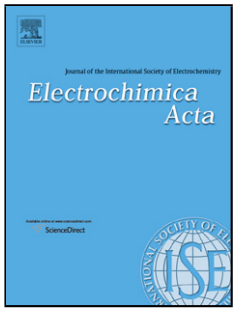

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Author: F.M.A. Lino L.Z. de Sá I.M.S. Torres M.L. Rocha T.C.P. Dinis P.C. Ghedini V.S. Somerset E.S. Gil<ce:footnote id="fn1"><ce:note-para id="npar0005">[1]ISE menbers</ce:note-para></ce:footnote>

PII: S0013-4686(13)01639-3
DOI: http://dx.doi.org/doi:10.1016/j.electacta.2013.08.109
Reference: EA 21134

To appear in: *Electrochimica Acta*

Received date: 6-5-2013
Revised date: 19-8-2013
Accepted date: 20-8-2013

Please cite this article as: F.M.A. Lino, L.Z. de Sá, I.M.S. Torres, M.L. Rocha, T.C.P. Dinis, P.C. Ghedini, V.S. Somerset, E.S. Gil, Voltammetric and spectrometric determination of antioxidant capacity of selected wines, *Electrochimica Acta* (2013), http://dx.doi.org/10.1016/j.electacta.2013.08.109

Voltammetric and spectrometric determination of antioxidant capacity of selected wines

F.M.A. Lino[a], L.Z. de Sá[a], I.M.S. Torres[a], M.L. Rocha[a], T.C.P. Dinis[b], P.C.Ghedini[a], V.S. Somerset[c,1], *E.S. Gil[a,1]

[a]Faculdade de Farmácia, Universidade Federal de Goiás, Campus Colemar Natal e Silva, Praça Universitária, CEP: 74605-220, Goiânia, Goiás, Brasil.

[b]Faculdade de Farmácia, Universidade de Coimbra, Coimbra, Portugal.

[c]Natural Resources and the Environment, CSIR, Stellenbosch, South Africa

[*]To whom correspondence should be addressed
Tel/FAX: +5562-3209-6042
e-mail: ericsgil@farmacia.ufg.br; ericsgil@gmail.com
Departamento de Controle de Qualidade,
Faculdade de Farmácia, Universidade Federal de Goias,
Av Universitaria s/n, Setor Universitário
74605-220 Goiânia, Brasil

[1]ISE menbers

Abstract: Considering the presence of phenolics in grapes and wines, as well as their importance for health promoting properties, the DPPH (1,1-diphenyl-2-picrylhidrazine) assay and a novel electroanalytical approach (Differential Pulse Voltammetry – DPV) for analysis have been performed in order to compare the antioxidant activity of different grape beverages. A total of fifty-two wine samples from different regions around the world were analyzed. The antioxidant activity of the different wines analyzed was expresses as the amount of wine required to produce 50% of decolorization of DPPH relative to the blank control (EC50) and as an Electrochemical index (EI), obtained by summing the ratios between peak current and peak potential values. Red wines presented higher antioxidant capacity than rose and white ones or red juices, evidencing the influence of the overall process of fabrication in phenolic extraction from the skin of grapes. A negative Pearson´s correlation was found (-0.9110) and this result is consistent with what was expected due to the different principles inherent to these methods.

Key Words: Total phenolics, wine, grape beverages, radical scavenging assays, electrochemical index.

1. Introduction

The consumption of wine has long been associated with health benefits. Many studies have observed a lower incidence of cardiovascular diseases in the French population when compared to other countries such as USA and England. Though the diet in France is also rich in saturated fat, owing to the daily wine ingestion, the occurrence of arteriosclerosis is lower than the expected [1].

The health promoting properties of wines are due to vast quantities of phenolic compounds, claimed to be the most important natural antioxidants [2]. Antioxidants are molecules that can inhibit or stop oxidation reactions promoted by free radicals, which are related to DNA degradation, membrane peroxidation and protein denaturation. The reactions promoted by the free radicals lead to the aging process and are also responsible for the occurrence of many diseases such as cancer, diabetes, neurological problems, as well as cardiovascular problems [3,4].

Wines represent better sources of antioxidants when compared to other dietary sources due to the fact that, in this beverage, phenols are already solubilized, facilitating the absorption process. It is interesting to highlight that wine´s phenolic profile is different from grapes, a fact that can be attributed to the extraction process, which increases the content, and also to the fermentation process, that modifies substances [6,7,8]. Thus, owing to the health properties of wines, in which the phenolic antioxidants play a crucial role, it is indispensable to have methods capable of measuring wine´s antioxidant activity [9,10].

Many different methods can be used to measure the antioxidant activity, with the DPPH (1,1-diphenyl-2-picrylhydrazine) approach as one of the most popular. $DPPH^{\cdot}$ is a commercial radical

that can be reduced by antioxidant molecules, changing its colour from purple to yellow after the reaction, causing a decrease in the absorbance at the wavelength of 517 nm [11-14].

The DPPH$^{\cdot}$ method presents some advantages when compared to other spectrophotometric methods such as ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), in which it is necessary for the generation of the radical, while the DPPH$^{\cdot}$ does not require this procedure. However, a major limitation of this method, especially when analyzing wines, is the interference of substances that absorb in the same wavelength as the DPPH$^{\cdot}$, leading to difficulties with the precision and accuracy of the results [11,12].

Therefore, complementary methods that specifically present a different analytical principle for the same type of analysis are often recommended. In this context, the development of novel techniques to evaluate the antioxidant activity of wines is an area of interest of many research groups. Electroanalytical methods, mostly voltammetric techniques, represent one of these novel tools, presenting many advantages such as speed, low cost, simplicity and low consumption of reagents when compared to other methods [9,13]. Another great advantage is the fact that they do not rely on the use of oxidizable compounds to measure the antioxidant capacity of the sample, but instead depend only on the inherent electrochemical properties of antioxidants in the sample [14].

