Electrochimica Acta 120 (2014) 180–186

Contents lists available at ScienceDirect

Electrochimica Acta

journal homepage: www.elsevier.com/locate/electacta

Evaluation of red wines antioxidant capacity by means of a voltammetric e-tongue with an optimized sensor array

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ARTICLE INFO

Article history:
Received 17 October 2013
Received in revised form
11 December 2013
Accepted 12 December 2013
Available online 27 December 2013

Keywords:
Electronic tongue
voltammetric sensors
wine
polyphenols
antioxidants ;

ABSTRACT

In this work, two sets of voltammetric sensors -prepared using different strategies- have been combined in an electronic tongue to evaluate the complete antioxidant profile of red wines. To this aim, wine samples were analyzed with the whole set of sensors. In order to reduce the large dimensionality of the data set while keeping the relevant information provided by the sensors, two different methods of feature selection and data compression were used (the *kernels method* and Discrete Wavelet Transform feature extraction method). Then, the coefficients obtained were used as the input variables of Principal Component Analysis (to evaluate the capability of discrimination. Partial-least squares regression (PLS) and artificial neural networks (ANNs) were performer to build the quantitative prediction models that allowed the quantification of the antioxidant capacity of the tested wines.

1. Introduction

Wine is an essential component of the Mediterranean diet and might be one of the factors responsible for the low incidence of heart disease in Mediterranean populations [1]. In this sense, the Mediterranean diet has largely demonstrated its health benefits which are related with the intake of foods and beverages rich in antioxidants [2], such as apples, olive oil and wine. A part from the health benefits, the antioxidant capacity is closely related with the quality of foods and beverages because it contributes to their organoleptic characteristics. It also plays a key role in the preservation of foods [3]. In the case of wine, those effects are mainly related with their content in phenolic compounds [4], which also affect their quality and organoleptic features.

The methods to assess the antioxidant activity are usually based on the evaluation of the capability of an oxidizing agent to induce an oxidative damage to a substrate; in presence of an antioxidant compound, these capabilities are inhibited or reduced. The main elements of any test for the evaluation of the antioxidant capacity are an appropriate substrate to monitor the inhibition of the oxidation, an initiator of the oxidation (free radical) and an appropriate measure of the endpoint of the oxidation [5].

When approaching the study of the antioxidant activity of wines, it has been recommended to use more than one method. The reason is that each method gives different information: certain antioxidants do not react with certain oxidizing species, but they do react with some others. As a consequence, different methods provide complementary information [6].

Among several other methods, the antioxidant activity can be evaluated by means of the measure of the absorbance capacity of the radical oxygen (ORAC) or the trolox equivalent antioxidant capacity (TEAC) [7]. On the other hand, the measure of phenolic compounds is usually achieved through the Folin-Ciocalteu method [8] or the I_{280} index [9]; the Folin method measures the reducing capacity of the sample, while the I_{280} index provides a measure of the sample absorbance at 280 nm. Although these absorbance indexes are related with the total phenolic content, they are also an accepted measure of the antioxidant activity of foods, given the role of phenolic compounds as antioxidants [10].

Taking into account that the antioxidant activity of wines is mainly related with the phenolic content, Folin index is even preferred by some authors, as it also evaluates the reducing power of wines. However, in some recent works related with the determination of their antioxidant capacity [11], it is stated that a complete antioxidant profile of red wines could be established by coupling (1) evaluation using ABTS (2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) to obtain a measure of total antioxidant capacity, (2) estimation of scavengers activities which

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http://dx.doi.org/10.1016/j.electacta.2013.12.079

give a complementary information, and (3) use of some type of biomarker methods to provide a measure of the oxidative stress.

In spite of their advantages, all these techniques have been developed for the analysis of samples at the laboratory level and require complex and time-consuming sample pre-treatment procedures.

The use of Electronic Tongues (ETs) is growing as a promising approach to analyze liquid samples [12,13], and can represent a suitable alternative to tackle the determination of antioxidant capacity of wines. Such analytical systems are formed by an array of sensors where several sensing units, which exhibit different responses to various compounds, are coupled with advanced signal processing methods based on pattern recognition or multivariate response models, which allow for the qualitative or quantitative analysis of different sample parameters. To this aim, sensors that might be used are mainly of electrochemical nature, specially of the potentiometric, voltammetric, even of the impedimetric type [14,15]. Voltammetric sensors chemically modified with a variety of sensing materials and biomaterials have demonstrated to be particularly suitable for the analysis of complex samples because they provide sensors with high cross-selectivity [16,17]. In particular, sensors based on phthalocyanines and conducting polymers provide chemical responses related with both the ions and the electroactive molecules (i.e. polyphenols) present in the solution, being particularly sensitive to pH and antioxidants [18,19]. In turn, biosensors based on tyrosinase are highly sensitive to phenols [20]. The use of tyrosinase and phthalocyanines (or conducting polymers) as electron mediators provides sensors with an increased sensitivity and selectivity towards phenols [21].

