



Cleberton Correia Santos
(Organizador)

**Estudos Interdisciplinares
nas Ciências e da Terra
e Engenharias 5**

Cleberton Correia Santos
(Organizador)

Estudos Interdisciplinares nas Ciências Exatas e da Terra e Engenharias 5

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APRESENTAÇÃO

O livro “Estudos Interdisciplinares nas Ciências Exatas e da Terra e Engenharias” de publicação da Atena Editora apresenta em seu 5º volume 37 capítulos com temáticas voltadas à Educação, Agronomia, Arquitetura, Matemática, Geografia, Ciências, Física, Química, Sistemas de Informação e Engenharias.

No âmbito geral, diversas áreas de atuação no mercado necessitam ser elucidadas e articuladas de modo a ampliar sua aplicabilidade aos setores econômicos e sociais por meio de inovações tecnológicas. Neste volume encontram-se estudos com temáticas variadas, dentre elas: estratégias regionais de inovação, aprendizagem significativa, caracterização fitoquímica de plantas medicinais, gestão de riscos, acessibilidade, análises sensoriais e termodinâmicas, redes neurais e computacionais, entre outras, visando agregar informações e conhecimentos para a sociedade.

Os agradecimentos do Organizador e da Atena Editora aos estimados autores que empenharam-se em desenvolver os trabalhos de qualidade e consistência, visando potencializar o progresso da ciência, tecnologia e informação a fim de estabelecer estratégias e técnicas para as dificuldades dos diversos cenários mundiais.

Espera-se com esse livro incentivar alunos de redes do ensino básico, graduação e pós-graduação, bem como outros pesquisadores de instituições de ensino, pesquisa e extensão ao desenvolvimento estudos de casos e inovações científicas, contribuindo na aprendizagem significativa e desenvolvimento socioeconômico rumo à sustentabilidade e avanços tecnológicos.

Cleberton Correia Santos

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AVALIAÇÃO DA CAPACIDADE ANTIOXIDANTE DE VINHOS UTILIZANDO TÉCNICAS ELETROANALÍTICAS E ESPECTROFOTOMÉTRICAS

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RESUMO: A composição fenólica e a capacidade antioxidante total dos vinhos portugueses tintos e brancos foram avaliadas por técnicas eletroquímicas e espectrofotométricas. Os diferentes compostos fenólicos presentes nos dezessete vinhos tintos e brancos, com

diferentes castas e de diferentes localizações geográficas, foram identificados e detectados com sucesso. Determinou-se a capacidade antioxidante total dos vinhos, utilizando-se o índice quantitativo eletroquímico (IE), e o método de captura da “concentração eficiente” (CE_{50}) de radicais livres 1,1-difenil-2-picrilhidrazil (DPPH). O vinho com maior capacidade antioxidante total apresentou o menor poder antioxidante, e foi obtida uma correlação muito boa entre os ensaios EI e EC_{50} .

PALAVRAS-CHAVE: Vinhos tintos e brancos, eletrodo de carbono vítreo, compostos fenólicos

EVALUATION OF THE ANTIOXIDANT CAPACITY OF WINES USING ELECTROANALYTICAL AND SPECTROPHOTOMETRIC TECHNIQUES

ABSTRACT: Red and white Portuguese wines phenolic composition and total antioxidant capacity have been evaluated by electrochemical, spectrophotometric. The different phenolic compounds present in the seventeen red and white wines, with different grape varieties, and from different geographical locations, were successfully identified and detected. The wines total antioxidant capacity, using the electrochemical quantitative index (EI), and the method of

capture of diphenilpicrilhydrazil (DPPH) free radical “efficient concentration” (EC_{50}), was determined. The wine with the highest total antioxidant capacity exhibited the lowest antioxidant power, and the EI and EC_{50} assays had a very good correlation. **KEYWORDS:** Red and white wines· glassy carbon electrode· phenolic compounds in vitro, total antioxidant capacity.

