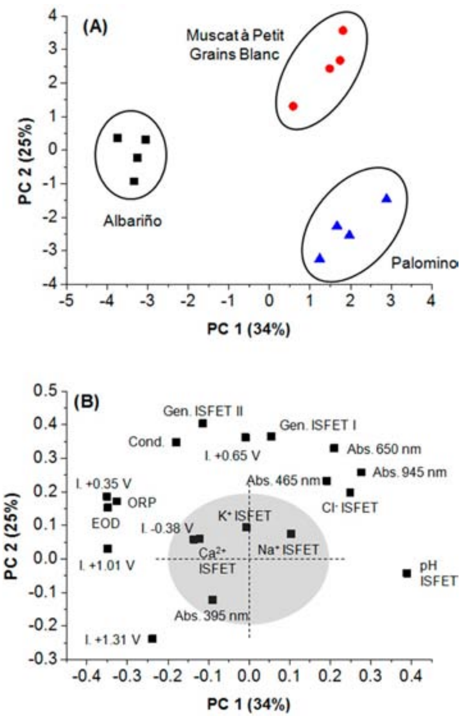

Figure 3. PCA results for the reference samples of *Albariño*, *Muscat à Petit Grains Blanc* and *Palomino*: (A) scores plot and (B) loadings plot.

Characterization of the Grape Juice Samples Using PCA. Once the response model using well-known varieties was constructed, the obtained values from the selected variables for the rest of samples were interpolated in the PCA. The idea was to interpret the characteristics of the grape juices by using the different position of these samples inside the response model generated by the reference genotypes (*Albariño, Muscat à Petit Grains Blanc* and *Palomino*). As can be observed in Figure 4, the interpolated samples are distributed in the two-dimension space. First, there is one sample, *Catalan Blanco*, with extreme values, considering this sample an outlier of the population. As mentioned before, this genotype is a direct producer hybrid (DPH), obtained from crossing the *V. vinifera* L. variety and an American species. Therefore, its characteristics are clearly differentiated from the remaining genotypes (all belonging to the *Vitis vinifera* L. species). On the other hand, the majority of the grape juices are situated in the third quadrant, forming a new group inside the new axes. This group is formed by the varieties *Albarín Blanco*, *Albillo Galicia*, *Blanca*, *Bastardo Blanco*,

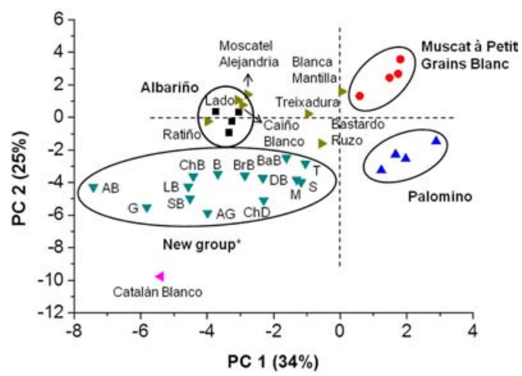

Figure 4. Interpolation of the grape juices in the PCA model formed by *Albariño*, *Muscat à Petit Grains Blanc* and *Palomino*. New group: *Albarín Blanco* (AB), *Albillo Galicia* (AG), *Blanca* (B), *Bastardo Blanco* (BaB), *Brancellao Blanco* (BrB), *Chasselas Doré* (ChD), *Chenin Blanc* (ChB), *Doña Blanca* (DB), *Godello* (G), *Loureiro Blanco* (LB), *Monstruosa* (M), *Savagnin Blanc* (SB), *Silveiriña* (S) and *Torrontés* (T).