The need of an electrochemical index for natural antioxidants has already been proposed [15]. However, the proposed index has only a qualitative approach, classifying antioxidants as ones of "high antioxidant power" or "low antioxidant power" accordingly to the potential in which the redox reaction occurs. The total polyphenol index of wines was also determined by using an electronic tongue and multivariate calibration, but the relationship between polyphenol content and antioxidant capacity was not discussed in this approach [16]. Furthermore, most papers have

focused on a low number of samples, mostly red and white wines, from a single geographical area [6,11,14,16,17]. Thus, the aim of this work was to evaluate the antioxidant activity of a range of wines from geographically distinct locations, different quality levels and varied production techniques.

A traditional method ($DPPH^{\cdot}$) and a novel electroanalytical approach (differential pulse voltammetry – DPV) was performed and an electrochemical quantitative index was proposed in order to compare the antioxidant activity of different wines.

2. Experimental

2.1 Reagents and Standards

1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\cdot}$) reagent was purchased from Sigma Chemical Co. (St. Louis, MO, USA). ABTS(2,2-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid), Gallic acid (GA), Trolox and Folin-Ciocalteu phenol reagent were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All supporting solutions were prepared using analytical grade reagents and purified water from a Millipore Milli-Q system (conductivity ≤ 0.1 μScm^{-1}) (Millipore S. A., Molsheim, France), in accordance with well-established procedures.

The electrochemical analyses were carried out in 0.1 M phosphate buffer solutions (pH 5.0). All electrolyte solutions were of the highest analytical grade and were prepared using double-distilled Milli-Q water.

2.2 Samples

A total of fifty-two wine samples, obtained from different regions worldwide, five commercial grape juices and a wine brandy sample were analyzed.

All of them were purchased from local markets (Goiânia – GO, Brazil). Prices of the selected wines listed in Table 1, 2 and 3 varied from US$ 4.00 to $ 120.00 dollars, the pH from 3.3 to 3.7, whereas the alcohol content varied from 7 to 21 % v/v.

2.3 *Sample Preparation*

Spectrophotometric assays: The wines and juices were diluted in alcohol of analytical grade in order to reach 10% v/v. The analytical samples were prepared by leaving the former solution in resting for two hours and then taking aliquots from 10 to 500 μL of its supernatant, followed by further dilution in ethanol in order to reach 0.5 mL.

Electroanalytical assays: Both the wine and juice samples were diluted in pH 5.0 0.1M Phosphate buffer solution in order to reach the proportion 2:3 mL. The resulting pH of the samples was 4.5. All samples were stored at 4°C until analysis.

2.4 Spectrophotometric Assays

2.4.1 Apparatus

The absorbance measurements were recorded with a spectrometer Q798U2VS (Quimis Aparelhos Científicos, São Paulo, Brazil). All samples were analyzed in a glassy cell of a 1 cm at room temperature (21 ± 1 ºC).

2.4.2 DPPH Radical Scavenging Assay

Radical scavenging activity of different fractions of wines was measured based on the conversion (decolorization) of stable 1,1-diphenyl-2-picrylhydrazyl (DPPH$^{\cdot}$) radical in DPPH by wine´s antioxidants. The ability of samples to scavenge DPPH$^{\cdot}$radicals was determined by the method of Blois (1958) [10,19,20]. Briefly, to 2.5 mL of DPPH$^{\cdot}$ ethanolic solution (0.1 mM) an aliquot of 0.5 mL of ethanol (blank) was added to reach a final volume of 3.0 mL that was repeated for all analytical samples. The reaction solution was incubated for 30 min in the dark at room temperature and measured at 517 nm, against the blank (A ~ 0.7), whereas ethanol, the solvent used to prepare all solutions, was used in order to adjust the baseline (A = 0.000). Antioxidant activity was expressed as EC_{50}, representing the amount of wine to produce 50% of decolorization of DPPH$^{\cdot}$ relative to the blank control.

2.4.3 ABTS Radical Scavenging Assay

The antioxidant capacity of the samples was evaluated by the ABTS radical cation decolorization assay as described [11,18-20]. Briefly, ABTS radical cation ($ABTS^{\bullet+}$) was generated by reacting 7 mM ABTS with 2.45 mM potassium persulfate (final concentration) for 16 h in the dark at room temperature. The $ABTS^{\bullet+}$ solution was then diluted with ethanol to reach an absorbance of 0.70 (± 0.02) at 734 nm. The antioxidant capacity of the samples were calculated as Trolox equivalent (TE) based on the percentage of inhibition of the blank absorbance by samples at 7 min and expressed as μmole of Trolox per mL of wine.

2.4.4 Determination of Total Phenols

The total phenolic content was estimated using the Folin-Ciocalteau reaction as described in the literature [19,20]. Briefly, 2.5 ml of 10% Folin-Ciocalteau reagent was added to small volume of sample (usually between 25 and 100 μL) and then treated with sodium carbonate solution. The absorbance was measured at 760 nm and the total phenolic content was calculated as gallic acid equivalent based on a standard curve of gallic acid. All the experiments were performed in triplicate. Results are expressed as the milligram gallic acid equivalent (GAE)/mL of fermented beverage [19,20].

2.5 Electroanalytical Methods

2.5.1 Apparatus

Voltammetric experiments were performed in a potentiostat/galvanostat μAutolab III® integrated to the GPES 4.9® software, Eco-Chemie, Utrecht, The Netherlands. Measurements were performed in a 5.0 mL one-compartment electrochemical cell, with a three-electrode system consisting of a carbon paste electrode (prepared as a piston-driven holder containing 70% of graphite powder and 30% of Nujol®, Ø = 2 mm), a Pt wire and the Ag/AgCl/KCl_{sat} (both purchased from Analyser, São Paulo), representing the working electrode, the counter electrode and the reference electrode respectively. The surface of the carbon paste electrode (CPE) was mechanically renewed before the start of a new set of experiments by extruding approximately 0.5 mm of carbon paste out of the electrode holder and smoothing it with filter paper. This procedure ensured very reproducible experimental results. The experimental conditions for differential pulse voltammetry (DPV) were: pulse amplitude 50 mV, pulse width 0.4 s and scan rate 5 mV s^{-1}. All experiments were done at room temperature (21 ± 1 ºC) in triplicate ($n = 3$).

