

# Prediction of Italian red wine sensorial descriptors from electronic nose, electronic tongue and spectrophotometric measurements by means of Genetic Algorithm regression models

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## Abstract

In the present work, innovative analytical techniques, such as an amperometric electronic tongue and a commercial electronic nose were used, together with spectrophotometric methods, to predict sensorial descriptors of Italian red dry wines of different denominations of origin. Genetic Algorithms were employed to select variables and build predictive regression models. On the selected models, an accurate validation technique (the Bootstrap procedure) and a procedure for the detection of outliers (Williams plot) were applied.

The results obtained demonstrate the possibility of using these innovative techniques in order to describe and predict a large part of the selected sensorial information. It was not possible to build an acceptable regression model for only one descriptor, sourness.

The proposed analytical methods have the advantage of being rapid and objective; furthermore, the statistical methods applied could be considered a rational operative procedure for building regression models with real predictive capability.

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*Keywords:* Electronic nose; Electronic tongue; Spectrophotometric methods; Italian red wines; Sensorial descriptors; Genetic algorithms; Bootstrap procedure

## 1. Introduction

It is well known that the sensory characteristics of wine are influenced by a broad spectrum of factors, such as type of grape, soil, enological and climatic conditions. Generally, sensory analysis, based on trained expert panellists, is useful for wine classification and quality control but it is of high-cost, time-consuming and sometimes without any objective value. In recent decades many efforts have been made to establish a relationship between sensory attributes and chemical composition of wine, in order to understand which components influence the sensory properties and the final quality of the product. These are based on traditional chemical measurements and on spectrophotometric or chromatographic determinations, coupled with

classical chemometric techniques, such as principal component analysis (PCA) and partial least square (PLS) analysis (Aznar, Lopez, Cacho, & Ferreira, 2003; Bertuccioli, Clementi, Giulietti, & Montedoro, 1989; Boselli, Boulton, Thorngate, & Frega, 2004; Cliff, Brau, King, & Mazza, 2002). However, these analytical schemes cannot fully replace sensorial examinations and often they are time-consuming and require skilled personnel. It is, therefore, of great interest to develop low-cost, rapid and non-destructive analytical procedures, able to quantify the sensorial descriptors and the overall quality of wines.

In this paper, innovative, rapid and objective analytical techniques, such as the electronic nose (e-nose) and the electronic tongue (e-tongue), coupled with spectrophotometric methods, were used for objective sensorial evaluation of wine. Signals from the instruments were used to build predictive models of sensorial descriptors by means of Genetic Algorithms (GAs).

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The e-nose consists of an array of gas sensors useful for the analysis of the head space of liquid or solid food samples (Dickinson, White, Kauer, & Walt, 1998; Shaller, Bosset, & Escher, 1998); the e-tongue comprises an array of sensors that are specific for liquids and based on conductimetric, potentiometric and voltammetric techniques (Winquist, Holmin, Krantz-Rulker, Wide, & Lundstrom, 2000). In this work, a commercial e-nose and a home-made e-tongue, based on a Flow Injection system, with two amperometric detectors, were used.

Genetic Algorithms (GAs) were proposed as an alternative to the mostly used PLS analysis and were employed to select subsets of variables that maximize the predictive power of regression models. The regression models selected were subsequently validated by the Bootstrap procedure, that provides a more certain evaluation of their predictive capability while the Williams plots was used to check the presence of outliers.

## 2. Materials and methods

### 2.1. Wine samples

Measurements were performed on 15 Italian dry red wines of different denominations and vineyards (Table 1). An expert wine taster selected the wines, on the basis of their well-known sensorial characteristics, e.g. astringency, bitterness, acidity, body, aroma and colour.

### 2.2. Electronic nose

Analyses were performed with a Portable Electronic Nose (PEN2) operating with the Enrichment and Desorption Unit (EDU). The system was from WMA (Win Muster Airsense) Analytics Inc. (Germany). PEN2 consists of a sampling apparatus, a detector unit containing the array of sensors, and pattern recognition software (Win Muster v.3.0) for data recording. The sensor array is composed of 10 Metal Oxide Semiconductor (MOS)-type chemical sensors: MOS1 (aromatic) MOS2 (broadrange), MOS3

(aromatic), MOS4 (hydrogen), MOSS (arom-aliph), MOS6 (broad-methane), MOS7 (sulphur-organic), MOS8 (broad-alcohol), MOS9 (sulph-chlor), MOS10 (methane-aliph).