Brancellao Blanco, *Chasselas*, *Chenin Blanc*, *Doña Blanca*, *Godello*, *Loureiro*, *Monstruosa*, *Savagnin Blanc*, *Silveiriña* and *Torrontés*. Having in consideration their position and the variables that form the model, it is probable that these grape juices present a high acidity and high concentration of sugars and polyphenols, even though the variation along the PC 1 is large. Besides, the position in the PC 2 indicates that they have in general low color intensity and diluted species. Finally, some of the samples are inside the reference groups, and therefore they present very similar characteristics regarding the variables used in the response model. It is the case of the *Ratiño*, *Lado*, *Caíño Blanco* and *Moscatel de Alejandría* grape juices, which are included in the *Albariño* group, or *Blanca Mantilla*, which is close to the *Muscat à Petit Grains* group. The inclusion of *Caíño Blanco* in the *Albariño* reference group is not surprising. In fact these two genotypes show morphological similarities (leaves and berries) and are genetically close, sharing at least one allele at each microsatellite locus.[34−36] The explanation for the inclusion of *Ratiño*, *Lado* or *Moscatel de Alejandría* (Table grape) in the reference group of *Albariño* is related mainly to the pH ISFET or the oxido-reduction proprieties of their grape juices among other variables used to construct the response model and not to morphological characteristics. On the other hand, as commented above, *Palomino* genotype is not autochthonous from northwestern Spain. Under the climatic conditions of this area, it is very productive but the wines obtained have less quality ("washy", in the opinion of the winemakers) than in south Spain, where *Palomino* has been traditionally grown to produce the famous Sherry wines.[37] Finally, the grape juice samples of *Treixadura* and *Bastardo Ruzo* are clearly situated halfway between the three reference groups, showing intermediate characteristics.

Classification of the Grape Juice Samples Using SIMCA. In order to study in more detail the feasibility of the hybrid electronic tongue to distinguish between different grape juices, the SIMCA method was applied. SIMCA is a multivariate chemometric tool based on class modeling algorithms for classification. Once constructed, any other sample could be interpolated in the SIMCA model, even if this sample has not participated in the initial setup. Recently, this type of pattern-recognition method has been recommended in food-authenticity applications given its capacity of characterizing individual classes in a totally independent way and defining an enclosed class space based on a statistical confidence level.[38]

Three different models based on PCA were built using the values of the four samples of *Albariño*, *Muscat à Petit Grains Blanc* and *Palomino*. All three models were formed by two PCs which explain 86%, 83% and 86% of the total variance, respectively. In Figure 5, the Coomans diagrams obtained for the classification of the rest of grape juices among the combination of these models are depicted. In the three plots, the prediction values are shown with a probability of 95% (significance of 5%). In the x-axis of Figure 5A,B, the distance to the *Albariño* model is shown. Thus, grape juices that are placed between 0 and 3 on the x-axis belong to the *Albariño* model. On the y-axis, the distance of the samples to the *Muscat à Petit Grains Blanc* (Figure 5A) or to the *Palomino* (Figure 5B) models is shown. Hence grape juices that are placed between 0 and 3 on the y-axis belong to one of these models. Juices that are placed in the area corresponding to $x,y > 3$ do not belong to any of the represented models and can be distinguished. On the other hand, juices that are located in the area $x,y < 3$ of the plot

