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ELECTROANALYSIS

DOI: 10.1002/elan.201700652

Fast and Simultaneous Determination of Antioxidant Activity, Total Phenols and Bitterness of Red Wines by a Multichannel Amperometric Electronic Tongue

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Abstract: An electronic tongue based on a flow injection system equipped with a multichannel amperometric detector was used to determine the antioxidant activity, total phenols and bitter taste of red wines. The detector composed of four glassy carbon electrodes, arranged in a square configuration, where two parallel electrodes (poised at $E_1 = +400$ and $E_2 = +800$ mV) were followed by other two electrodes (poised at $E_3 = +800$ and $E_4 = -400$ mV). This configuration allowed to gain information on the content of the antioxidants activity (E_1) and total phenols (E_2 and E_3). E_1/E_2 was used to express the proportion of strong vs. weak antioxidants. E_3/E_4 was used to express an index of reversibility. Finally, the ratio E_2/E_3 was used to control the system. Overall, the four electrodes led to seven variables, which provided a characteristic pattern profile. The eleven variables in total were analyzed by principal component analysis (PCA) to determine, the antioxidant activity, total phenol content and bitterness value of red wine samples. The results of antioxidant activity were correlated with the Folin Ciocalteu index ($R^2 = 0.94$). Bitterness was correlated with the descriptors of the e-tongue ($R^2 = 0.81$). Overall, the analysis with the proposed electronic tongue is simple (only a dilution is required), fast (the analysis takes less than 20 seconds per sample), objective (precision within 5%, expressed as RSD%) and cheap compared to classical sensory analysis.

Keywords: Red wine · electronic tongue · amperometry · antioxidants · bitterness

1 Introduction

Phenolic compounds have been studied a lot recently due to their benefits on human health [1–4]. Red wine is characterized by a high polyphenolic content [3,4]. Wine's phenolic compounds like flavonoids and phenolic acids are responsible for important sensory properties including color, flavor and bitterness [4,5,13]. Also, moderate consumption may have some beneficial effects on human health due to the high antioxidant activity of wine [4,6,7,8]. However, the phenolic content in wine is quite variable depending on a series of factors, including grape variety [9], grape ripeness [10], environment [1], processing [11] and storage conditions [12]. Because of such variability and its significant effect on the bitter taste of wine, wineries are routinely faced with the measurement of total phenol content and bitterness. The knowledge of total phenol content and bitterness becomes an essential information, for instance, during wine blending. Blending different wines is a common operation used to equilibrate the resulting pH, alcohol level, aroma, flavor (e.g. sweetness, acidity) or color of wine. Therefore, the knowledge on the total phenol content and bitterness value is important for the enologist during the decision-making process.

The evaluation of bitter taste is typically obtained with the sensory analysis. Although sensory analysis is widespread used during the winemaking process, this kind of approach can be expensive and time-consuming. Moreover, high levels of phenols may block receptors of the tongue, which may affect the ability of the taster to evaluate the samples in a short time. Other methods for the determination of phenolic compounds in wine are based on liquid chromatography or photometric assays [14]. One of the most common procedure is the Folin-Ciocalteu index [10,15–18]. This spectrophotometric method is simple, cheap, although lacks specificity; moreover, the results can be affected by interfering substances (e.g. sugars, sulphur dioxide, organic acids, nitrogen compounds) [15,16].

Thus, there is a growing interest in developing rapid methods for the detection and analysis of phenolic compounds, especially in wines. In the last years, electronic tongues based on electrochemical sensors have emerged as rapid and sensitive devices that can become an alternative to classical sensory analyses [21,24,25]. Such e-tongues were successfully applied to the process classification and identification of several types of beverages, e.g. wine, juices, milk and oil [20,24]. In wine analysis, e-tongues were previously used for the classifica-

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tion [21,24], and to predict chemical parameters [22,24] or taste descriptors [23,24]. Moreover, the voltammetric method was applied to study reaction mechanisms and reversibility [26]. However, some electronic tongue systems show certain weaknesses due to complexity of some matrices [27]. One of the main problems is the necessity to fill the sample manually before each measurement [28,29] and frequently stop the analysis to clean the working electrode due to passivation [29,30,31].

Accordingly, the aim of the current study was to develop a method for the simultaneous determination of the antioxidant activity, total phenols and bitterness of red wine. The procedure involves an electronic tongue based on flow injection and equipped with a multichannel amperometric detector composed by four glassy carbon electrodes.