1 | INTRODUCTION

The origin of wines in Portugal is more than two thousand years old, with the influence, from Phoenicians, Greeks and Celts, been consolidated by the Romans. The enormous number, over 2₅₀ *Vitis vinifera* Portuguese native grape varieties, a Portuguese treasure, enable the great diversity and the different personalities found in the Portuguese wines. Although each variety has different characteristics, the same grape variety can also produce different wines according with the terroir, soil, topography, and climate, where it is cultivated. The most important red wine varieties being: *Touriga Nacional*, *Baga*, *Castelão*, *Touriga Franca*, *Alfrocheiro*, *Jaen* and *Trincadeira*; and the most important white varieties: *Alvarinho*, *Loureiro*, *Arinto*, *Encruzado*, *Bical*, *Fernão Pires*, *Moscatel* and *Malvasia Fina*.

The red and white Portuguese wine samples natural phenolic composition, include flavonoids, which are ubiquitous in the vegetal kingdom, occurring mainly as secondary metabolites in a wide variety of structures, each one presenting interesting chemical and biological properties, being members of the food antioxidants group. The evaluation of the mechanisms involved in the natural flavonoids’ antioxidant and pro-oxidant role in our daily nutrition is of great relevance.

The flavonoids are effective antioxidants because of their sequestering properties of free radicals and chelate metal ions (KANDASWAMI, C.; MIDDLETON JR, 1994), thus protecting tissues from free radicals and lipid peroxidation. They are the main antioxidants in natural products and the basic structure present an A-ring connected to a benzene B-ring via a heterocyclic pyrene C-ring.

The flavonoids antioxidant properties aim to protect from highly reactive species, mainly the hydroxyl radical ($\cdot OH$) and the superoxide anion ($O_2^{\cdot -}$), which are involved in tissue damage by initiating lipid peroxidation and matrix interstitial disruption (KAHRAMAN, A. et al. 2003). In addition, flavonoids have membrane stabilizing properties, which can affect some intermediary metabolic processes (GALATI, G. et al. 2002). In particular, quercetin sequesters oxygen radicals ($\cdot OH$ and $O_2^{\cdot -}$), inhibits xanthine oxidase and lipid peroxidation, and has iron chelating and stabilizing properties (SORATA, Y., TAKAHAMA, U., KIMURA, M., 1984).

Although flavonoids present important antioxidant properties in the prevention of diseases, studies have demonstrated that, depending on concentration, they also have *in vitro* a pro-oxidant activity. Concentrated extracts of plants rich in flavonoids as green tea leaves, isolated isoflavones from soya beans, and grape seeds, are

widely diffused as nutraceuticals for the treatment of cardiovascular diseases, cancer, and chronic inflammation. Thus, mutagenicity reports based on the oxidative damage caused by flavonoids became of great interest (HEIM, K.E., TAGLIAFERRO, A.R., BOBILYA, D.J. 2002)

The phenolic compounds importance led to the development of analytical methods, such as chemiluminescence, (CHEN, R.L.C., LIN,C.H., CHUNG,C.Y., CHENG,T.J. 2005), spectrophotometry (MAGALHÃES, L.M., SANTOS, M., SEGUNDO, M.A., REIS, S., LIMA, J. L. F. C. 2009), capillary electrophoresis (FERNANDEZ-PACHON M.S., VILANO, D., TRANCOSO, A.M., GARCIA-PARRILLA, M.C. 2006; BERLI, F.; D'ANGELO, J.; CAVAGNARO, B.; BOTTINI, R.; WUILLOUD, R.; SILVA, M. F. 2008)., and chromatography (SPÍNOLA, V.; PINTO, J.; CASTILHO, P.C. 2015; CASSANO, A.; CAIAZZO, F.; DRIOLI, E. 2017) for their determination in different types of samples.

Due to their high sensitivity, voltammetric methods have been successfully used for the investigation of the oxidation mechanism of biological active substituted phenols, providing valuable insights into their redox behaviour and their detection in various samples. The electrochemical characterization of the mostly widespread flavonoids has already been investigated at different carbon electrode materials: glassy carbon, carbon paste, *etc.* (J. B. HE, Y. WANG, N. DENG, X. Q. LIN. 2007; H. P. HENDRICKSON, A. D. KAUFMAN, C. E. LUNTE. 1994) and their oxidation mechanisms were correlated with the electroactive groups: phenol, resorcinol and catechol.