EDU is a microprocessor-controlled device capable of automatically trapping and thermally desorbing the samples. The adsorbent material is Tenax-TA<sup>®</sup> polymer, 150 mg.

The e-nose operating procedures are the same as reported in a previous work (Buratti, Benedetti, Scampicchio, & Pangerod, 2004).

### 2.3. Electronic tongue

A measurement system based on Flow Injection Analysis (FIA) with two amperometric detectors was employed.

The FIA apparatus consisted of a Jasco (Tokyo, Japan) model 880 PU pump and two EG&G Princeton Applied Research (Princeton, NJ, USA) Model 400 thin-layer electrochemical detectors connected in series. Each detector was equipped with a working electrode (a dual glassy carbon electrode and a gold electrode), a reference (Ag/AgCl saturated) electrode and a platinum counter electrode. The connecting tubes were of PEEK (1.5 mm o.d. × 0.5 mm i.d.). Data were recorded using a Philips (Eindhoven, Netherlands) PM 8252 recorder.

In the flow system, a carrier solution is continuously pumped through the amperometric detectors and the samples are injected into the flow stream. Data are obtained by measuring the current resulting from the oxidation or reduction of the electroactive compounds present in the samples.

The overall configuration of the system and the operating procedures are the same as reported in a previous work (Buratti et al., 2004).

### 2.4. Spectrophotometric determinations

#### 2.4.1. Analyses of phenolics

A modified version of the Glories method was used to estimate the phenolic contents of wine samples (Romani, Mancini, Tatti, & Vinceri, 1996). Samples were diluted 1:10 with ethanol 10% and 0.25 ml were placed in a cuvette. After the addition of 0.25 ml of 0.1% HCl in 95% ethanol and 4.5 ml of 2% HCl the solution was mixed and the absorbances at 280 nm ( $A_{280}$ ), 320 nm ( $A_{320}$ ), 360 nm ( $A_{360}$ ) and 520 nm ( $A_{520}$ ) were measured.

Total phenols were also determined using the Folin Ciocalteu method (Singleton & Rossi, 1965).

Anthocyanins were determined using the procedure described by Mazza, Fukumoto, Delaquis, Girard, and Ewert (1999). In this procedure, total anthocyanins were evaluated by adding 20 µl of 10% acetaldehyde to 2 ml of wine and by reading the absorbance at 520 nm ( $A_{acet}$ ). Polymeric anthocyanins were evaluated by adding to another 2 ml sample, 160 µl of 5% SO<sub>2</sub> and determining the absorbance at 520 nm ( $A_{SO_2}$ ). At the same wavelength, also, the wine absorbance was evaluated ( $A_{wine}$ ) and

Table 1  
Italian dry red wines considered in the experimentation

Wines	Vineyard
Pinot nero alto adige DOC	Pinot Nero
Brusco dei barbi IGT	Sangiovese
Morellino di scansano DOC	Sangiovese
Primitivo del salento IGT	Primitivo Nero
Val calepio rosso DOC	Merlot – Cabernet Sauvignon
Aglianico IGT	Aglianico Rosso
Inferno DOC	Nebbiolo
Vallegorina	Negrara
Santa costanza novello IGT	Sangiovese, Gamay
Lacrima di morro DOC	Lacrima Nero
Dolcetto DOC	Dolcetto Nero
Bonarda oltrepò DOC	Corvina Nero
La segreta rosso IGT	Nero d'Avola, Merlot
Colline novaresi DOC	Nebbiolo, Bonarda
Val di cornia suvereto rosso DOC	Sangiovese

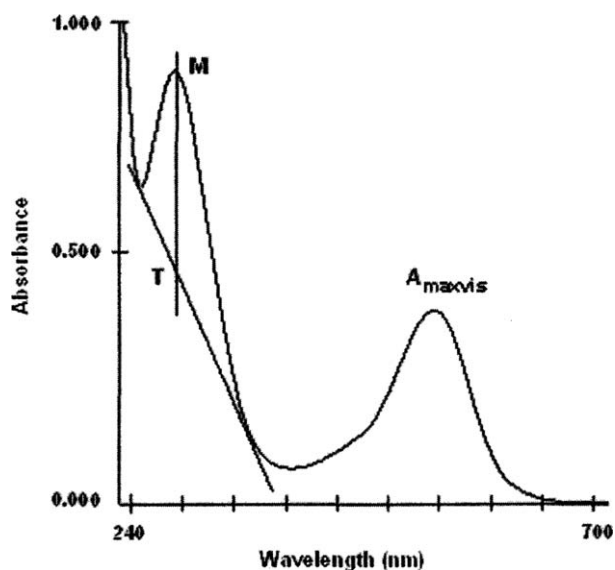


Fig. 1. Wine spectrum for the evaluation of total flavonols and non-anthocyanic flavonols.