2 Materials and Methods

2.1 Wine Samples

Three types of red wines (Lagrein, St. Magdalener and Vernatsch) produced in the area of Bolzano and Appiano (South Tyrol, Italy) were purchased in a local shop. All the wines were obtained from grapes harvested in 2015. Lagrein and Vernatsch are both obtained with grapes of a single variety, respectively, whereas St.Magdalener (sample M) is traditionally obtained from vineyards where the Vernatsch grape variety is grown together with about 10–15% of the Lagrein variety. Lagrein and Vernatsch wines were mixed in the following proportions: wine A, 100% Vernatsch; wine B, 75% Vernatsch and 25% Lagrein; wine C, 50% Vernatsch and 50% Lagrein; wine D, 25% Vernatsch and 75% Lagrein; wine E, 100% Lagrein. All samples were sonicated for min and stored at 6 °C until the analysis.

2.2 Chemicals

Folin-Ciocalteu reagent (FCR) and ethanol (EtOH) were purchased from Merck. Acetonitrile (ACN) was purchased from VWR Chemicals. Lithium perchlorate ($LiClO_4$), anhydrous sodium carbonate (Na_2CO_3), ferrocene methanol (FeMeOH), gallic acid and caffeic acid were purchased from Sigma Aldrich. Aluminum oxide (Al_2O_3) was purchased from Riedel-de Haën. All chemicals were of analytical grade and were used without purification.

2.3 Measurement of the Total Phenol Content (TPC)

The total phenolic content was measured using the Folin-Ciocalteu method [32]. Briefly, 1 mL of the red wine diluted 1:5 with water was transferred into a 100-mL volumetric flask. Then, 50 mL of distilled water, 5 mL of the Folin-Ciocalteu reagent and 20 mL of 20% anhydrous Na_2CO_3 solution were added. Finally, all flasks were brought to 100 mL with distilled water. Samples were left for 30 min and after that, the total quantity of phenolic compounds was determined at 750 nm using the UV-Vis spectrophotometer (Agilent Technologies). The blank solution was made with 1 mL of water instead of wine. The results were reported as gallic acid equivalent per mL of wine (GAE/mL).

2.4 Electronic Tongue

Flow measurements were performed using a Thermo Fischer Ultimate 3000 LC Autosampler and a thin-layer cell (ALS, Japan) equipped with a non-aqueous reference electrode (Ag/Ag+), a platinum counter electrode and four working electrodes made of glassy carbon of 2 mm diameter operating at potentials of +400 mV, +800 mV, +800 mV and −400 mV; the thin layer of the cell had a thickness of 30 μm thanks to the use of Teflon gasket as spacer.The following measurement settings were used: flow rate of 1 mL/min and a 20 μL injection volume. The data was recorded with Autolab analyzer PGSTAT128N (Metrohm, Netherlands). E-tongue experiments were performed at room temperature using a 0.1 M $LiClO_4$ in ACN:EtOH as carrier solution. For the flow injection analysis wine samples were diluted 1000 times with the 0.1 M $LiClO_4$ in ACN:EtOH buffer before injection. Before each series of measurements, the quadruple electrode was polished mechanically with Al_2O_3 slurry (50 nm) on a micro polish cloth. The calibration of the four electrodes was performed with standard solutions of caffeic acid in the range 10–90 μM and the results were reported as caffeic acid equivalent per mL (CAE/mL).

2.5 Sensory Analysis of Bitterness

Bitterness was evaluated by a sensory panel of experts. Twelve panelists with previous sensory experience in wine were first trained with tannic acid solutions. Then, each panelist evaluated the bitterness intensity by tasting each wine sample for about 15 s and reporting the result using an unstructured scale, ranging from “not bitter” (corresponding to 0) to “very bitter” (corresponding to 10). Between each sample, a 4-min break was taken during which the panelists were required to rinse the mouth with water in order to minimize any carry-over effect.

