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Electrochemical determination of the antioxidant capacity of Brazilian woods as alternative materials for the aging of cachaça

Determinação eletroquímica da capacidade antioxidante de madeiras brasileiras como materiais alternativos para o envelhecimento de cachaça

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Summary

Brazilian flora presents a vast biodiversity with great chemical and pharmacological potentialities. Particularly, the local industries of distilled spirits (*cachaça*) are actively searching for a type of wood that can substitute the oak in the aging process of those beverages. The great challenge is to find a wood with a phenolic content similar to oak and, at the same time, can furnish analogous sensorial characteristics to the final product. As a possible attempt, the antioxidant capacity of some local woods can be measured as a preliminary indicative of its phenolic composition. Therefore, the antioxidant capacity of four samples of Brazilian woods extracts, namely, cabreúva (*Myrocarpus frondosus*), cabreúva-vermelha (*Myroxylon balsamum*), imbuia (*Octea porosa*) and pequi (*Caryocar brasiliense*), as well as, of the traditionally used oak (*Quercus* sp.) were determined using the recently developed CRAC (ceric reducing/antioxidant capacity) assay. The CRAC methodology uses chronoamperometric measurements to monitor the diminishing of the Ce^{4+} species concentration, using the Cottrell equation before and after four minutes of reaction between each antioxidant sample and the oxidant solution (Ce^{4+}). Due to the high redox potential of the Ce^{4+}/Ce^{3+} couple, this system allows measurements for the majority of the known antioxidant molecules using a boron-doped diamond film as the cathode. The experimental results are commonly expressed in terms of Trolox Equivalent (TE), a non-dimensional value of the relative antioxidant capacity. In the present study, the antioxidant hierarchy was determined as being: oak (1.73) > cabreúva-vermelha (1.05) > cabreúva (0.90) > imbuia (0.71) > pequi (0.31). These results show that among the analyzed woods, the cabreúva-vermelha stands out with an antioxidant capacity closer to the oak and might become a proper alternative after the necessary sensorial tests are carried out. They also show that the CRAC assay is a powerful tool in the determination of the antioxidant capacity of real samples.

Key words: *Antioxidant capacity; Brazilian woods; Cachaça; Chronoamperometry, CRAC assay.*

Resumo

A flora brasileira apresenta uma vasta biodiversidade com grandes potencialidades químicas e farmacológicas. Particularmente, as indústrias locais de destilados (cachaça) estão procurando ativamente por um tipo de madeira que possa substituir o carvalho no processo de envelhecimento dessas bebidas. O grande desafio é encontrar uma madeira com um conteúdo fenólico similar ao do carvalho e que, ao mesmo tempo, possa fornecer características sensoriais análogas às do produto final. Como uma possível alternativa, a capacidade antioxidante de algumas madeiras locais pode ser avaliado como um indicativo preliminar de sua composição fenólica. Portanto, a capacidade antioxidante de quatro amostras de extratos de madeiras brasileiras, isto é, cabreúva (*Myrocarpus frondosus*), cabreúva-vermelha (*Myroxylon balsamum*), imbuia (*Octea porosa*) e pequi (*Caryocar brasiliense*) assim como a do carvalho (*Quercus* sp.) tradicionalmente usado, foram determinadas empregando-se o recentemente desenvolvido ensaio CRAC (ceric reducing/antioxidant capacity). A metodologia CRAC usa medidas cronoamperométricas para monitorar a diminuição na concentração das espécies Ce^{4+}, empregando a equação de Cottrell antes e após quatro min de reação entre cada amostra antioxidante e a solução oxidante (Ce^{4+}). Devido ao elevado potencial redox do par Ce^{4+}/Ce^{3+}, esse sistema permite determinar a maioria das moléculas antioxidantes conhecidas usando um filme de diamante dopado com boro como cátodo. Os resultados experimentais são comumente expressos em termos de Trolox Equivalent (TE), um valor adimensional da relativa capacidade antioxidante. No presente estudo, a hierarquia antioxidante foi determinada como sendo: carvalho (1,73) > cabreúva-vermelha (1,05) > cabreúva (0,90) > imbuia (0,71) > pequi (0,31). Esses resultados mostram que, dentre as madeiras analisadas, a cabreúva-vermelha destaca-se com uma capacidade antioxidante próxima a do carvalho e pode ser uma alternativa apropriada após necessários testes sensoriais serem realizados. Eles também demonstraram que o ensaio CRAC é uma ferramenta poderosa na determinação da capacidade antioxidante de amostras reais.

Palavras-chave: *Capacidade antioxidante; Madeiras brasileiras; Cachaça; Cronoamperometria; Ensaio CRAC.*

1 Introduction

Sugar-cane spirit, locally known as *cachaça*, is the most typical Brazilian alcoholic beverage. It is produced from the fermentation of sugar cane (*Saccharum officinarum*) followed by aging during at least one year (in agreement with the Brazilian legislation) in wood casks. Its production is around 1.3 billion L per year of which less than 1% is destined for exportation (BOSCOLO et al., 1995; FARIA et al., 2003).