2.5.2 Acquisition and Presentation of Voltammetric Data

In order to improve the visualization and the identification of peaks over the baseline, all the voltammograms presented were background-subtracted and baseline-corrected using the moving average application with a step window of 2 mV included in GPES version 4.0 software. This Mathematical treatment does not introduce any artifact; however, the peak intensity is in some cases reduced (< 10%) in relation to the untreated curve. Nevertheless, this mathematical treatment was used in the presentation of all experimental voltammograms after subtraction of the baseline.

The replicates ($n = 3$) of each wine sample were analyzed and treated with the software Origin 8®. Furthermore, such treated DPV voltammograms were compiled accordingly to the wine category in order to get the average of the readings and to obtain more reliable results about the electrochemical profile of each class.

2.5.3 Electrochemical Index Determination

An Electrochemical Index (EI) was proposed taking into account the main voltammetric parameters, peak potential (E_{pa}) and peak current (I_{pa}). Thus, since the lower the potential (thermodynamic parameter) is, the higher is the electron donor ability, as well as the higher is the peak current (kinetic parameter), the higher is the amount of electroactive species, the EI was calculated by using the equation [10]:

□□□□ =

„□□-□□□□□.-,□□-□□□□□..+„□□-□□□□□.-,□□-□□□□□..+⋯+„□□-□□□□□.-,□□-

$$EI = \frac{I_{pa1}}{E_{pa1}} + \frac{I_{pa2}}{E_{pa2}} + \cdots + \frac{I_{pan}}{E_{pan}}$$

In which I_{pa} and E_{pa} correspond to current and potential value for each major anodic peak observed in the DPV voltammograms.

2.6 Statistical Analysis

Statistical analysis was performed using GraphPad Prism® version 5.0 (GraphPad Software, San Diego, CA, USA). Comparisons among groups were made using ANOVA. Post hoc comparisons were performed using Newman-Keels' comparison test. The significance level considered was 0.05.

3. Results and Discussion

Tables 1 to 3 presents the **EC_{50}** and **EI** values obtained for some selected wines and other grape beverages. In order to investigate some factors that can influence the antioxidant power and related health benefits of different wines, the sampling was carefully performed to include wines from different types, i.e. grape varietal, quality level, production techniques and geographical locations.

TABLE 1

TABLE 2

The first observation that can be taken from Tables 1 and 2 is that red wines present higher antioxidant capacity than white ones, which is in agreement with the expected results [16,21,22,23]. For instance, the average **EC_{50}** for red and white dry wines were 20.1 μL (*n = 27*) and 98.4 μL (*n = 6*), respectively. In fact, the worst (higher) **EC_{50}** value among dry red wines, 26.9 μL was even lower than 56.4 μL, the best value obtained among the white wines evaluated in this study. Indeed, with respect to the DPPH radical scavenging activity, it could be implied that red wines are around five times stronger than white wines.

In turn, no statistically relevant difference was found for different grape varieties used in wine production, as well as among different wine regions. On the other hand, the processing features of some specific types of wines showed to exert major influence on the phenolic content and as consequence on the final antioxidant power. This is similar to the results found in a study by [20,23,24].

For instance, fortified wines, *i.e. Porto (Portugal), Madeira (Portugal), Jerez (Spain), Vermouth (Italy), Commandaria (Greece)* and *Marsala (Italy)* type wines, as well as sparkling wines, *i.e. espumante (Brazil), Champagne (France), Vinho verde (Portugal)* and *frizzant (Italy)* may also present higher **EC_{50}** values. Thus, the addition of brandy and sugar, exert a dilution effect and

perhaps also, the natural oxidation that occurs during the aging process may explain the lower scavenging activity obtained for the eight fortified wines, *Porto (Portugal), Madeira (Portugal), Jerez (Spain), Marsala (Italy)* and *Vermouth (Italy)*, evaluated in this study. Yet, in the case of sparkling wines, the higher $\mathbf{EC_{50}}$ values when compared to their white and rose analogues may be also related to the inherent dilution effect of bubbles. For example, the average values obtained for white and rose wines were 98.4 μL (*n = 6*) and 80.6 μL (*n = 3*) respectively, which are lower than the overall average of 134.9 μL (*n = 5*) obtained for the sparkling wines (Table 3).

Though the table wines presented lower average value than the analogues of higher commercial value, the quality level was better evidenced when dry red wines were compared to sweet ones. For instance, the sweet red wines presented an average value of 45.9 μL (*n = 3*), which is more than two-fold the value observed for dry red wines.

In turn, it was also observed that red wines present higher antioxidant activity than juices (Tables 1 and 3), demonstrating that the overall process of fabrication of such wines may really promote higher phenolic extraction from the skin of grapes, as it was already demonstrated in previous studies [23-25].

TABLE 3

Furthermore, as it would be expected, the whole grape juice presented higher antioxidant activity than grape juice nectar, both commonly found in supermarkets. In fact, the whole natural juices have higher pulp content than grape juice nectars. Though sugars are generally recognized as weak reducing agents, such a property is hidden by the stronger electron donor characteristic of phenolic compounds. Therefore, the higher amount of sugar in nectar juice or in sweet wine may not have great influence on their radical scavenger activity. In fact, even in honey, in which the

sugar content reaches more than 80%, the antioxidant activity is mostly driven by polyphenol composition [26].

3.1 Electrochemical Index

Antioxidants are electroactive compounds; hence electroanalysis emerges as a useful tool for their evaluation. In fact, good electron donor agents may oxidize reversibly at lower peak potentials (E_{pa}< 0.5 mV, pH = 7). Following this idea, the concept of an Electrochemical Index (EI) was previously proposed to qualify pure compounds [15]. Besides the direct information that peak potentials can provide about the reduction potential, another electrochemical parameter, the peak current, I_{pa}, can also be very useful. According to the Faraday Law, in which the amount of electroactive species can be stoichiometrically correlated to the quantities of transferred electrons, the peak current indicates the concentration of antioxidants in the analytical sample [10,13].