The aim of the present work is to examine the potential of an optimized voltammetric electronic tongue to provide a complete antioxidant profile of wine samples. To such purposes, two sets of voltammetric sensors prepared using different strategies were evaluated. After registering the voltammetric responses of the sensors exposed to wines with different oxidation states, a feature selection and data compression stage was performed employing the Discrete Wavelet Transform (DWT) and *kernel* feature extraction. This step is necessary to reduce the large dimensionality of the data set, while keeping the relevant information from the measurements. Finally, the obtained responses were analyzed by means of Principal Component Analysis for visualization of samples dis(similarities), and using PLS and ANNs to achieve the quantification of wine antioxidant capacity.

2. Experimental

2.1. Reagents and solutions

All reagents used were of analytical grade and all solutions were prepared using deionized water from a Milli-Q system (Millipore, MA, USA). Copper and platinum nanoparticles (<50 nm), polyaniline and polypyrrole, cobalt phthalocyanine (CoPc), tyrosinase from mushroom (EC 1.14.18.1, 5370 $U{\cdot}mg^{-1}$), gallic acid, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium persulfate (di-potassium peroxidisulfate) and 6-hydroxy-2,5,7,8-tetramethychroman-carboxylic acid (Trolox) were purchased from Sigma-Aldrich (St. Louis, MO, USA). KCl was purchased from Merck KGaA (Darmstadt, Germany). Folin-Ciocalteu's reagent and sodium carbonate were purchased from Panreac Química (Barcelona, Spain). HPLC grade ethanol was obtained from Scharlau (Barcelona, Spain).

The lutetium (III) bisphthalocyaninate ($LuPc_2$) was synthesized and purified in neutral radical state following earlier published procedure [22].

2.2. Wine samples under study

A total of 9 red wine samples from Tempranillo grapes, with different oxidation levels were provided by the *Matarromera* group (D.O. Ribera del Duero, Spain) in 2011. Wine oxidation was established according to the results provided by a panel of experts following the established regulations [23,24]; moreover, antioxidant capacity of wine samples was assessed by different standard methods (section 2.3). Table 1 summarizes the information about the wines used. Three replicas of each sample were analyzed.

2.3. Spectrophotometric measurements

For comparison purposes, the antioxidant capacity and the polyphenolic content of wines were assessed spectrophotometrically with three different methods: Trolox Equivalent Antioxidant Capacity (TEAC), Folin-Ciocalteu index (FC) and UV Polyphenol Index (I_{280}).

Spectrophotometric measurements were registered in a Schimadzu-UV-1601 spectrophotometer (Kyoto, Japan) and a 1 cm path quartz cell. In all cases, determinations were carried out in triplicate and using a hydro-alcoholic solution (12%, v/v ethanol) of tartaric acid (3 $g.L^{-1}$) as the blank solution.

2.3.1. TEAC

TEAC measures the antioxidant capacity of a given substance, as compared with the standard, Trolox (a water-soluble vitamin E analogue). This assay is based on the scavenging of long-lived radical ions (such as $ABTS^{\bullet+}$). Firstly, radicals, which can easily be detected spectrophotometrically at 734 nm, are generated. Then, antioxidants are added and the scavenging capacity is measured, providing the TEAC value by comparing the previous value to that of Trolox.

The ABTS assay was performed according to a previously reported procedure [25]. First, a ABTS stock solution in water (7 mM) was prepared; followed by the generation of ABTS radical cation ($ABTS^{\bullet+}$) by reacting the stock solution with a potassium persulfate solution (final concentration 2.45 mM). The resulting solution was kept in dark at room temperature for 12 hours prior to its use. $ABTS^{\bullet+}$ solution was diluted with ethanol to an absorbance of 0.70 (±0.02) at 734 nm.

For the assay, 4 mL of $ABTS^{\bullet+}$ solution were added to a 1 cm spectrophotometer cuvette followed by the addition of 10 μl, 20 μl, 30 μl and 40 μl of previously diluted wine, respectively. The absorbance reading was taken exactly 1 min after initial mixing and up to 10 minutes. The inhibition percentage for the absorbance at 734 nm was calculated as the ratio between the decrease of absorbance due to sample addition (A_C-A_S) and the control absorbance (A_C) multiplied by 100, and afterwards, plotted as a function of the added volume:

$$\%I = \frac{A_C - A_S}{A_C} \cdot 100 \quad (1)$$

Prior to wine samples measurement, they were diluted such that, after addition of 10-40 μL aliquot of the diluted wine into the assay, they produced between 20%-80% inhibition of the blank absorbance. In our case, the dilution necessary to achieve these inhibition percentages was 1:15 (wine:blank solution).

The same procedure was followed using Trolox standard (2.5 mM prepared in ethanol absolute) instead of diluted wine samples; with a concentration range for the assay from 2.5 μM to 15 μM (including also the 0). Then, as before, the absorbance inhibition percentage vs. concentration plot was built and the slope was calculated.