Due to the well-known and valuable characteristics such as its high antioxidant capacity, oak casks have been extensively and preferentially employed for the aging process (MOSEDALE and PUECH, 1998). In Brazil, however, since no sufficient oak is available, an alternative is to use native woods. As there is a growing interest among Brazilian distillers in improving the quality of *cachaça*, efforts have been directed towards increasing the knowledge of its chemical composition (NASCIMENTO et al., 1997, 1998; BETTIN et al., 2002; CARDOSO et al., 2004; SOUZA et al., 2007). A detailed knowledge of the chemical composition of *cachaça* should provide the necessary background for technological improvements and new regulations for quality control.

As a consequence, the antioxidant capacity of *cachaça* mainly deriving from the wood employed in the aging process is a parameter that contributes in a significant way to the characterization of that product. Moreover, this parameter indirectly informs on the presence of several phenolics compounds with antioxidant action.

The use of electroanalytical techniques for the determination of the antioxidant capacity of compounds contained in woods is of fundamental importance since the process of maturation and aging of the *cachaça* is characterized by changes in the color of the distillate. This fact severely limits the use of spectrophotometric techniques such as the ferric ion reducing antioxidant power (FRAP), the ABTS (2,2´-azino-bis(3-ethyl-benzthiazoline 6-sulfonic acid) and the DPPH (1,1-diphenyl-2-picrylhydrazyl) assays, among others (MILARDOVIC et al., 2005, 2007) for the determination of antioxidant capacity.

Similarly to the spectrophotometric assays, the use of electroanalytical techniques also involves a single electron transfer between a given oxidant and the antioxidant present in the sample, as follows: oxidant (ox) + e^- (from antioxidant) → reduced ox + oxidized antioxidant

Thus, a decrease of the initial oxidant concentration reflects the antioxidant's reducing capacity. At the present time, several different oxidants have been used for the determination of antioxidant capacities.

However, Ce^{4+} is the most powerful oxidant agent in acid medium (H_2SO_4 0.5 mol.L^{-1}) because have a redox couple Ce^{4+}/Ce^{3+} at high potentials (1.29 V *versus* Ag/AgCl, KCl 3.0 mol.L^{-1}), superior to the potential observed for the main antioxidant assays (ABTS – 0.90 V and FRAP – 0.92 V).

This high potential allows a proper reactivity with the majority of the antioxidants currently under investigation and also guarantees that any oxidation product of the antioxidants compounds could be reduced together with the Ce^{4+} (FERREIRA and AVACA, 2008a). The Ce^{4+} was used as oxidant by Ozyurt et al. (2007) in a spectrophotometric assay denominated CeRAC (ceric ion reducing antioxidant capacity). At the same time, Ferreira and Avaca (2008a, b) developed a chronoamperometric assay denominated CRAC (ceric reducing/antioxidant capacity) which was successfully applied for the determination of the antioxidant capacity of several substances in ethanol standard solutions and for industrialized fruit juices samples.

Therefore, this study describes the use of a new simple electron transfer methodology (the CRAC assay) for the determination of the antioxidant capacity of four Brazilian woods extracts (cabreúva, cabreúva-vermelha, imbuia and pequi) and oak extracts, used as standard. It was observed that the antioxidant capacity of cabreúva-vermelha was higher than the other Brazilian wood extracts and near to the detected in an oak extracts, thus suggesting a potential application of this Brazilian wood in the aging of *cachaça*.

2 Material and methods

2.1 Chemicals and solutions

Chemicals of high purity, without any pre-treatment, were used in the experiments. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a water-soluble analogue of vitamin E, was from Aldrich Chemical Co. and cerium (IV) sulfate tetrahydrate ($Ce(SO_4)_2.4H_2O$) from E. Merck.

An ethanolic stock solution of Trolox was freshly prepared before the measurements in the concentration of 4.25 × 10^{-3} mol.L^{-1}. Sulfuric acid 97.99% (H_2SO_4) from Mallinckrodt was used as supporting electrolyte at a concentration of 0.5 mol L^{-1} while a 1.01 × 10^{-3} mol.L^{-1} stock solution of $Ce(SO_4)_2.4H_2O$ in 0.5 mol.L^{-1} H_2SO_4 (CRAC reagent) was used as oxidant. All solutions were prepared using double deionized water (18.2 MΩ – cm 25 °C) from Millipore – MilliQ system (USA).

Four certified Brazilian wood species, namely, cabreúva (*Myrocarpus frondosus*), cabreúva-vermelha (*Myroxylon balsamum*), imbuia (*Octea porosa*) and pequi (*Caryocar brasiliense*), were provided by the *Instituto de Pesquisas Tecnológicas* (IPT-USP, São Paulo, Brazil) and by the *Laboratório de Estruturas de Madeira* at the *Universidade de São Paulo* (EESC-USP, São Carlos, Brazil). An oak sample (*Quercus* sp.) that was used for

comparison was provided by the Department of Bioscience at Strathclyde University (Glasgow, Scotland).