Nevertheless, the electrochemical oxidation of phenolic compounds is often followed by a strong adsorption process. Such uncontrolled behavior leads to blockade of the electrode and, as result to the lack of repeatability of quantitative analysis.

In order to solve this problem, carbon paste electrodes were employed in all DPV determinations. Figure 1 show six DP voltammograms obtained from the same red wine sample, for which each assay a different carbon paste electrode was employed.

FIGURE 1

With the ease of renewing the surface of the CPE as described earlier in the study, the repeatability obtained (CV < 5%; n = 5) was rather satisfactory.

The voltammetric profile of the different wine varieties showed some typical features. It was observed in our experiments that red wines presented a shoulder peak labeled ***1a,s*** at $E_{p1a,s}$~ 0.3 V (*vs.* Ag/AgCl), followed by three well defined anodic peaks labeled ***1a***, ***2a*** and ***3a***, at 0.37, 0.57 and 0.80 V (*vs.* Ag/AgCl), respectively. In turn, the white wines presented two very well defined peaks (Figure 2, dashed line), labeled ***1a*** and ***3a***, at E_{p1a}~ 0.37 V (*vs.* Ag/AgCl) and E_{p3a}~ 0.83 V (*vs.* Ag/AgCl). In the case of the rose wine (Figure 3, dashed line), the DPV results presented three distinct peaks, labeled ***1a***, ***2a*** and ***3a***, at 0.38, 0.58 and 0.80 V (*vs.* Ag/AgCl), with a less defined peak ***2a***. In Figure 2 the dashed line **(**- - -) for the white wine and the continuous line **(** —) for the red wine, shows the average simulated voltammogram calculated by Origin 8® software for all red and white wines characterized in this study. These results are very close to those ones obtained in previous assays for red and white wines, in pH 3 model wine solutions characterized at a glassy carbon electrode [21]. In this study it was observed that peaks 1a (Figure 1) are related to orto-diphenolic compounds, *i.e.* quercetin, rutin, caffeic acid, as well as gallic acid, while the peaks 2a and 3a (Figure 1), may be associated to the presence of resorcinol and mono-phenol-pattern, such as the major compounds ferullic acid, resveratrol, malvidin, coumaric acid [21,24].

FIGURE 2

In fact, despite some small variations of resolution, such as the appearance of shoulders and outliers, the pattern of two or three major peaks for white and red wines respectively is like a fingerprint of those wines. Some of the most distinct DPV voltammograms profiles are shown is Figures 2 and 3.

FIGURE 3

The appearance of shoulder peaks (Figure 3) is related to the inherent complexity of these samples, but can be also influenced by experimental conditions. Hence, natural phenolic and non-phenolic compounds and also some wine additives present native electroactivity, which could lead to the observed voltammetric profile. For instance, it was observed that sulfur dioxide may cause some distortions on cyclic voltammograms obtained for wine or phenolic compounds in model wine solutions [6,23,24]. However, such distortions might be corrected and minimized in differential pulse voltammetry. Though sugar and ethanol, when present at higher concentrations leads to an ohmic drop, they do not show any great effect on the redox behavior of phenolic compounds and wines at the assay conditions [6,24,27]. However, it is possible to standardize the electrolyte and sample´s dilution in order to reproduce some results and to avoid comparative errors. An important limitation when using voltammetric methods is the fact that this standardization is not possible for the electrode´s surface, as it can be modified during electro-oxidation by adsorption processes, even for diluted samples [24]. Therefore, taking into account that shoulders and minor peaks are more difficult to reproduce they were not considered in the EI calculations.

The average **EI** values of 30.6 μA/mV (*n = 27*) and 16.5 μA/mV (*n = 6*), ranging respectively from 24.3 to 43.5 μA/mV for dry red wine and from 8.5 to 20.2 μA/mV in the case of the white ones, follow an negative correlation with DPPH assays. The same tendency was observed for the other wine categories analyzed in this study. The resulting Pearson´s correlation was -0.9110 and is illustrated in Figure 4.

FIGURE 4

Finally, any deviation on the correlation between spectrometric and electroanalytical approaches is consistent with the different principles of methods. Moreover, small correlations have been

found even between similar methodologies [4,13,18]. Such fact reinforces the use of electroanalytical approach, which is less expensive and simple, thus it showed to be at least two times faster.

Although the production of wines follows some basic concepts, the varieties of grapes and some processing technologies differ widely, leading to a great diversity of wines available. Since the main biological actions of wines are related to their antioxidant capacity, such property has been extensively studied. Nevertheless, to the best of our knowledge no study involving a wide and diverse variety of wines has been conducted. Thus, other researchers have shown that red wines has far greater antioxidant capacity than white wines, but the ageing of the grape variety may have no expressive influence [6,11,14,16,17,24].

It was also found that the varieties of grapes, as well as the geographic location of the world in which the wine was produced, do not have great influence on antioxidant capacity. By contrast, such property is greatly influenced by the category and/or quality of wine, with the dry red wines being the most powerful antioxidant beverages. Despite few exceptions, the quality label was found to have minor but important influence on the antioxidant power of wines. The use of distinct methodologies in order to get more accurate results about the real antioxidant power is thus worthwhile investigating.

In this context, the ABTS and the Folin-Ciocalteu assays, widely applied on the evaluation of foodstuffs and wines were applied in this study in order to establish additional comparisons. Owing to the time constraints generally experienced with the aforementioned techniques, such assays were carried out with pooled samples of each wine class as shown in Table 3.

The results showed good agreement, thus inferring that the use of simpler methods such as DPPH and/or EI are fast and suitable tools to express the antioxidant activity of wines, which may be also a quality criteria [11,20].