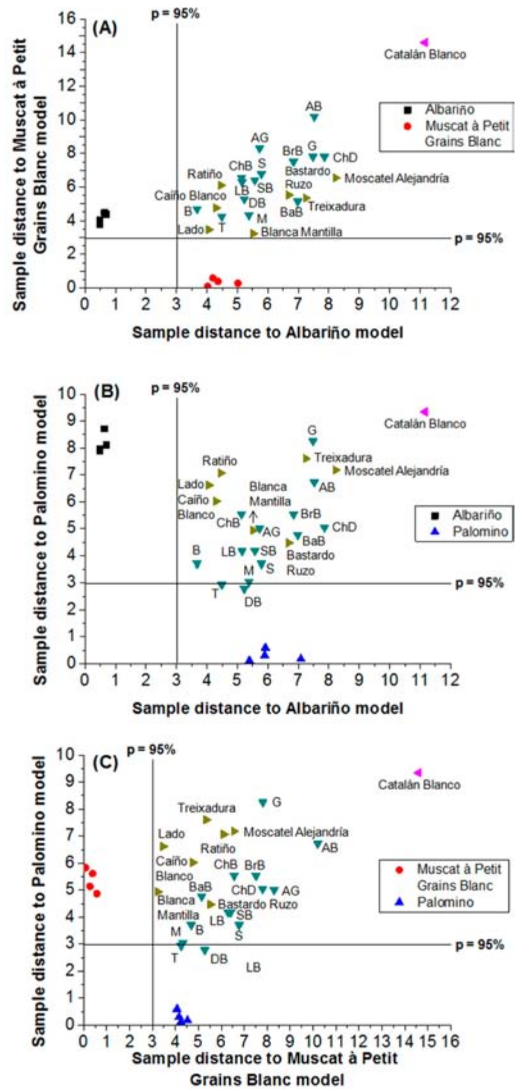

Figure 5. Coomans diagrams for the three combinations of models: (A) *Albariño-Muscat à Petit Grains Blanc*, (B) *Albariño-Palomino* and (C) *Muscat à Petit Grains Blanc-Palomino*. All are represented with a probability of 95%. *Albarín Blanco* (AB), *Albillo Galicia* (AG), *Blanca* (B), *Bastardo Blanco* (BaB), *Brancellao Blanco* (BrB), *Chasselas Doré* (ChD), *Chenin Blanc* (ChB), *Doña Blanca* (DB), *Godello* (G), *Loureiro Blanco* (LB), *Monstruosa* (M), *Savagnin Blanc* (SB), *Silveiriña* (S) and *Torrontés* (T).

belong to both represented models. As can be observed in Figure 5A,B, the *Albariño* grape juices are located inside their model and are well separated from the rest of the samples, with 95% probability. Using the SIMCA no grape juice coincided with the *Albariño*. Some samples presented as similar using the PCA (*Ratiño*, *Lado* and *Caíño Blanco*) now are the closest to the model (distance between 3.5 and 5). However, *Moscatel de Alejandría*, the unique Table grape genotype included in the study, is now, as expected, one of the most distant from the *Albariño* model. This new classification is due to the different algorithm used by PCA and SIMCA. PCA builds a model based in the variables that differentiate the reference grape juice samples, whereas SIMCA focuses on those variables that make an established group homogeneous. In the case of the *Muscat à Petit Grains Blanc* model (Figure 5C), it is also well distinguished from the rest of the grape juices. The *Blanca Mantilla* sample is the most similar to the model with a distance of 3.2, very near to the limit of the *Muscat à Petit Grains Blanc* group. On the other hand, *Palomino* produces neutral grape juices under the edaphoclimatic conditions of the northwestern Spain and therefore is more likely to be confused with other grape juice samples obtained from genotypes with neutral berry flavor. In this case, the *Doña Blanca*, *Torrontés* and *Monstruosa* grape juice samples are indistinguishable from *Palomino* samples, with 95% probability. Besides, it is remarkable that any grape juice belongs to two models at the same time, proving the validity of the genotypes selected as reference for training the hybrid electronic tongue. As happened in the PCA, the position of the *Catalán Blanco* in the Coomans diagrams is very far from the three referenced models, confirming its hybrid nature, with overall characteristics like high acidity, low alcohol degree or *foxè* flavor (not present in *Vinifera*) that clearly differentiate the grape juice of this genotype from the others.

In conclusion, the good results obtained both for characterization and for classification of grape juices confirm the viability of the multisensor system. This system differentiates with a high resolution the three reference varieties and is able to classify using the different grape juice samples according to their similarities with the reference varieties. Also the PCA model constructed allows rapid and global information about their basic characteristics to be obtained: aromatic quality, total acidity, pH, global content of sugars, color intensity, etc. Besides, SIMCA technique allowsthe reference varieties and the rest of grape juice samples to be distinguished. With a 95% probability, no grape juice is confused with the *Albariño* or *Muscat à Petit Grains Blanc* models, while the *Palomino* model is wider and some samples are not differentiated.

This system could be applied for fraud detection as demonstrated with the results for the non-*vinifera* genotype *Catalán Blanco*. This grape juice has been considered by the electronic tongue as an outlier among all other grape juices analyzed. This agrees with the consideration of this genotype as a direct producer hybrid the use of which for wine production is prohibited in several European countries. Besides, this multisensor system could also be a very useful tool for the producers during wine-making according to the grape juice characteristics reported by the electronic tongue.