monomeric and copigmented anthocyanins were determined as follows:

$$\text{Monomeric anthocyanins} = A_{\text{wine}} - A_{\text{SO}_2},$$

$$\text{Copigmented anthocyanins} = A_{\text{acet}} - A_{\text{wine}}.$$

Flavonoids were determined using the procedure described by Di Stefano, Cravero, and Gentilini (1989). In this procedure, wine samples were diluted 1:50 with a solution of water (30%), ethanol (70%) and HCl (1%) and the spectrum in the range between 230 and 700 nm was registered. As shown in the spectrum in Fig. 1, the length of the segment MT was measured and transformed into absorbance units corresponding to the absorbance at 280 nm of total flavonoids ( $A'_{280}$ ). The maximum of absorbance in the visible ( $A_{\text{maxvis}}$ ) was also evaluated and the absorbance of non-anthocyanic flavonoids ( $A''_{280}$ ) was expressed as:

$$A''_{280} = A'_{280} - A_{\text{maxvis}}/3.5.$$

#### 2.4.2. Colour evaluation

The colour measurements were performed using a GARY 100 BIO UV–visible spectrophotometer (Varian) equipped with GARY UV WIN Software, which is able to convert the transmittance into  $L$  and  $a/b$  values.  $L$  represents the difference between light (where  $L = 100$ ) and dark (where  $L = 0$ ),  $a$  represents the difference between green ( $-a$ ) and red ( $+a$ ) while  $b$  represents the difference between yellow ( $+b$ ) and blue ( $-b$ ) (Wyszecki & Stiles, 1967). For all measurements, glass cells of 1 mm path length were used. The readings were taken at intervals of 10 nm in the wavelength range 400–700 nm.

#### 2.5. Sensory analysis

The sensory evaluation was conducted according to UNI U590A1950 (1998). Eight expert wine judges (having

an O.N.A.V. – Organizzazione Nazionale Assaggiatori Vini – certificate) evaluated 15 Italian dry red wines on two days. Wines were presented in random order and each sample was evaluated twice on the same day. No information was given to the assessors about the origin of the samples. The panel tasting took place in an air-conditioned room (21 °C) with isolated booths. Sensory descriptors were selected from those more reported in literature. Judges assessed the aroma (total fruits, wood, spicy), the flavour (total fruits, wood, spicy) and the taste (sourness and bitterness). Also astringency, alcohol and body descriptors were evaluated. Judges scored the magnitude of each attribute from 1 to 9 where 1 was “low” and 9 was “high”. The sensory evaluation was given also in terms of “overall quality”; the eight judges scored the magnitude of this attribute from 1 to 100. All scorecards were collected at the end of each section, and the average values given by all eight judges for all the descriptors were used for the multivariate statistical analysis.

#### 2.6. Data analysis

The regression models were built by means of Genetic Algorithms (Goldberg, 1989). GAs select subsets of variables that maximize the predictive power of regression models and perform this selection by considering populations of models generated with an evolution process and optimised according to an objective function (in this case  $Q^2$  leave-one-out, calculated with the ordinary least square regression) (Leardi, Boggia, & Terrile, 1992). A population is made of a series of chromosomes. Each chromosome is a binary vector, where each position (a gene) corresponds to a variable (i.e. a chromosome represents a model made up of a subset of selected variables).

The evolution process is based on three main steps: initially the model population is randomly built. The value of the objective function of each model is calculated and the models are then ordered with respect to this objective function. After that, the reproduction step selects pairs of models (parents) and, from each pair of models, a new model (son) is generated preserving the common characteristics of the parents (i.e. variables excluded in both models remain excluded; variables included in both models remain included) and mixing the opposite characteristics. If the generated son coincides with one of the individuals already present in the actual population, it is rejected; otherwise, it is evaluated. If the objective function value is better than the worst value in the population, the model is included in the population, in the place corresponding to its rank; otherwise, it is no longer considered. This procedure is repeated for several pairs. The mutation step instead changes every gene of each chromosome into its opposite according to a defined probability. If the objective function of each mutated model is better than the worst value in the population, the model is included in the population. Reproduction and mutation steps are alternatively repeated until a stop condition has occurred or the evolution process is ended arbitrarily.