4. Conclusions

In this study an electroanalytical approach was proposed for the evaluation of antioxidant character of different wines. The new method presented good correlation with traditional methods used for the evaluation of the antioxidant activity and total phenol content. In addition, the results obtained by means of spectroscopy and electroanalytical techniques were all consistent with the expected results for different categories of wine evaluated in this study. The results obtained for the order of antioxidant activity was: dry red wine > sweet and/or table red wine >> rose wine >> white wine >>> sparkling wine ≥ fortified wines. Therefore, it can be concluded that the use of the electrochemical index (EI) approach is a fast and suitable tool to express the antioxidant activity of wines, which can also be applied as a quality criteria for the evaluation of wines.

Acknowledgments: The authors are grateful for the financial support from CNPq and Capes.

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Table 1. The results for EC50 and EI obtained for mono and multi-varieties of red wines from different geographical locations evaluated in this study.

Table 2. The results for EC50 and EI obtained for rose and white wines from different geographical locations evaluated in this study.

Table 3. The results for EC50 and EI obtained for other grape beverages evaluated in this study.

Table 4 .Antioxidant assays obtained for pooled samples of different categories.

Figure 1. DP voltammograms obtained for 2 mL of red wine in 3 mL of 0.1 M phosphate buffer (pH 5.0) solution characterized at six different carbon paste electrodes (Ø = 2 mm). Other parameters included a pulse width of 5 mV, pulse amplitude of 50 mV, scan rate of 5 mV s^{-1}.

Figure 2. Average DP voltammograms obtained for 2 mL of red (—), n= 25 and white (- - -), n = 5 wines in 3 mL of 0.1 M phosphate buffer (pH 5.0) solution characterized at carbon paste electrodes (Ø = 2 mm). Other parameters included a pulse width of 5 mV, pulse amplitude of 50 mV, scan rate of 5 mV s^{-1}.

Figure 3. DP voltammograms obtained for 2 mL of white wine (—), rose sparkling wine (- - -) and red nectar juice (-···-) in 3 mL of 0.1 M phosphate buffer (pH 5.0) solution characterized at carbon paste electrodes (Ø = 2 mm). Other parameters included a pulse width of 5 mV, pulse amplitude of 50 mV, scan rate of 5 mV s^{-1}.

Figure 4. Graphic representation of EC_{50} (A) and EI (B) values for different classes of wines (R = red; r = rose; W = white; F = fortified; S = sparkling). **P*<0.001 compared with R. # Denotes difference between the two groups (*P*<0.05). Note: The EC_{50} represents the amount of wine (in μL) to produce 50% of decolorization of DPPH˙ relative to the blank control, whereas EI, electrochemical Index, represents the sum of the ratios, I_{pa}/E_{pa} .

Table 1. The results for EC_{50} and EI obtained for mono and multi-varieties of red wines from different geographical locations evaluated in this study.

Varietal(s) / Country code**	**EC_{50}** (μL)	**EI** (μA/mV)
Cabernet Sauvingnon/CL	16 ± 4	33 ± 3
Cabernet Sauvingnon/FR	17 ± 3	27 ± 1
Pinotage/AU	22 ± 3	29 ± 3
Pinotage/ZA	18 ± 2	33 ± 3
Tannat/UY	16 ± 3	34 ± 2
Tannat/BR	17 ± 3	29 ± 1
Merlot/BR	22 ± 4	27 ± 3
Merlot/CL	19 ± 3	28 ± 3
Merlot/US	22 ± 2	26 ± 1
Syrah/US	21 ± 3	28 ± 2
Syrah/AU	23 ± 3	29 ± 4
Tempranilho/ES	20 ± 2	25 ± 2
Carmenere/CL	15 ± 2	36 ± 3
Pinot Noir/NZ	20 ± 4	31 ± 3
Malbec/AR	22 ± 3	33 ± 4
Barbera/IT	21 ± 3	32 ± 2
Agiorghjitiko/GR	19 ± 4	28 ± 4
Zwiegelt/AT	16 ± 4	30 ± 3
Monovarietal Red Average(*n = 18*)	30.1 ± 5.5 (sd = 3.0)	19.2 ± 4.0 (sd = 2.5)
Cabernet-Merlot/CL	17 ± 3	33 ± 3
Cabernet-Merlot[s]/BR	**25** ± 3	**22** ± 3
Malbec-Bonarda[t]/AR	26 ± 5	28 ± 3
Malbec-Tempranillo[t]/AR	22 ± 2	33 ± 4
Izabel-Tannat[t]/BR	21 ± 2	32 ± 2
Local vine grapes[ts]/BR	**38** ± 3	**19** ± 1
Syrah-Tempranillo-TN/PT	17 ± 3	28 ± 3
Trincadeira-Touriga Franca-Touriga Nacional-Tinta Roriz/PT	19 ± 4	25 ± 2
Trincadeira-Periquita-Touriga Nacional/PT	18 ± 3	27 ± 3
Multivarietal Red Average (*n = 9*)	23 ± 10 (sd = 6.9)	27 ± 6.5 (sd = 4.7)

Legend: [t](table wine); [s](sweet wine)

**ISO 3166-1-alpha-2 code: http://www.iso.org/iso/home/standards/country_codes.htm

Argentina (AR); Australia (AU); Austria (AT); Brazil (BR); CL (Chile); FR (France); Germany (DE); Greece (GR); Italy (IT); New Zealand (NZ); Portugal (PT); South Africa (ZA); Spain (ES); United States (US); Uruguay (UY)

Table 2. The results for EC_{50} and EI obtained for rose and white wines from different geographical locations evaluated in this study.