2.6 Cyclic Voltammetry

Cyclic voltammetry was performed by using the Autolab PGSTAT 128N potentiostat/galvanostat (Eco Chemie, Utrecht, Netherlands). A three-electrode system was used throughout the study. A glassy carbon (GC disk, 3 mm in diameter), a platinum wire and a saturated Ag/Ag+ (in Ag/AgNO3, 0.1 M) (non-aqueous silver/silver ion reference electrode kit, MF-2026, BASi, West Lafayette, IN-USA) electrode were used as working, auxiliary and reference electrode, respectively. Before analysis, the electrode was activated by cycling at 0.1 $V s^{-1}$ from 0.1 to 1.2 V until a repeatable voltammogram was achieved (typically within 20–30 cycles). The wine measurements were performed with 10 mL of ethanol:acetonitrile solution (50:50) with $LiClO_4$ (0.1 M) as supporting electrolyte. Wines were diluted 100 times before the measurement.

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2.7 Statistical Analysis

Linear correlation and Principal Component Analysis (PCA) were performed using the XLSTAT software Ver. 2016.02.28014 from Addinsoft (Paris, France).

3 Results and Discussion

3.1 Flow Injection Based on a Four-electrode System

A simplified scheme of flow injection analysis (FIA) equipped with a multichannel amperometric detector based on four glassy carbon electrodes deployed in a square configuration was illustrated in Figure 1 (upper part). In details, two parallel sensors (E1 and E2) were followed by other two sensors (E3 and E4). The detection potential of each of the four electrodes was fixed at E_1: + 400 mV; E_2: + 800 mV; E_3: + 800 mV and E_4: −400 mV. These potentials were chosen based on previous works [21] and optimized according to the hydrodynamic voltammogram of a wine sample (not shown). Briefly, the potentials of + 800 was selected in order to oxidize most of the phenolic present in the wine sample. The applied potential at −400 mV was low enough to reduce back the phenolic compounds perviously oxidized. The potential of + 400 mV was selected as index of antioxidant activity, similarly to previous work [21].

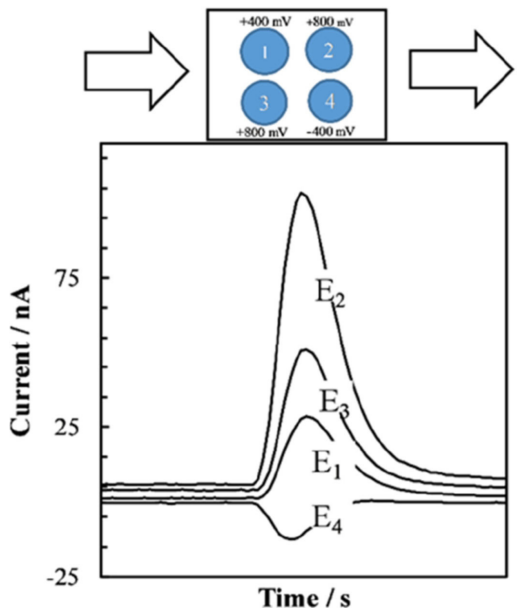

Fig. 1. Injection of Vernatsch wine sample into the electronic tongue, equipped with four electrodes, each poised at: E_1: + 400 mV; E_2: + 800 mV; E_3: + 800 mV; E_4: -400 mV.

Figure 1 shows the resulting amperometric signals subsequent to the injection of a red wine sample (20 µL of the sample previously diluted 1:100 with the acetonitrile:ethanol (1:1) solution, containing 0.1 M $LiClO_4$ as supporting electrolyte). The electrodes were poised at specific potentials to gain signal only from the antioxidant activity (E_1) and the total phenol content (E_2). Moreover, the ratio E_1/E_2 was conveniently used to express the proportion of weak antioxidants vs total phenols, as this is the ratio between the sensors detecting only strong antioxidant (E_1) and the sensor detecting all phenol species (E_2) in the sample. Furthermore, the ratio E_3/E_4 was also used to express an index of reversibility, as this is the ratio between the maximum anodic and cathodic current signal. In a reversible reaction, reactants react to form new products, but the original products can be obtained by applying a negative potential, whereas in case of irreversible reactions, it is impossible to get back to original chemical compounds. Finally, the ratio E_2/E_3 was used to control the system, as it reflects the reproducibility of the sensor 's response. Briefly, this ratio tends to 1 when both electrodes have the same performance. Overall, these indices were used to provide a characteristic pattern profile of each wine samples.