Since the quality of a regression model can be evaluated by its predictive capability, the final models were subsequently validated by the Bootstrap procedure. By this validation technique, the original size of the data set ( $n$ ) is preserved for the training set: the training set usually consists of repeated objects and the evaluation set of the objects left out (Efron, 1982). The model is calculated on the training set and responses are predicted on the evaluation set. This procedure of building training sets and evaluation sets is repeated thousands of times and the average predictive power is calculated ( $Q^2$  bootstrap). Thus, the validation is performed by randomly generating training sets with sample repetitions and then evaluating the predicted responses of the samples not included in the training set. This validation technique is more time-consuming than the leave-one-out method (generally used in the GAs), but it provides a more certain evaluation of the predictive power of regression models (Efron, 1983).

Each model has been analysed to detect the presence of outliers. An object that is atypical (different from the average) of the rest of the objects in a data set is deemed an outlier. An object may be an outlier with respect to the independent variables and/or with respect to the response variable. Regarding the first aspect, the leverage matrix,  $H$ , also called influence matrix, is an important tool in regression diagnostics containing information on the independent variables on which the model is built (Cook & Weisberg, 1982). The leverage matrix,  $H$ , is a symmetric matrix defined as:

$$H = X(X^T X)^{-1} X,$$

where the matrix  $X$  is the model matrix, i.e. a matrix with  $n$  rows (where  $n$  is the number of samples) and  $p'$  columns (where  $p'$  is the number of model parameters). Samples whose  $h_{ii}$  value is greater than a critical value  $h^*$  can be con-

sidered as having a great influence (leverage) on the model. The critical value  $h^*$  is defined as:

$$h^* = \frac{3p'}{n}.$$

Regarding the second aspect, the standardised residuals in prediction (Jackknife) (Johnson & Wichern, 1982) can be calculated as the ordinary residuals in prediction divided by the residual standard deviation:

$$\hat{e}_i = \frac{\hat{y}_{i/i} - y_i}{s \cdot \sqrt{1 - h_{ii}}},$$

where  $\hat{e}_i$  is the standardised residual in prediction of the  $i$ th object,  $\hat{y}_{i/i}$  and  $y_i$  are, respectively, the predicted and the observed response of the  $i$ th object,  $h_{ii}$  is the leverage value of the  $i$ th object and  $s$  is the standard error of the estimate:

$$s = \sqrt{\frac{\sum_{i=1}^n (\hat{y}_i - y_i)^2}{n - p'}},$$

Table 2  
Variables considered in the experimentation

	Variables
Spectrophotometric determinations	$A_{280}$ ; $A_{320}$ ; $A_{360}$ ; $A_{520}$ ; total phenols; total anthocyanins; polymeric anthocyanins; monomeric anthocyanins; copigmented anthocyanins; total flavonoids; non-anthocyanic flavonoids; Colour ( <i>L</i> ); Colour ( <i>alb</i> )
E-nose	MOS1 ; MOS2; MOS3; MOS4; MOS5; MOS6; MOS7; MOS8; MOS9; MOS10
E-tongue	Carbon electrode 0.8 V ( $C_{0.8V}$ ); Carbon electrode 0.6 V ( $C_{0.6V}$ ); Carbon electrode 0.4 V ( $C_{0.4V}$ ); Carbon electrode -0.2 V ( $C_{-0.2V}$ ); Gold electrode 0.4 V ( $G_{0.4V}$ )