	Varietal(s)/ Country code**	**EC_{50}** (μL)	**EI** (μA/mV)
Rose Wines	Tempranillo-Touriga Nacional/PT	93 ± 6	18 ± 2
	Syrah-Merlot/FR	68 ± 3	20 ± 3
	Niagara-Isabel[t]/BR	81 ± 4	19 ± 2
	Rose Average (*n = 3*)	81 (sd = 13)	19 (sd = 1)
White Wines	Chardonnay/AR	75 ± 4	18 ± 1
	Chardonnay/US	64 ± 3	16 ± 3
	Riesling/DE	200 ± 9	9 ± 3
	Riesling/CL	55 ± 4	19 ± 2
	Niagara-Lorena[t]/BR	56 ± 3	18 ± 3
	Niagara- Lorena[t]/BR	102 ± 3	15 ± 3
	White Average (*n=6*)	92 (sd = 56)	16 (sd =3.6)

Legend: [t](table wine)

**ISO 3166-1-alpha-2 code: http://www.iso.org/iso/home/standards/country_codes.htm

Table 3. The results for EC_{50} and EI obtained for other grape beverages evaluated in this study.

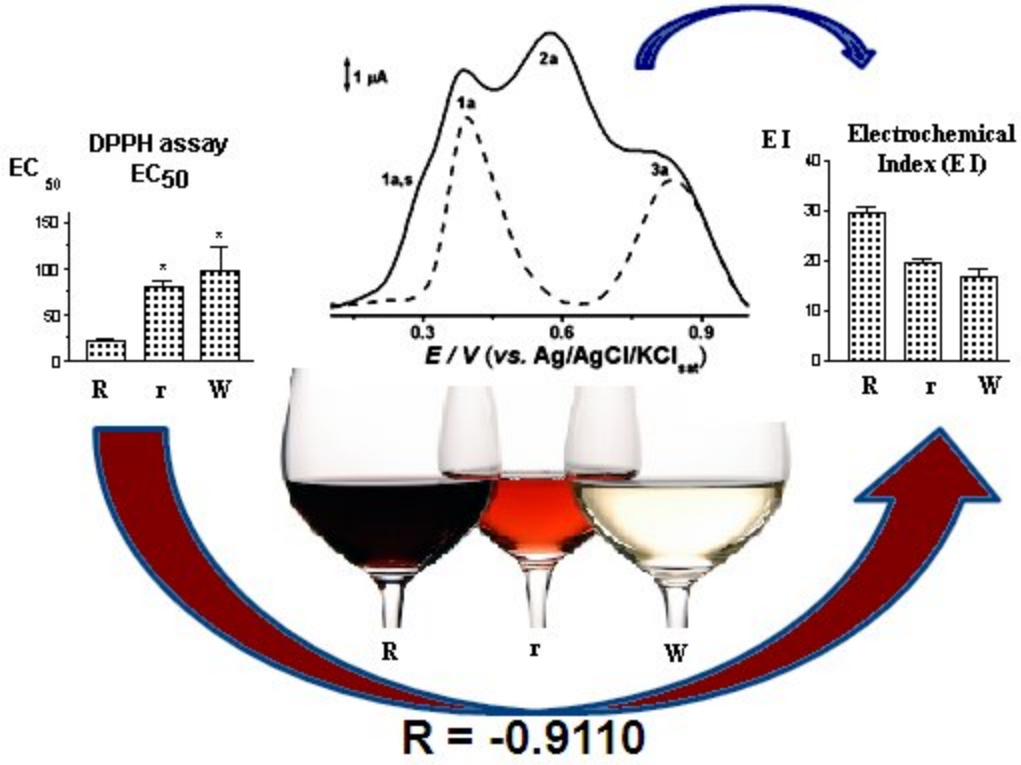
Varietal(s) / Country code**	**Grape Beverages**	**EC_{50} (µL)**	**EI (µA/mV)**
Barbera-Moscatel/IT	Sparkling$^{\mathbf{rose}}$	79 ± 3	16 ± 3
Moscatel-Lambrusco/IT	Sparkling$^{\mathbf{white}}$	97 ± 2	14 ± 2
Moscatel-Pinot bianco/IT	Sparkling$^{\mathbf{white}}$	95 ± 3	15 ± 2
Barcelo/PT	Sparkling$^{\mathbf{green}}$	118 ± 4	16 ± 2
Local vinigrapes/BR	Sparkling$^{\mathbf{white(ts)}}$	290 ± 9	9 ± 2
Average(*n=6*)		**136 (sd = 87)**	**14 (sd = 2.9)**
Grillo-Ct-In/IT	Marsala^{red}	104± 3	14 ± 2
Palomino-PX/ES	Sherry^{white}	255± 3	11 ± 2
Ct-Tb-Cb-Pe/IT	Vermouth^{rose}	304± 3	11 ± 2
TN- TF -TB /PT	Port rose^{rose}	100± 3	11 ± 2
TN- TF -TR-TC-TB/PT	Port tawny^{red}	97± 3	12 ± 2
TN- TF -TR-TC-TB/PT	Port ruby^{red}	91± 3	16 ± 2
Malvasia-TNM/PT	Madeira dry^{red}	105± 3	9 ± 2
Malvasia-TNM/ PT	Madeira medium dry^{red}	117± 3	8 ± 2
Average (*n= 8*)		**146 (sd = 83)**	**11.5 (sd = 2.6)**
Local vini grapes/PT	brandy	**> 1000**	**0**
Red Grapes/BR	Whole Juice	37 ± 1	18 ± 2
Red Grapes/BR	Whole Juice	40 ± 3	16 ± 1
Green Grapes/BR	Whole Juice	25 ± 3	15 ± 3
Red Grapes/BR	Nectar Juice	65± 2	8 ± 3
Red Grapes/BR	Nectar Juice	75 ± 3	9 ± 2
Average(*n= 5*)		**48 (sd =21)**	**13 (sd = 4.2)**

Legend: $^{\mathbf{ts}}$(table sweet wine); Ct (Catarrato); In (Inzolia); Tb (Trebbiano); Pq (Piquepoul); PX (Pedro Ximenes); TN (Touriga Nacional); TF (Touriga Franca); TB (Tinta Barroca); TR (Tinta Roriz); TC (Tinta Cão); TNM (Tinta Negra Mole).

**ISO 3166-1-alpha-2 code: http://www.iso.org/iso/home/standards/country_codes.htm

Table 4. Antioxidant assays obtained for pooled samples of different categories.