3.2 Comparison of Vernatsch and Lagrein Wines

To assess the performance of the electronic tongue on real wine samples, two red wines from South Tyrol region (Italy), respectively, Lagrein and Vernatsch, were selected. Lagrein is a red wine obtained from an autochthonous grapes variety and characterized by a high polyphenol content. This was confirmed by the analysis of total phenols with the Folin-Ciocalteu method. The result was 2.11 mg/mL, expressed as gallic acid equivalent (Table 1). Conversely, Vernatsch is another local grape variety from South Tyrol, but characterized by a lower phenol content (1.26 mg/mL) than Lagrein. Such differences were also reported previously by other authors [33,34]. Mattivi et al. [35] reported that Lagrein shows higher amount of flavonols (29.9 mg/kg) compared to Vernatsch (21.5 mg/kg). In our case, both wines showed significant differences in bitterness, as measured by sensory analysis: 2.1 ± 0.1 for Vernatsch and 6.6 ± 0.3 on the scale from 0 (min.) to 10 (max.).

To test the suitability of the proposed electronic tongue, 10 samples of Lagrein and Vernatsch wines were injected into the system. A representative amperometric response of each electrode was reported in Figure 2. As expected, due to the lower amount of phenols in Vernatsch, the current signals of all electrodes were much smaller than those observed in Lagrein. As reported in the spider plot of Figure 2, the measured current for Vernatsch and Lagrein were respectively: E_1: 27.5 ± 0.8 and 42 ± 2 nA; E_2: 99 ± 4 ± 3 nA and 127 ± 4 nA; E_4: −12.3 ± 0.7 and −17.1 ± 0.8 nA.

3.3 Analysis of Red Wine Blends

Vernatsch and Lagrein samples were analyzed in purity and after blending (25 % Lagrein and 75 % Vernatsch; 50 % Lagrein and 50 % Vernatsch; 75 % Lagrein and

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Table 1. Results of the wine blends measurements by quadrupole electronic tongue (CAE, mg/L), total phenol content (TPC) expressed in gallic acid equivalent (GAE), mg/L) and cyclic voltammetry (I_a – anodic current, I_c – cathodic current, CAE, mg/L). A = 100 % Vernatsch; B = 25 % Lagrein, 75 % Vernatsch; C = 50 % Lagrein, 50 % Vernatsch; D = 75 % Lagrein, 25 % Vernatsch; E = 100 % Lagrein; M = 100 % St. Magdalener. Results are expressed as the average of ten samples ± the standard deviation.

Wines and blends	+400 mV (E_1)	+800 mV (E_2)	+800 mV (E_3)	−400 mV (E_4)	TPC	I_a	I_c
M	73 ± 11	888 ± 55	1183 ± 31	853 ± 27	1043 ± 1	152 ± 29	506 ± 11
A	85 ± 4	1037 ± 26	1235 ± 17	871 ± 17	1257 ± 2	165 ± 7	680 ± 36
B	117 ± 12	1076 ± 53	1243 ± 22	900 ± 23	1435 ± 1	180 ± 4	691 ± 27
C	140 ± 10	1175 ± 51	1287 ± 23	934 ± 22	1687 ± 5	229 ± 13	740 ± 26
D	149 ± 10	1212 ± 46	1305 ± 25	941 ± 14	1969 ± 2	298 ± 8	766 ± 30
E	166 ± 9	1233 ± 33	1332 ± 16	974 ± 19	2106 ± 3	422 ± 18	955 ± 13

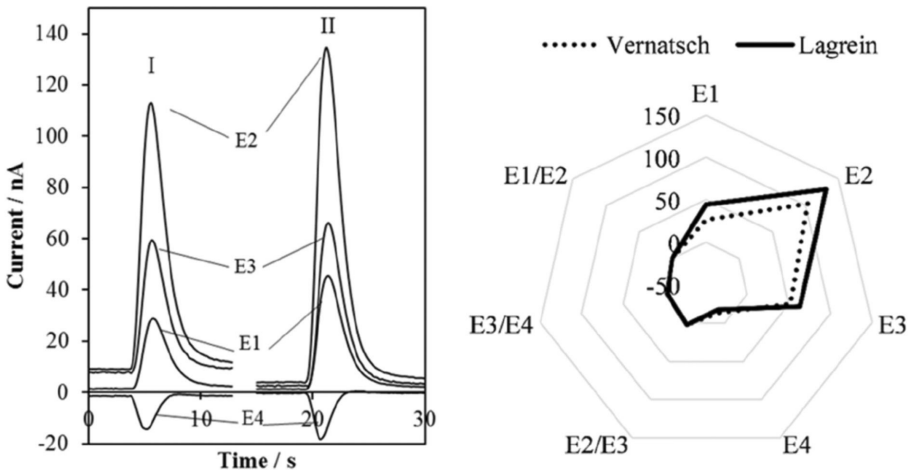

Fig. 2. Electronic tongue measurements of (I) Vernatsch and (II) Lagrein. E_1, +400 mV; E_2, +800 mV; E_3, +800 mV; E_4, −400 mV.