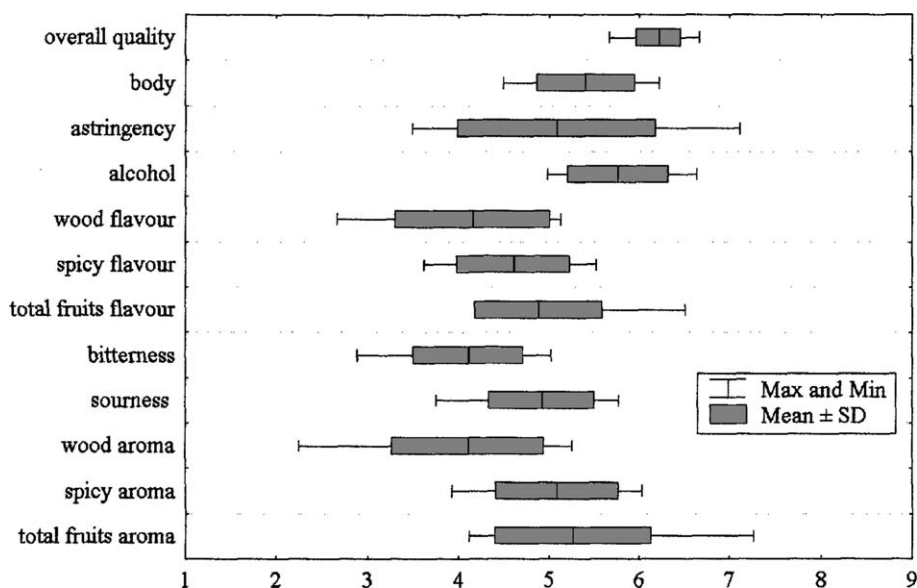


Fig. 2. Box plot of wine sensorial descriptors and overall quality. For each sensorial attribute mean, standard deviation, maximum and minimum values are shown.