AUTHOR INFORMATION

Corresponding Authors

*Tel: +34 986 854800. Fax: +34 986 841362. E-mail: carmenmartinez@mbg.csic.es.

*Tel: +34 93 594 7700. Fax: +34 93 580 1496. E-mail: manuel.gutierrez@imb-cnm.csic.es.

Author Contributions

The first two authors contributed equally to this research.

Funding

We acknowledge funding from the Spanish R & D National Program (MINECO Project TEC2011-29045-C04-01), the INIA-MICINN National Program (Project RF2008-00002-C02) and the Juan de la Cierva scholarship (M.G.-C.).

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The technical assistance of Nidia Santamaría, Alfredo Cadarso, Iván González, Elena Zubiaurre and Mª Sol Taboada is highly appreciated.

ABBREVIATIONS USED

CPE, composite planar electrode; CV, cyclic voltammogram; DBP, dibutyl phthalate; DOS, dioctyl sebacate; DPH, direct producer hybrid; EOD, electrochemical oxygen demand; ETH 129, *N*,*N*,*N*′,*N*′-tetracyclohexyl-3-oxapentanediamide; ISFET, ion-selective field effect transistor; KpClPB, potassium tetrakis-(4-chlorophenyl) borate; MIR, multiple internal reflection; ORP, redox potential; PCA, principal component analysis; PDMS, poly(dimethylsiloxane); SIMCA, soft independent modeling class analogy; TBCTA, 4-*tert*-butylcalix[4]arene tetraacetic acid tetraethyl ester; TDMACl, tridodecylmethylammonium chloride; THF, tetrahydrofuran; TOAB, tetraoctylammonium bromide

REFERENCES

(1) Alleweldt, G.; Dettweiler, E. *The Genetic Resources of Vitis: World List of Grapevine Collections*, 2nd ed.; Federal Research Centre for Grape Breeding Geilweilergof: Siebeldingen, Germany, 1994.

(2) Le Bourse, D.; Jegou, S.; Conreux, A.; Villaume, S.; Jeandet, P. Review of preparative and analytical procedures for the study of proteins in grape juice and wine. *Anal. Chim. Acta* **2010**, *667*, 33−42.

(3) Siret, R.; Gigaud, O.; Rosec, J. P.; This, P. Analysis of grape Vitis vinifera L. DNA in must mixtures and experimental mixed wines using microsatellite markers. *J. Agric. Food. Chem.* **2002**, *50*, 3822−3827.

(4) Rodriguez-Plaza, P.; Gonzalez, R.; Moreno-Arribas, M. V.; Polo, M. C.; Bravo, G.; Martinez-Zapater, J. M.; Martinez, M. C.; Cifuentes, A. Combining microsatellite markers and capillary gel electrophoresis with laser-induced fluorescence to identify the grape (Vitis vinifera) variety of musts. *Eur. Food Res. Technol.* **2006**, *223*, 625−631.

(5) Escuder-Gilabert, L.; Peris, M. Review: Highlights in recent applications of electronic tongues in food analysis. *Anal. Chim. Acta* **2010**, *665*, 15−25.

(6) Holmberg, M.; Eriksson, M.; Krantz-Rülcker, C.; Artursson, T.; Winquist, F.; Lloyd-Spetz, A.; Lundström, I., 2nd Workshop of the Second Network on Artificial Olfactory Sensing (NOSE II). *Sens. Actuators, B* **2004**, *101*, 213−223.

(7) Vlasov, Y.; Legin, A.; Rudnitskaya, A.; Di Natale, C.; D'Amico, A. Nonspecific sensor arrays ("electronic tongue") for chemical analysis of liquids (IUPAC Technical Report). *Pure Appl. Chem.* **2005**, *77*, 1965−1983.

(8) Arrieta, A. A.; Rodriguez-Mendez, M. L.; de Saja, J. A.; Blanco, C. A.; Nimubona, D. Prediction of bitterness and alcoholic strength in beer using an electronic tongue. *Food Chem.* **2010**, *123*, 642−646.