25 % Vernatsch). For comparison, St. Magdalener wine samples were also analyzed as this is a typical South Tyrolean red wine made from Vernatsch and Lagrein grapes. Overall, for each sample, 10 independent measurements were performed and from the resulting current signals of the electronic tongue, seven variables were collected (E_1, E_2, E_3, E_4, E_1/E_2, E_2/E_3 and E_3/E_4) and combined with variables from other measurements: cyclic voltammetry (*Ic*, *Ia*), total phenol content by Folin-Ciocalteu (FC) and bitterness. Such dataset was finally analyzed by Principal Component Analysis (PCA) and the score plot was reported in Figure 3. The first two principal components (F1 and F2) retained 84.37 % of the total variance, most of which (69.26 %) was accounted by F1. The most important variables contributing to the F1 were the amperometric signals of the sensors E_1, E_2, E_3, anodic current I_a, total phenols FC and bitterness. The amperometric signal of sensor E_4 as well as the cathodic current I_c by cyclic voltammetry were inversely related to the other variables. First five sensors provided information on the antioxidant capacity of wines, which is mainly attributed to the total phenol content. Instead, the most important variables of the second principal component (accounting for 15.11 % of the total variance) were the ratio E_1/E_2, which provides information on the proportion of strong antioxidants vs total phenols, and the ratio E_3/E_4, which provides information on the system reversibility.

According to the resulting score plot, the Vernatsch samples were positioned on the negative side of the first principle component. The other blended samples were shifted to the right of the first score plot, proportionally to the content of Lagrein. Such trend can be easily explained with the phenol content of these blends. Indeed, the electronic tongue measurements showed strong correlation with the Folin-Cocalteu index ($R^2 > 0.94$). Also, wine samples with increasing concentration of Lagrein wine showed higher anodic (*Ia*) and cathodic (*Ic*) currents, as measured by cyclic voltammetry (Table 1). St. Magdalener samples were placed on the negative side of the F1 close to the Vernatsch samples, which was expected. According to the denomination rules, this wine can only contain up to 15 % of Lagrein grape variety.

3.4 Correlation with Bitterness Evaluated by Sensory Analysis

The prediction of the bitter taste of red wines with the electronic tongue is a challenging issue. According to the literature, bitter taste in a wine is based on the content of monomeric flavan-3-ol fractions, containing (+)-catechin, (−)-epicatechin, and (−)-epicatechin-3-gallate and con-

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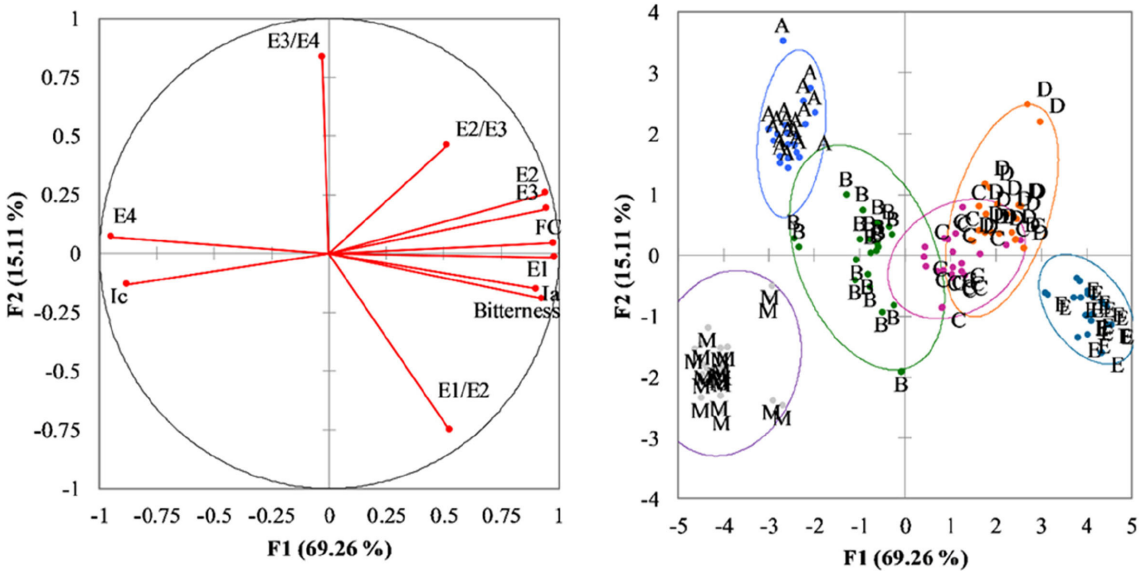