The electrochemical behaviour and “antioxidant capacity” can be linked through the relationship: the lower the oxidation potential the greater the antioxidant power (H. P. HENDRICKSON, A. D. KAUFMAN, C. E. LUNTE. 1994). Therefore, the presence of voltammetric currents at low anodic potentials indicates the presence of polyphenolic compounds of higher antioxidant capacity, while oxidation at higher potentials denotes polyphenolic compounds of low antioxidant activity (ARRIBAS, A.S., MARTÍNEZ-FERNÁNDEZ, M., CHICHARRO, M. 2012).

The electrochemical index (EI) is defined as the total phenolic concentration. The EI is obtained using differential pulse voltammetry, taking into account the compound peak potential (E_p) and peak current (I_p), using the equation [26].

$$EI = (I_{p1} / E_{p1}) + (I_{p2} / E_{p2}) + \dots + (I_{pn} / E_{pn}) \quad (1)$$

The spectrophotometric methods have also been applied for the determination of phenolic compounds total antioxidant capacity, using different compounds: DPPH• (1,1-diphenyl-2-picrylhydrazine) (VILLAÑO, D. ET AL 2007; P. MOLYNEUX. 2004; K. MISHRA, H. OJHA, N. K. CHAUDHURY. 2012)., ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (L. P. LEONG, G. SHUI. 2002; KATALINIC,

V., MILOS, M., KULISIC, T. AND JUKIC, M. 2006) and DMPD (*N,N*-dimethyl-*p*-phenylendiamine) (RAO, P.S.; E. HAYON, E. 1975); AL-ABACHI, W.Q., HADDI, H.; AL-ABACHI, A.M. 2005; ÇEKIÇ, S.M.; AVAN, A.N.; UZUNBOY, S.; APAK, R. 2015).

The DPPH• free radical scavenging assay involves a stable and commercially available radical, and it is a determination easy to perform, that leads to highly reproducible and accurate results. The decrease in absorbance is monitored at $\lambda = 516$ nm and the DPPH• scavenging radical efficiency is calculated using the equation:

$$\text{DPPH}^\bullet \text{ scavenging effect (\%)} = [(A_0 - A_1) / A_0] \times 100 \quad (2)$$

where A_0 and A_1 correspond to the absorbance at $\lambda = 516$ nm of the DPPH• radical in the absence and in the presence of antioxidant, respectively. The total antioxidant capacity is expressed as “efficient concentration” or EC_{50} , representing the amount of extract to produce 50% of decolourization of DPPH• relative to the methanol blank control.

The present work focuses on the evaluation of the phenolic composition and total antioxidant capacity of seventeen wines, three white and fourteen red, from different wine demarcated regions (Controlled Denomination of Origin - DOC), in Portugal, using electrochemical and DPPH• radical scavenging quantification. The phenolic compounds with a glassy carbon electrode (GCE) and electrochemical index (EI) was determined, and compared with the total antioxidant capacity expressed as “efficient concentration” or (EC_{50}), evaluated by DPPH• free radical scavenging assay.

2 | EXPERIMENTAL

2.1 Reagents and solutions

Standard of delphinidin chloride, (-)-epigallocatechin gallate, ferrulic acid, hesperidin, hyperoside, kuromanin chloride, malvidin chloride, morin, quercetin dihydrate, quercetin-3-O-glucopyranoside, peonidin chloride, peonidin-3-O-rutinoside chloride, pellargonidin chloride, procyanidin A2, rutin, and resveratrol, were from Extrasynthese, Genay, France. Catechin hydrate, 2,2-diphenyl-1-picrylhydrazyl, and methanol (99.8 %), were from Sigma-Aldrich, Germany, and formic acid (98-100 %) was from Merck, Darmstadt, Germany.

All solutions were prepared using analytical grade reagents and purified water from a Millipore Milli-Q system (conductivity $\leq 0.1 \mu\text{S cm}^{-1}$) and the experiments were performed at room temperature, $25 \pm 1^\circ\text{C}$.

The volumes were measured using Pipetman pipettes, from Gilson, France. The pH measurements were performed with a CRISON 2001 micro pH-meter, with an Ingold-combined glass electrode, from Spain. The ultrasound bath used was from

Bandelin Sonorex, Germany. The microwave was a Miele, M625EG, Germany.