Pooled Samples	**DPPH** (μmol TE/mL)	**ABTS** (μmol TE/mL)	**Folin-Ciocalteu** (μg GAE/mL)	**EI** (μA/mV)
Red wines (*n = 27*)	5.2 ± 0.5	6.9 ± 0.5	1105 ± 57	30.6 ± 0.7
Rose wines (*n = 3*)	2.9 ± 0.6	4.1 ± 0.3	406 ± 13	16.5 ± 0.5
White wines (*n = 5*)	2.6 ± 0.7	3.8 ± 0.4	378 ± 19	15.8 ± 0.6
Sparkling wines (*n = 5*)	1.5 ± 0.5	2.8 ± 0.4	273 ± 15	14.1 ± 0.8
Fortified wine (*n = 8*)	1.4 ± 0.3	2.7 ± 0.4	273 ± 15	11.3 ± 0.9
Grape Juice (*n = 3*)	3.8 ± 0.7	5.2 ± 0.4	880.5 ± 48	22.5 ± 4.0
Nectar Juice (*n = 2*)	2.6 ± 0.7	3.9 ± 0.8	680.5 ± 48	15.5 ± 4.0
Wine brandy (*n = 2*)	0.7 ± 0.4	1.1 ± 0.3	19 ± 11	0



Graphical Abstract

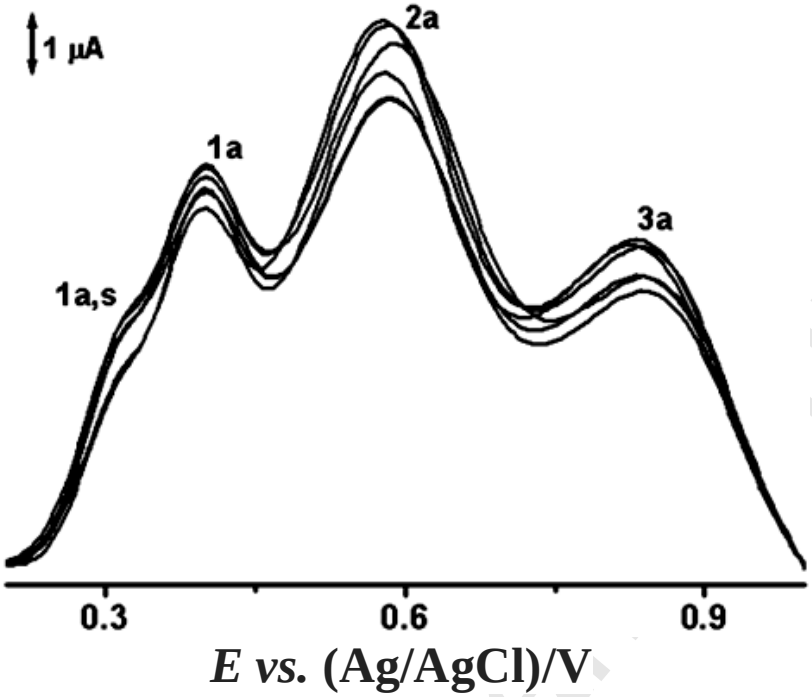


Figure 1. DP voltammograms obtained for 2 mL of red wine in 3 mL of 0.1 M phosphate buffer (pH 5.0) solution characterized at six different carbon paste electrodes (Ø = 2 mm). Other parameters included a pulse width of 5 mV, pulse amplitude of 50 mV, scan rate of 5 mV s^{-1}.

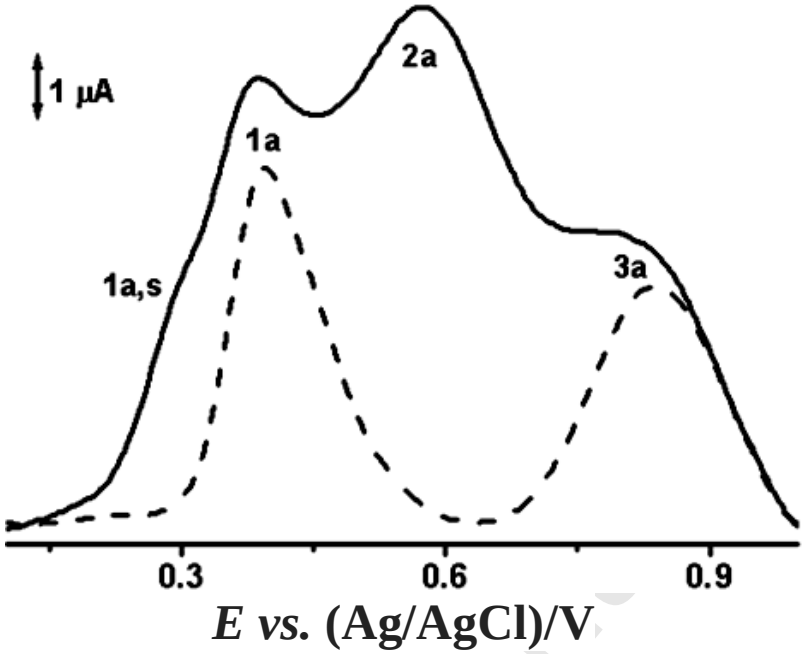
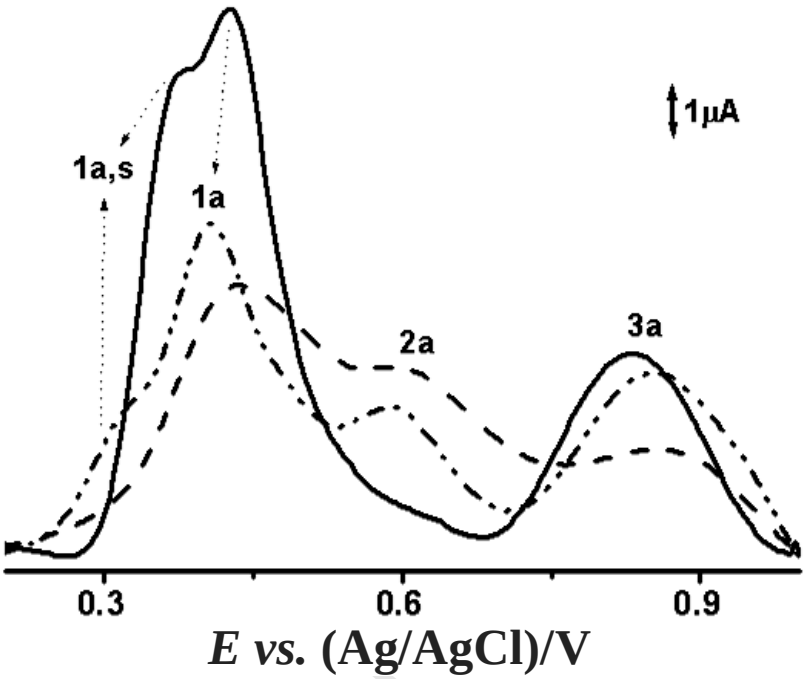