Fig. 3. PCA variable and score plots showing a relative position of the wine samples: A = 100 % Vernatsch; B = 25 % Lagrein, 75 % Vernatsch; C = 50 % Lagrein, 50 % Vernatsch; D = 75 % Lagrein, 25 % Vernatsch; E = 100 % Lagrein; M = 100 % St.Magdalener.

densed tannins [36,37,38]. Condensed tannins are also responsible for the astringency–shrinking, drawing, puckering sensations of epithelium [39,40]. Since wine bitterness and astringency are mainly correlated with the phenolic profile, the proposed multichannel amperometric system should provide, in principle, a pattern signal that could be suitable for the determination of the bitter taste intensity and probably the sensation of astringency. In this paper, however; only bitterness was evaluated by the sensory panel.

The correlation between the first principal component with the bitterness of wine samples evaluated by sensory analysis is described in Figure 4. As expected, the perceived bitterness of red wine blends was linearly increasing from Vernatsch to Lagrein. This trend corresponded relatively well to the results of the electronic tongue measurements ($R^2 = 0.81$). Such correlation is in agreement with previously published studies on wines [41] and also extra virgin olive oils [42], where also the bitter taste was correlated with an amperometric signal. Scampicchio et al. [43] evaluated the astringent taste of tea using eight electrodes connected in parallel in a positive range of potentials. However, the results obtained in our work demonstrated not only the possibility of using the electronic tongue to predict bitterness, but also to get information on the antioxidant activity and total phenolic content by using the configuration with four electrodes. This result shows a clear advantage compared to previously reported systems.

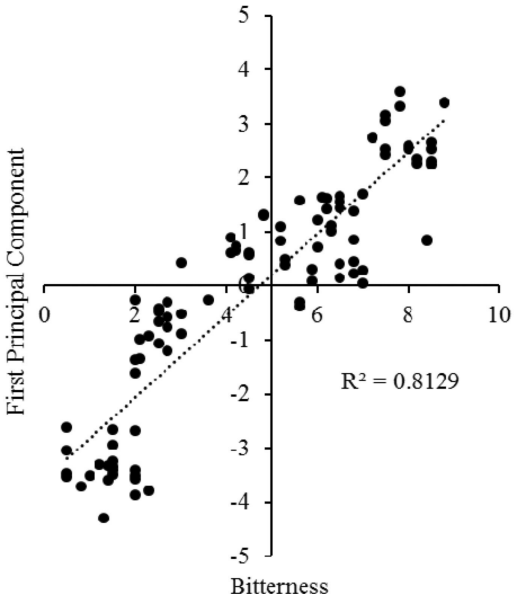

Fig. 4. Correlation between electronic tongue measurements (first principal component) and the bitterness of red wine blends measured by sensory analysis.

4 Conclusion

The proposed procedure based on a multichannel amperometric electronic tongue is a fast and reliable method to measure the antioxidant activity, the total phenolic content and to predict the bitter taste of red wines. The results of the electronic tongue measurements were consistent with the Folin-Ciocalteu index and phenolic content determined by cyclic voltammetry. Moreover, bitterness of the samples was proportional to the phenol content of red wines. Accordingly, the electronic tongue with a four-electrode system was proven to be a sensitive and rapid method for the characterization of wine blends. Overall, this system could become a simple, cheap and rapid alternative to sensory analysis for quality control in

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wineries. However, further experiments are needed to validate the method also for wines produced using different grape varieties and their blends.

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Received: October 20, 2017
Accepted: December 1, 2017
Published online on December 14, 2017

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