2.2 Methods, instruments and cells

The electrochemical cell was a VT-03 flow cell from Antec Leyden, Zoetewoude, Netherlands, in a three-electrode wall-jet configuration: a glassy carbon working electrode (GCE) with 2 mm diameter, an in situ Ag/AgCl reference electrode and a stainless steel auxiliary electrode. The in situ Ag/AgCl reference electrode, referred as ISAAC (in situ **Ag/AgCl**) is in direct contact with the mobile phase that contains 2 mM chloride ions (2 mM KCl). There is a difference of + 0.2 V between the potential of the Ag/AgCl (saturated KCl) reference electrode and the ISAAC reference electrode in contact with 2 mM KCl. Thus, for an experiment running at + 0.7 V vs. Ag/AgCl with saturated KCl, the potential setting using ISAAC should be + 0.5 V. All measurements were performed at the applied cell potential of $E_{ap} = + 0.5 \text{ V vs. ISAAC}$ ($E_{ap} \sim + 0.7 \text{ V vs. Ag/AgCl}$ (saturated KCl) reference electrode). The photodiode array detection (PDA) experimental conditions were $190 \text{ nm} < \lambda < 600 \text{ nm}$.

2.2.1 Electrochemistry

Differential pulse (DP) voltammetric experiments were carried out using an Ivium CompactStat potentiostat, Ivium, The Netherlands. Measurements were carried out using a glassy carbon working electrode (GCE) with 1 mm diameter, a platinum wire counter electrode and an Ag/AgCl (3 M KCl) reference electrode, in a 1 mL one compartment electrochemical cell (eDAQ Products, Poland).

In order to ensure reproducible results, the GCE was submitted to a cleaning procedure before each electrochemical assay, consisting in polishing with diamond spray (particle size 1 μM , Kement, Kent, UK). After polishing, the GCE was rinsed thoroughly with Milli-Q water, placed in buffer supporting electrolyte and various DP voltammograms were recorded until a constant baseline voltammogram was obtained. The experimental conditions for DP voltammetry were: pulse amplitude 50 mV, pulse width 100 ms, step potential 2 mV, and scan rate $\nu = 5 \text{ mV s}^{-1}$.

2.2.2 Spectrophotometry

The absorbance measurements were recorded on a U-2810 Spectrophotometer Digilab® Hitachi with UV Solutions Program. The experimental conditions for absorption spectra were: scan speed 400 nm/min, sampling interval 1.50 nm, and path length 1 cm. All UV-Vis spectra were measured for $200 < \lambda < 800 \text{ nm}$.

2.3 Sample preparation

Seventeen wine samples, from different red and white wine demarcated Portuguese regions (Controlled Denomination of Origin - DOC), were purchased from

local stores in Portugal, stored in the freezer at -20°C , and the analyses carried out within a few days. The wine samples were diluted to 2, 4, 6, 8 and 10% concentrations. These concentrations were further diluted in the UV-Vis cuvette due to the addition of the DPPH \cdot solution and/or methanol during absorbance measurements resulting in 11 different concentrations which varied from 0.13 to 1.7 %. Each wine sample was diluted (1:1) in the mobile phase A for the DP voltammetric measurements.

2.4 UV-Vis spectrophotometry and DPPH \cdot assay

The radical scavenging activity of different fractions of wine, based on the conversion (decolourization) of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH \cdot) radical by wine antioxidants, was measured. The ability of samples to scavenge DPPH \cdot radicals was determined by the Brand-Williams assay [28].

The DPPH \cdot stock solution was prepared daily, and a concentration of 90 μM , corresponding to 0.9 absorption at $\lambda = 516 \text{ nm}$, was used. Eleven different concentrations of each wine, have been assayed in order to check the response linearity, and to establish the antioxidant capacity in the suitable linear range. Briefly, to 2.5 mL of DPPH \cdot methanolic solution, an aliquot of 0.5 mL of methanol blank control was added in order to reach a final volume of 3.0 mL. This procedure was repeated for all analytical samples. The reaction solution was incubated for 15 min in the dark at room temperature and measured at $\lambda = 516 \text{ nm}$. The total antioxidant capacity was expressed as “efficient concentration” or EC_{50} , representing the amount of extract to produce 50 % of decolourization of DPPH \cdot relative to the methanol blank control.