(9) Labrador, R. H.; Masot, R.; Alcaniz, M.; Baigts, D.; Soto, J.; Martinez-Manez, R.; Garcia-Breijo, E.; Gil, L.; Barat, J. M. Prediction of NaCl, nitrate and nitrite contents in minced meat by using a voltammetric electronic tongue and an impedimetric sensor. *Food Chem.* **2010**, *122*, 864−870.

(10) Cosio, M. S.; Ballabio, D.; Benedetti, S.; Gigliotti, C. Evaluation of different storage conditions of extra virgin olive oils with an innovative recognition tool built by means of electronic nose and electronic tongue. *Food Chem.* **2007**, *101*, 485−491.

(11) Tran, T. U.; Suzuki, K.; Okadome, H.; Homma, S.; Ohtsubo, K. Analysis of different the tastes of brown rice and milled rice with milling yields using a taste sensing system. *Food Chem.* **2004**, *88*, 557−566.

(12) Urbano, M.; de Castro, M. D. L.; Perez, P. M.; Garcia-Olmo, J.; Gomez-Nieto, M. A. Ultraviolet-visible spectroscopy and pattern recognition methods for differentiation and classification of wines. *Food Chem.* **2006**, *97*, 166−175.

(13) Sayago, I.; Horrillo, M. D.; Ares, L.; Fernandez, M. J.; Gutierrez, J. Tin oxide multisensor for detection of grape juice and fermented wine varieties. *Sens. Mater.* **2003**, *15*, 165−176.

(14) Roussel, S.; Bellon-Maurel, W.; Roger, J. M.; Grenier, P. Authenticating white grape must variety with classification models based on aroma sensors, FT-IR and UV spectrometry. *J. Food Eng.* **2003**, *60*, 407−419.