Figure 2. Average DP voltammograms obtained for 2 mL of red (—), n= 25 and white (- - -), n = 5 wines in 3 mL of 0.1 M phosphate buffer (pH 5.0) solution characterized at carbon paste electrodes (Ø = 2 mm). Other parameters included a pulse width of 5 mV, pulse amplitude of 50 mV, scan rate of 5 mV s^{-1}.



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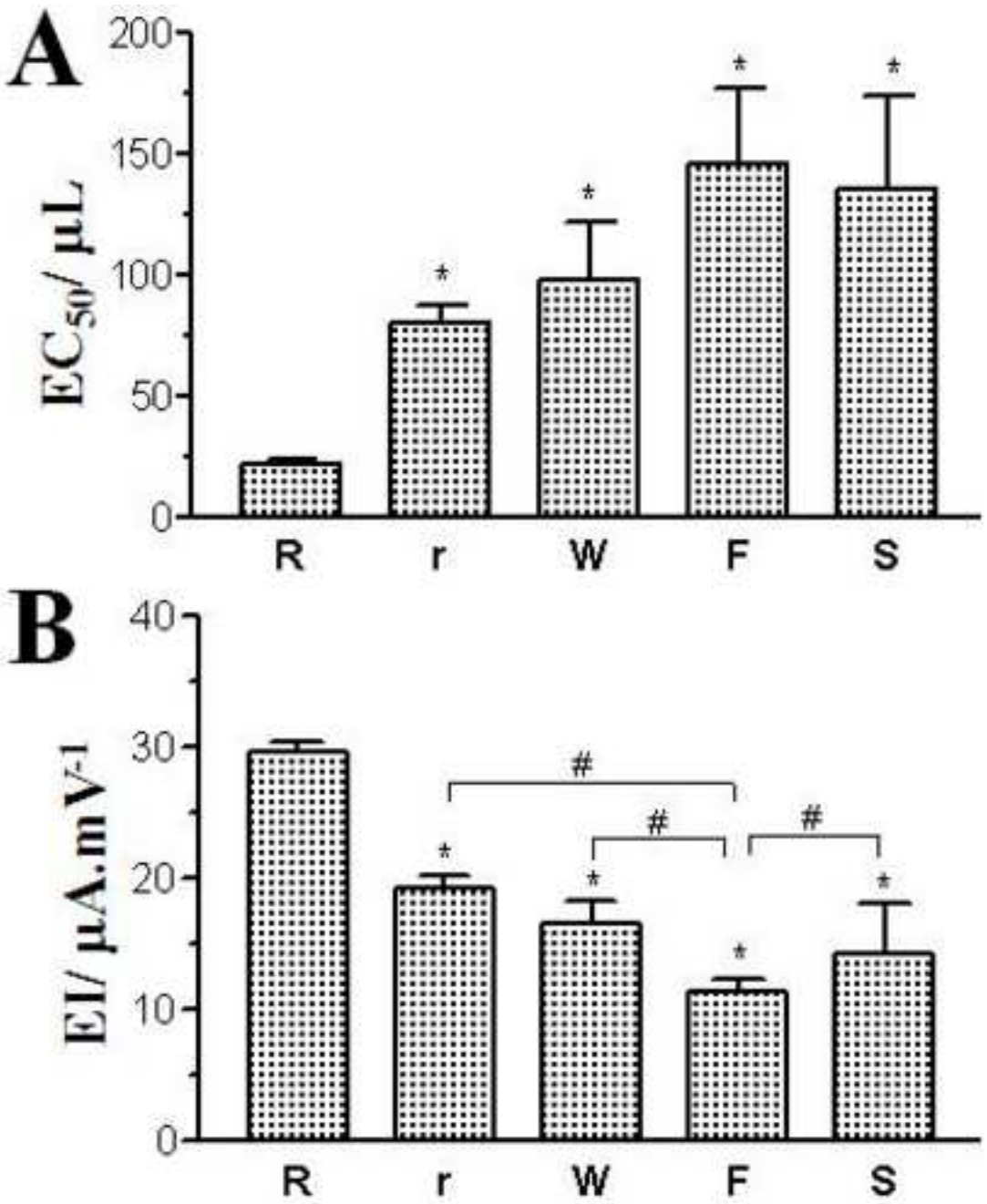

Figure 4. Graphic representation of EC_{50} (A) and EI (B) values for different classes of wines (R = red; r = rose; W = white; F = fortified; S = sparkling). *P<0.001 compared with R. # Denotes difference between the two groups (P<0.05). Note: The EC_{50} represents the amount of wine (in μL) to produce 50% of decolorization of DPPH· relative to the blank control, whereas EI, electrochemical Index, represents the sum of the ratios, I_{pa}/E_{pa}.

Highlights:

. The electrochemical index (EI) is a practical method for determination of antioxidant activity (AOA).

. AOA is a quality criteria for wines and other antioxidant beverages.

. The EI is a useful and complementary approach for AOA determination.