3 | RESULTS AND DISCUSSION

The aim of this work was the evaluation of the phenolic composition and total antioxidant capacity of seventeen wines, from different wine demarcated regions (Controlled Denomination of Origin - DOC), in Portugal, electrochemical and DPPH \cdot radical scavenging measurements. The phenolic composition, was carried out. The voltammetric method (EI) was used for the determination of the total antioxidant capacity, as well as the DPPH \cdot radical scavenging due to the correlation found between oxidation peak potential and EC_{50} . This methods are fast and cheap and allowed making the measurements under a variety of experimental conditions.

3.1 Electrochemical characterization of wine samples

The electrochemical behaviour of phenolic standard compounds was also investigated for control and the results obtained are summarized in **Table 1**.

Standard flavonoid compounds	Ep (V)			
	Peak 1	Peak 2	Peak 3	Peak 4
1-Delphinidin Chloride	-	0.5	0.6	0.9
2-Malvidin Chloride	-	0.5	0.85	-
3-Catechin	-	0.45	0.81	-
4-(-)-Epigallocatechin gallate	-	0.6	-	0.95
5-(-)-epicatechin	0.2	0.45	0.85	-
6-Kuromanin chloride	-	0.68	0.77	0.94
7-Peonidin-3-O-glucoside chloride	-	0.55	0.75	0.9
8-Ferulic acid	0.45	0.69	0.75	-
9-Procyanidin A2	-	-	0.7	0.95
10-Hyperoside	-	0,58	-	-
11-Quercetin-3-O-glucopyranoside	-	0.57	-	-
12-Rutin	0.31	0.36	-	1.01
13-Resveratrol	0.21	0.52	0.88	1.035
14-Fisetin	-	0.52	0.85	-
15-Morin	0.25	0.45	-	-
16-Quercetin dihydrate	0.23	0.47	0.58	-
17-Perlagodin Chloride	0.53	0.57	0.9	1.07
18-Peonidin Chloride	-	0.5	0.9	-
19-Hesperidin	0.3	0.5	-	-

Table 1: Standard phenolic compounds oxidation peak potentials, using GCE, obtained by DP voltammetry.

In order to evaluate the total antioxidant capacity, the electrochemical behaviour of red and white wine samples, in pH = 2.2, in order to maintain the flavylum cation at a higher concentration, by DP voltammetry, at a GCE, was investigated, Figure 1. The DP voltammetric assays, for each wine sample, under the same experimental conditions as for catechin, were carried out, Figure 2.

The wine oxidation peaks, in the potential range from 0.4 to 0.8 V, are independent of the wine type, corresponding to the oxidation of various polyphenolic compounds with catechol groups in the B-ring of their structure, flavonoids such as catechin, epicatechin, etc., Table 1, as well as, some phenolic acids, such as caffeic and ferulic acids. The oxidation peaks in the potential range from 0.6 to 0.9 V, for red wines, Figure 1A, correspond to the anthocyanins oxidation, as well as the second oxidation peak of catechin and aldehydes.