(15) Moreno i Codinachs, L.; Kloock, J. P.; Schoning, M. J.; Baldi, A.; Ipatov, A.; Bratov, A.; Jimenez-Jorquera, C. Electronic integrated multisensor tongue applied to grape juice and wine analysis. *Analyst* **2008**, *133*, 1440−8.
(16) Buratti, S.; Ballabio, D.; Giovanelli, G.; Dominguez, C. M. Z.; Moles, A.; Benedetti, S.; Sinelli, N. Monitoring of alcoholic fermentation using near infrared and mid infrared spectroscopies combined with electronic nose and electronic tongue. *Anal. Chim. Acta* **2011**, *697*, 67−74.
(17) Llobera, A.; Demming, S.; Wilke, R.; Buttgenbach, S. Multiple internal reflection poly(dimethylsiloxane) systems for optical sensing. *Lab Chip* **2007**, *7*, 1560−1566.
(18) Flanzy, C. *Enologǵia: Fundamentos científicos y tecnológicos*; Mundiprensa: Barcelona, 2003.
(19) Casares, A. *Observaciones sobre el cultivo de la vid en Galicia*; Imprenta de la Viuda e Hijos de Companel: Santiago de Compostela, Spain, 1843.
(20) Suarez-Canton, N. Asturias vinícola. Breves apuntes sobre el vino de Cangas de Tineo. *Revista de Asturias* **1879**, *14*, 219−221.
(21) Martinez, M. C. La colección de variedades de vid del Consejo Superior de Investigaciones Científicas (Misión Biológica de Galicia). *La Semana Vitivinícola* **2007**, *3198*, 3926−3929.
(22) Jimenez, C.; Bratov, A.; Abramova, N.; Baldi, A. ISFET based sensors: fundamentals and applications. In *Encyclopedia of Sensors*; Grimes, C. A., Dickey, E. C., Pishko, M. V., Eds.; American Scientific Publishers: New York, 2005.
(23) Bratov, A.; Abramova, N.; Dominguez, C. Investigation of chloride sensitive ISFETs with different membrane compositions suitable for medical applications. *Anal. Chim. Acta* **2004**, *514*, 99−106.
(24) Bratov, A.; Abramova, N.; Dominguez, C.; Baldi, A. Ion-selective field effect transistor (ISFET)-based calcium ion sensor with photocured polyurethane membrane suitable for ionised calcium determination in milk. *Anal. Chim. Acta* **2000**, *408*, 57−64.
(25) Bratov, A.; Abramova, N.; Munoz, J.; Dominguez, C.; Alegret, S.; Bartroli, J. Photocurable polymer matrices for potassium-sensitive ion-selective electrode membranes. *Anal. Chem.* **1995**, *67*, 3589−3595.
(26) Beltran, A.; Artigas, J.; Jimenez, C.; Mas, R.; Bartroli, J.; Alonso, J. Development of durable nitrate-selective membranes for all-solid state ISE and ISFET sensors based on photocurable compositions. *Electroanalysis* **2002**, *14*, 213−220.
(27) Orozco, J.; Baldi, A.; Baena, R.; Cadarso, A.; Bratov, A.; Jimenez, C. Portable system based on microsensors for environmental monitoring applications. *Meas. Sci. Technol.* **2007**, *18*, 935−940.
(28) Orozco, J.; Fernandez-Sanchez, C.; Mendoza, E.; Baeza, M.; Cespedes, F.; Jimenez-Jorquera, C. Composite planar electrode for sensing electrochemical oxygen demand. *Anal. Chim. Acta* **2008**, *607*, 176−182.
(29) Gutierrez, M.; Llobera, A.; Vila-Planas, J.; Capdevila, F.; Demming, S.; Buttgenbach, S.; Minguez, S.; Jimenez-Jorquera, C. Hybrid electronic tongue based on optical and electrochemical microsensors for quality control of wine. *Analyst* **2010**, *135*, 1718−1725.
(30) Blasco, A. J.; Rogerio, M. C.; Gonzalez, M. C.; Escarpa, A. "Electrochemical Index" as a screening method to determine "total polyphenolics" in foods: A proposal. *Anal. Chim. Acta* **2005**, *539*, 237−244.
(31) European Commission Regulation (EEC), N° 2676/90 Community methods for the analysis of wines, 17/09/1990.
(32) Zamuz, S.; Martinez, M. C.; Vilanova, M. Primary study of enological variability of wines from different clones of Vitis vinifera L cv. Albarino grown in Mision Biologica de Galicia (CSIC). *J. Food Compos. Anal.* **2007**, *20*, 591−595.
(33) Boso, S.; Rodriguez-Canas, E.; Alonso-Villaverde, V.; Gago, P.; Santiago, J. L.; Martinez, M. C. Variabilidad en vinos de diferentes clones de la variedad Albariño (Vitis viniÉfera L.). *Invest. Cultura, Cienc. Tecnol.* **2011**, *3*, 41−46.
(34) Santiago, J. L.; Boso, S.; Gago, P.; Alonso-Villaverde, V.; Martinez, M. C. Molecular and ampelographic characterisation of Vitis vinifera L.'Albariño','Savagnin Blanc' and 'Caíño Blanco' shows that they are different cultivars. *Span. J. Agric. Res.* **2007**, *5*, 333−340.
(35) Gago, P.; Santiago, J. L.; Boso, S.; Alonso-Villaverde, V.; Grando, M. S.; Martinez, M. C. Biodiversity and Characterization of Twenty-two Vitis vinifera L. Cultivars in the Northwestern Iberian Peninsula. *Am. J. Enol. Vitic.* **2009**, *60*, 293−301.
(36) Diaz-Losada, E.; Salgado, A. T.; Ramos-Cabrer, A. M.; Diaz-Hernandez, B.; Pereira-Lorenzo, S. Genetic and geographical structure in grapevines from northwestern Spain. *Ann. Appl. Biol.* **2012**, *161*, 24−35.
(37) Estevez, P.; Gil, M. L.; Falque, E. Effects of seven yeast strains on the volatile composition of Palomino wines. *Int. J. Food Sci. Technol.* **2004**, *39*, 61−69.
(38) Oliveri, P.; Downey, G. Multivariate class modeling for the verification of food-authenticity claims. *TrAC, Trends Anal. Chem.* **2012**, *35*, 74−86.