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Voltammetric and spectrometric determination of antioxidant capacity of selected wines

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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.

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 [3–5].

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 [6–8]. 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• is a commercial radical that can be reduced by antioxidant molecules, changing its color from purple to yellow after the reaction, causing a decrease in the absorbance at the wavelength of 517 nm [11–14].

The DPPH• 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• 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•, 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

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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•) and a novel electroanalytical approach (differential pulse voltammetry – DPV) were 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•) 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 $\leq 0.1\ \mu S\ cm^{-1}$) (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 Tables 1–3 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 2 h 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 0.1 M 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.

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[a]	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)

[t](Table wine); [s](sweet wine).
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).

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

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 °C).

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•) radical in DPPH by wine's antioxidants. The ability of samples to scavenge DPPH• radicals was determined by the method of Blois (1958) [10,19,20]. Briefly, to 2.5 mL of DPPH• 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• relative to the blank control.