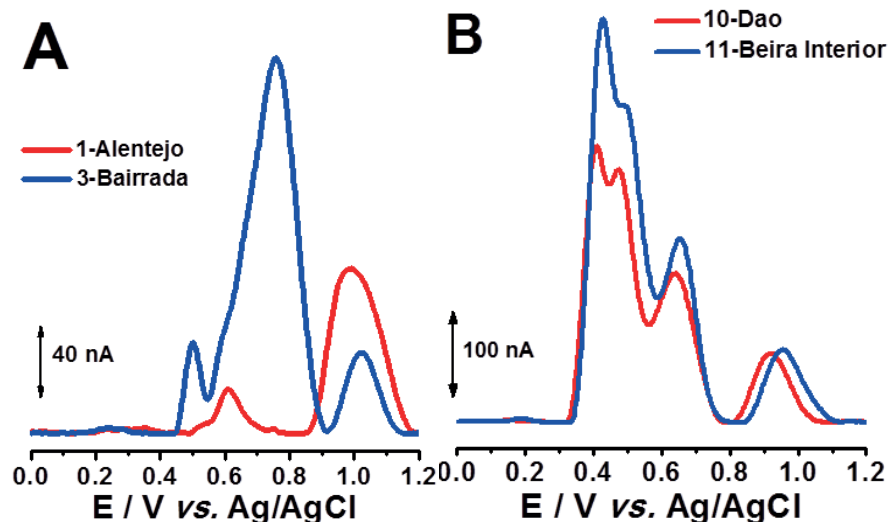


Figure 1. DP voltammograms in (A) white and (B) red wine samples, dilution (1:1), at GCE, in pH = 2.2. Scan rate 5 mV s⁻¹.

The first peak for white wines and the second peak for red wines are due to the oxidation of polyphenols with higher oxidation potentials, some phenolic acids (p-coumaric and vanillic acids). However, the wine oxidation peaks occurring from 0.8 to 0.9 V are due to flavonoids and tannins that can be oxidized, as well as, the hydroxyl group at position 3 in the flavonoids C-ring.

In the wines DP voltammograms three anodic peaks, were observed, Figure 1. In the case of red wines, the first oxidation peak current was much higher than for the white wines, this being in agreement with the catechin standard first oxidation peak, P_{1a}, Figure 2. The first oxidation peak current was used to quantify the red wines total antioxidant capacity. For red wines the second and third oxidation peak current, are related to other antioxidants present in each different grape varieties, and the currents were lower.

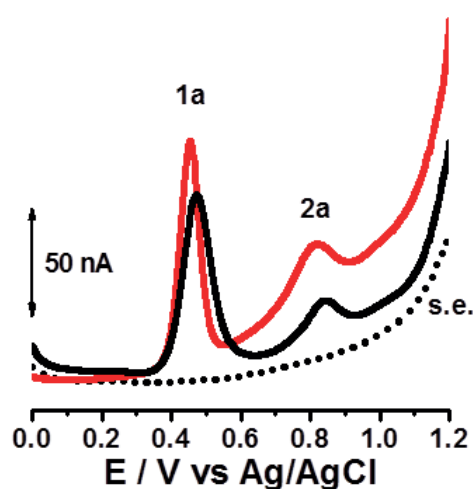


Figure 2: DP voltammograms in 10 μ M catechin, at GCE, in pH = 2.2: (-) first scan (-) second scan, and (•••) supporting electrolyte (s.e), scan rate 5mV s⁻¹.

In the case of white wines the highest oxidation peak occurred at a more positive potential than for red wines, and with a oxidation peak current three times lower, when compared to the first red wines oxidation peak currents, in agreement with white wines less total antioxidant capacity than red wines, Table 2. A comparison between the oxidation peak potentials obtained for the phenolic standards, Table 1, and those obtained for the red and white wines, Table 2, showed a correlation between oxidation peak potentials of the wine polyphenols and the phenolic standards.

Samples	Year	DOC Region	Ep (V)				EC50 (%)	EI (nA/mV)
			Peak 1	Peak 2	Peak 3	Peak 4		
Catechin	-	-	-	0.45	0.81	-	1.039 x 10 ⁻³ mg/mL	0.206
1	2010	Alentejo	0.59	0.69	-	-	0.55	0.317
2	2012	Alentejo	-	0.59	0.68	1.03	0.53	0.475
3	2013	Bairrada	0.36	0.6	-	1.01	0.56	0.280
4	2010	Douro	0.45	0.53	0.69	0.95	0.43	0.615
5	2012	Douro	0.48	0.54	0.77	1.03	0.40	0.110
6	2013	Douro	0.44	-	0.70	0.97	0.37	0.146
7	2008	Dão	0.44	0.50	0.68	0.94	0.37	0.403
8	2010	Dão	0.41	0.47	0.64	0.92	0.37	1.851
9	2011	Dão	0.39	0.44	0.70	0.95	0.41	0.998
10	2012	Dão	0.42	0.48	0.65	0.91	0.39	2.610
11	2008	Beira Interior	0.40	0.48	0.68	0.95	0.37	0.297
12	2011	Lisboa	0.48	0.54	0.77	1.0	0.37	0.622
13	2013	Lisboa	0.38	0.45	0.62	0.89	0.36	0.965
14	2010	Alentejo	0.38	0.43	0.69	0.94	0.49	1.066
15	2011	Alentejo	0.51	0.58	0.73	1.04	0.42	0.203
16	2012	Alentejo	-	0.47	0.66	0.89	0.45	0.647
17	2013	Alentejo	0.45	0.52	0.69	0.94	0.49	0.644