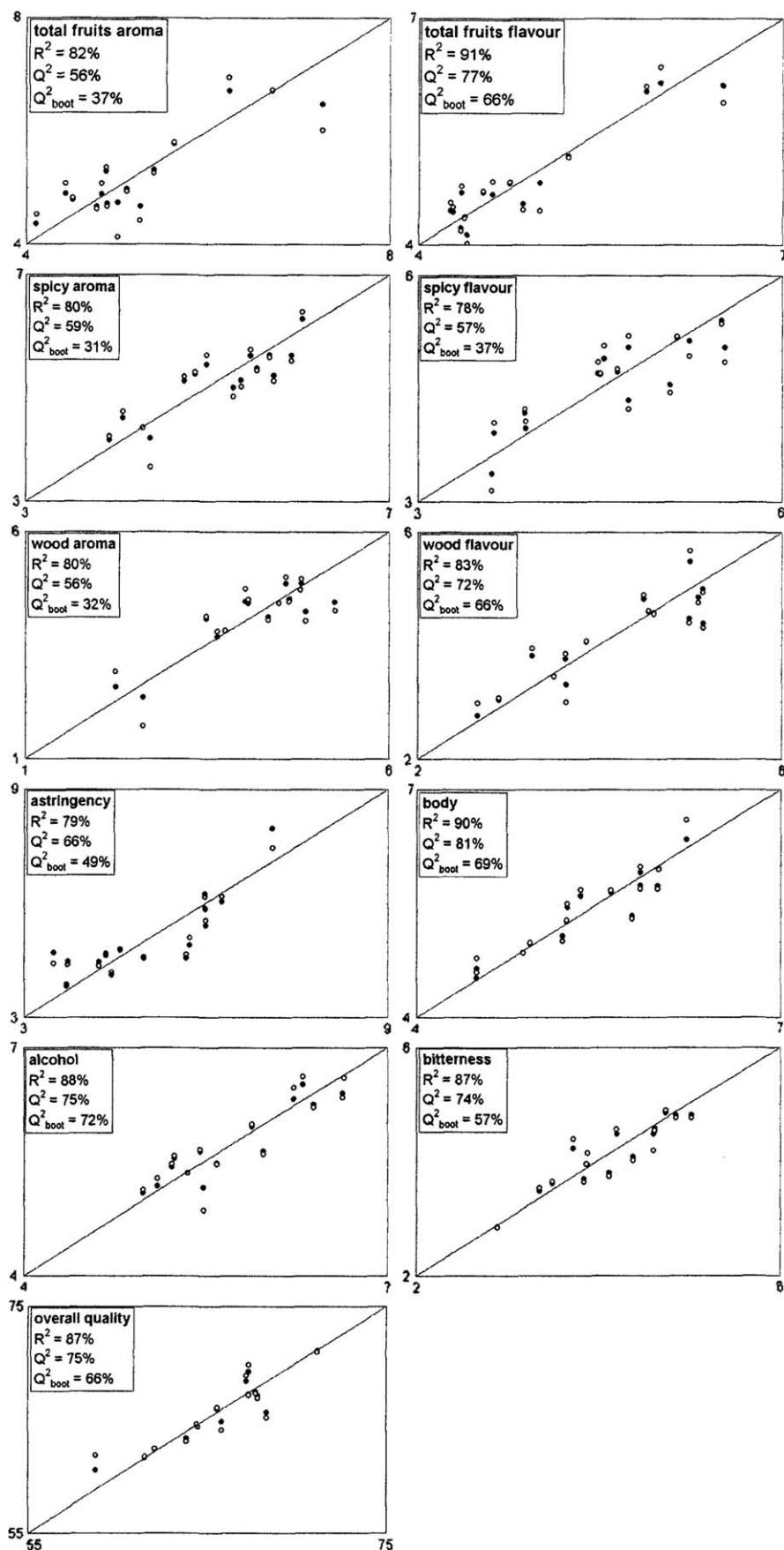


Fig. 3. Scatter plot of the sensorial descriptors and overall quality estimated by Genetic Algorithms built on e-nose, e-tongue and spectrophotometric data set. Plots show predicted (○) and calculated (●) versus experimental responses. The  $R^2$ ,  $Q^2$  leave-one-out and  $Q^2_{boot}$  values of each model are shown.

where  $\hat{y}_i$  is the estimated response of the  $i$ th object. Objects whose  $|\hat{e}_i|$  value is greater than 3 (Cook & Weisberg, 1982) can be considered as outliers with respect to the response variable. Leverage and standardised residuals in prediction are used to build graphics (Williams Plot) for the detection of outliers and/or objects with high influence on the results.

Genetic algorithms, bootstrap validation and Williams plots were performed by MobyDigs software (Todeschini, Ballabio, Consonni, Mauri, & Pavan, 2004).

### 3. Results and discussion

The selected wines were analysed by three independent techniques: electronic nose, amperometric electronic tongue and spectrophotometric determinations. These analytical methods were chosen principally for their rapidity, objectivity and reproducibility. A data matrix with 15 rows (wine samples) and 56 columns (variables) was built. Thirteen variables were obtained from spectrophotometric analyses, five from the electronic tongue and ten from the electronic nose (Table 2). The square value of each variable was also included in the matrix.

Since the main scope of this paper was to investigate the capability of extracting from these techniques, information about the wine sensory properties and quality, the sensorial analysis was considered as reference method. In Fig. 2, the box plot of wine sensorial descriptors and overall quality is shown. Since the overall quality had a magnitude from 1 to 100 and the sensorial descriptors from 1 to 9, the scores have been scaled in order to make them comparable. Taking into account how difficult it is to find wines scattered over the whole scale, the majority of the sensory scores can be considered widely distributed on the scale, showing that the wines were adequately chosen.

GAs were used to select the best subset of variables and to build predictive regression models in order to study the relationships between the signals obtained from the instruments (e-nose, e-tongue and spectrophotometer) and the sensory parameters. Regression models were also calculated, trying to explain the overall quality of wines. In these models, the signals represented the independent variables. Since a high number of independent variables was available, the selection of the best subset was required for two reasons: in a regression model, the ratio between the number of samples and variables should be high, as statisticians suggest (Frank & Friedman, 1993); the fitting performance of a regression model always rises when the number of independent variables increases, while the predictive performance decreases when non-useful variables are added to the regression model. The models were built setting the maximum number of variables to 4. This is a reasonable limit considering the available number of samples and the regression technique involved (ordinary least square regression).

For each sensory parameter, the models with the highest value of  $Q^2$  leave-one-out were taken into account and validated by the Bootstrap procedure. It is well known that a validation procedure is required to recognize stable regres-

sion models. Models with a good fitting performance (i.e. high  $R^2$  value) do not always have an acceptable predictive performance (i.e. high  $Q^2$  leave-one-out value). Furthermore,  $Q^2$  leave-one-out is often an overoptimistic predictive parameter. In fact, regression models often turned out not to be as predictive as expected if more severe validation was applied. In this work, the Bootstrap procedure was used and repeated 8000 times for each model and the model with the highest value of  $Q^2$  bootstrap was selected as the best for the analysed sensory descriptor.