Table 2. Oxidation peak potentials of catechin standard, white and red wines, using GCE, by DP voltammetry, efficient concentration (EC₅₀) obtained by the DPPH• free radical assay, and electrochemical quantitative index (EI).

3.2 Spectrophotometric characterization of wines samples

The total antioxidant capacity of the different red and white wines has been determined using the DPPH• free radical scavenging assay, for different extract concentrations, ranging from 0.13 to 1.7 %, and at different incubation times, from 0 to 15 minutes. Measuring the DPPH• absorbance decrease after 15 min incubation, for each wine concentration, the EC₅₀ was calculated, and the results were compared with the catechin standard, Table 2. The results obtained for red and white wines, for different concentrations, showed an increase of antioxidant capacity, and a decrease in the radical absorbance till completion, Figure 3, Table 2.

The red wines presented the lowest radical scavenging ability, corresponding

to the lowest EC_{50} , Table 2. On the other hand, the EC_{50} and the EI values differ within red wines due to variables, such as: grape varieties, DOC regions, terroir, alcohol content of wines, etc. It is very interesting finding that the three Dão wines consistently presented high EI values, as well as one Lisboa and one Alentejo wine. The three white wines presented the highest radical scavenging ability, with an $EC_{50} \sim 0.55$, Figure 3A, Table 2, corresponding to the lowest total antioxidant capacity.

In the case of the DPPH• assay, the results represented the concentration of wine, which contained the amount of antioxidants necessary for neutralization of 50% of DPPH•. The electrochemical results gave information about the total antioxidant capacity [38].

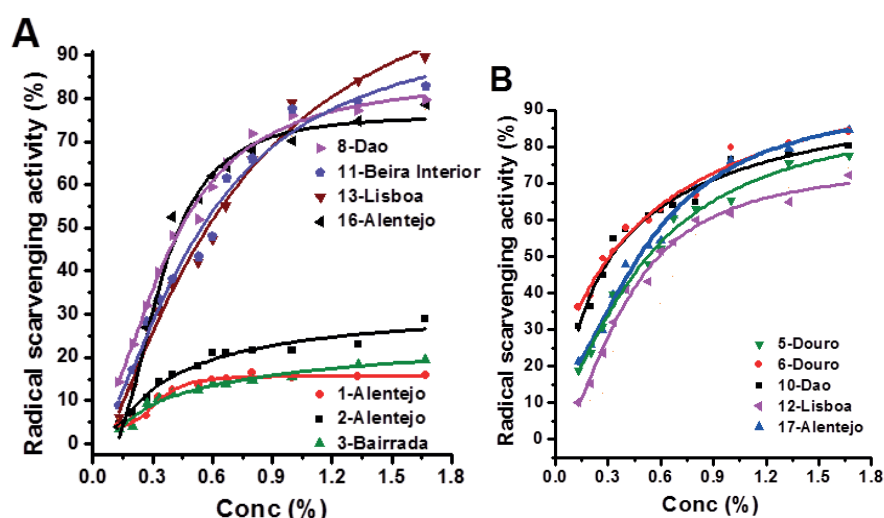


Figure 3. DPPH radical scavenging activity (%) versus wine samples concentrations (%).

4 | CONCLUSIONS

The Portuguese red and white wines total antioxidant capacity, by the electrochemical quantitative index (EI), and “efficient concentration” (EC_{50}), showed that the total antioxidant capacity of white wines is much lower compared with that of red wines, corresponding to the white wines lower content in phenolic compounds.

These results showed the excellent electrochemical detection sensitivity and the method suitability for the detection of low levels of electroactive phenolic compounds in red and white wines, and for the electrochemical determination of the total antioxidant capacity.

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