In Fig. 3, the scatter plots of the sensorial descriptors are shown. Predicted and calculated versus experimental values are plotted.  $R^2$  values describe the fitting performance of the regression model, while  $Q^2$  leave-one-out and  $Q^2$  bootstrap give information about the predictive capability of the model. As seen, an acceptable accuracy in prediction was obtained for global descriptors such as body, overall quality, alcohol and astringency. Also the acceptable regression of bitterness, total fruit flavour and wood flavour has to be recognized. The regression toward spicy flavour and aroma (spicy, total fruit and wood) was quite poor and, in particular, these models had a quite good fitting capability but their predictive performance was limited, as shown by  $Q^2$  bootstrap.

It was not possible to build a model for sourness since all the regression quality parameters were not acceptable.

In Table 3 the variables involved in each selected model are reported. As seen, the model built for the astringency is composed only of e-tongue variables, showing that this device can be useful for predicting in a rapid and simple way, an important quality parameter, as astringency is e-tongue variables were also involved in the predictive models of body, alcohol and overall quality; the spectrophotometric evaluation of colour ( $L$ ;  $alb$ ) and the non-anthocyanic

Table 3  
List of variables involved in the final models

Sensorial descriptors	Model size	Model variables
Total fruits aroma	4	Total phenols, colour ( $alb$ ), MOS1, non-anthocyanic flavonoids
Total fruit flavours	4	Total phenols, total flavonoids, colour ( $alb$ ), MOS1
Spicy aroma	4	Total phenols, colour ( $alb$ ), MOS 1, MOS 7
Spicy flavour	4	Non-anthocyanic flavonoids, total phenols, $A_{520}$ , MOS 9
Wood aroma	4	Total phenols, non-anthocyanic flavonoids, total Anthocyanins, colour ( $alb$ )
Wood flavour	3	Colour ( $alb$ ), colour ( $L$ ), MOS 7
Astringency	3	$C_{0.6V}$ , $C_{0.4V}$ , $G_{0.4V}$
Body	4	$C_{0.4V}$ , colour ( $alb$ ), colour ( $L$ ), non-anthocyanic flavonoids
Alcohol	3	$G_{0.4V}$ , total phenols, total flavonoids
Bitterness	4	Non-anthocyanic flavonoids, copigmented anthocyanins, Total anthocyanins, total phenols
Overall quality	4	$C_{0.8V}$ , total flavonoids, non-anthocyanic flavonoids, total anthocyanins

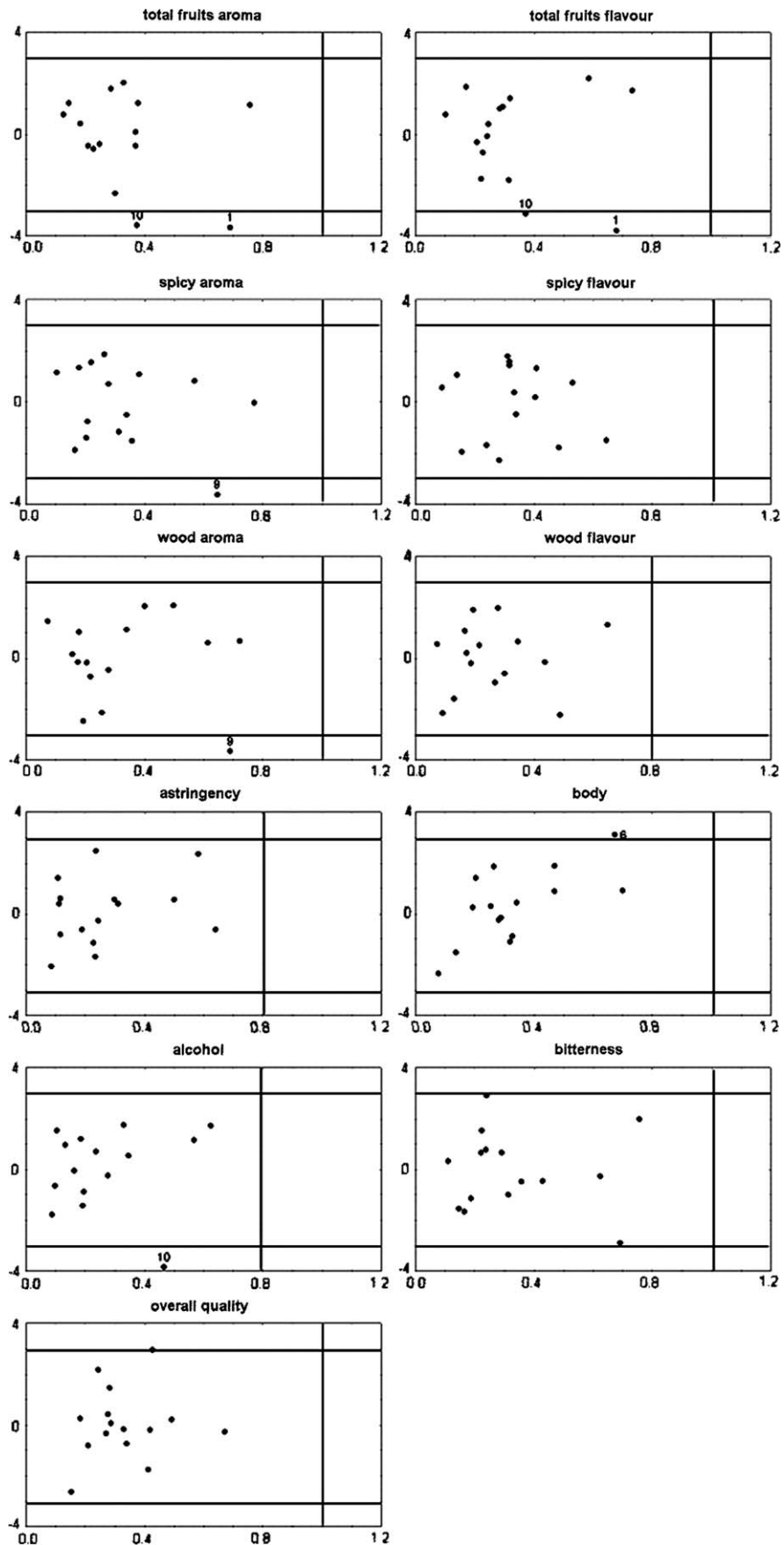


Fig. 4. Williams plots of the selected models showing the leverage values versus the standardised residuals in prediction. Reference lines are shown both for leverage critical value and for standardised residual in prediction critical value.

flavonols appeared to be important in defining the body, while total phenols and total flavonols were included in the predictive model of alcohol. It is interesting to note that the wine phenolic profile (total flavonols, non-anthocyanic flavonols and total anthocyanins) turned out to be important for predicting the wine overall quality. This result is particularly significant, since it is well known that the phenolic compounds are related to wine sensorial descriptors normally associated with wine preference.

Of importance for the predictive models of flavour and aroma descriptors were some e-nose variables (MOS 7; MOS 1 and MOS 9) and the spectrophotometric evaluation of total phenols, and phenolic fractions. Particularly interesting is the predictive model of wood flavour, composed only of an e-nose variable (MOS 7) and the colour variables (*L*, *alb*).

To better evaluate the selected models, the Williams plots were built to check the presence of outliers and/or objects with high influence on the results. Leverage values and standardised residuals in prediction are reported, respectively, on *x* and *y* axes. In each plot, reference lines are also reported both for leverage critical value and for standardised residuals in/prediction critical value. Objects with leverage greater than the critical value can be interpreted as samples with too much influence on the regression model. In the same way, samples with a standardised residual greater than the critical value are characterized by a poor prediction value.

As seen (Fig. 4), all samples had acceptable leverage values while few have standardised residuals in prediction higher than the critical value in more than one model (samples 1 and 10 for total fruits flavour and aroma, sample 10 also for alcohol, sample 9 for spicy and wood aroma).

From all these results it is possible to conclude that the proposed analytical methods (sensor arrays and spectrophotometric determinations) are able to describe and predict a large part of the dry red wine sensorial information produced by a panel. Only for one descriptor (sourness) was it not possible to build an acceptable regression model, while models of all aroma descriptors and spicy flavour could be improved to give a significant predictive capability.

#### 4. Conclusion

The results of the present work demonstrated the feasibility of the e-nose, e-tongue and spectrophotometric determinations for predicting with a good accuracy, some sensorial parameters and the overall quality of dry red wines.

GAs and Bootstrap techniques are computationally time-consuming but this is not to be considered a serious problem, since the sensorial data collection is more time-consuming than the proposed procedures and the predictive models obtained are stable.

Even if a larger number of wine samples are required to better validate the predictive models, the present results demonstrate the possibility of using these innovative analytical methods in order to obtain, in a rapid and objective

way, information about the sensorial properties of wines. Moreover, the statistical methods applied could represent a rational operative procedure for building regression models with real predictive capability and, consequently, applicable to unknown samples.

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