A non-aged sugar-cane spirit without sugar addition certified and provided by *Indústrias Müller de Bebidas Ltda.* (Pirassununga-SP, Brazil) was used as solvent for the extraction procedure (ethanol extracts ~40% v/v). Dry wood sawdust (air-dried) was extracted in non-aged sugar-cane spirit, in a ratio of 0.010 g of wood sawdust per mL, by shaking for 26 days at ambient temperature (25 ± 3 °C). Samples were protected against light throughout the process (CARDOSO et al., 2008).

2.2 Apparatus and instrumentation

Electrochemical experiments were carried out in one-compartment Pyrex® glass cell (30 mL) provided with three electrodes and degassing facilities for bubbling N_2. The reference system was the Ag/AgCl (3.0 mol.L^{-1} KCl) electrode and the counter one was a 2 cm^2 Pt foil. The working electrode was a boron-doped diamond (BDD) single-faced plate with an exposed area of 0.36 cm^2 and final boron content of the order of 8000 ppm. The BDD films were produced by Adamant Technologies S.A., La Chaux-de-Fonds, Switzerland, on silicon wafers using the hot-filament chemical vapor deposition (HF/CVD) technique. Prior to the experiments, the BDD electrode received a pre-treatment at +3.0 and −3.0 V during 15 and 45 s, respectively, in a H_2SO_4 (0.5 mol.L^{-1}) solution to ensure reliable and reproducible results (SUFFREDINI et al., 2004).

2.3. Methodology

Preliminary assays using cyclic voltammetry was carried out to characterize the systems under investigation. Thus, for each wood extract, 50 µL of sample were added to 10 mL of supporting electrolyte (H_2SO_4 0.5 mol.L^{-1}) and then cycled in the range between −0.7 V and 1.8 V (100 mV.s^{-1} scan rate) after 10 min of deoxygenation with N_2.

As described by Ferreira and Avaca (2008a), the CRAC assay consists in the monitoring of the decrease in the initial concentration of Ce^{4+} species after four minutes of reaction with the added antioxidant by means of chronoamperometric measurements. The results were correlated with the antioxidant/reductant capacity through of the Cottrell Equation 1 (BARD and FAULKNER, 2001):

$$I_t = \frac{nFAC_O^{\circ}D_O^{1/2}}{\pi^{1/2}t^{1/2}} = bt^{-1/2} \quad (1)$$

where: I_t is the instantaneous current at time t, n is the number of electrons involved in the process, F is the Faraday's constant, A is the electrode area, C°_O is the bulk concentration of the electroactive species (normally an oxidized entity) and D_O its diffusion coefficient.

Chronoamperometric results are commonly analyzed by means of the linear relationship existing between I and $t^{-1/2}$ (Cottrell lines) which, in turn, have a slope (b) directly proportional to the remaining bulk concentration of the Ce^{4+} species for a given antioxidant sample. Keeping all other parameters constant, the slope variation for experiments with different antioxidants will reveal the variations in the remaining Ce^{4+} concentration and consequently the relative antioxidant capacity. Meanwhile, for the later purpose it is necessary to know the mathematical dependence of the Cottrell slope (b) with the remaining Ce^{4+} concentration. Thus, chronoamperometric measurements under the same experimental conditions were initially carried out in solutions containing up to 1 × 10^{-3} mol.L^{-1} Ce^{4+} in H_2SO_4 0.5 mol.L^{-1}.

For a more precise comparison between different samples, the variation of the Cottrell slope (b) after the addition of different amounts of a given sample can be plotted against the volume of the sample, resulting in a new linear curve. A regression equation of this new curve can be used in conjunction with the one described above for the determination of the concentration of Ce^{4+} remaining in solution or the Ce^{3+} produced by the reaction, thus reflecting more accurately the reduction capacity of the antioxidant. These capacities can be eventually expressed as Trolox Equivalent (TE) after measurements carried out with such substance using the same procedures.

Chronoamperometric assays were carry out using 10 mL of CRAC reagent which were initially deoxygenated with N_2 for six minutes. Later, 50 µL of wood extract was added to the mixture keeping the N_2 stirring for additional four minutes (reaction time). At this point, the measured open circuit potential for the system (E_{OCP} ~ 1.29 V) was applied to the electrode for two seconds and then stepped to the reduction potential (E_{RED} = 0.8 V) where the variation of the current with time was recorded during 10 s. The same procedure was carried out with a 50 × 10^{-6} mol.L^{-1} Trolox solution used as standard in the determination of the Trolox equivalent (TE). All measurements were performed using a model PGSTAT30 potentiostat/galvanostat from AUTOLAB connected to a personal computer for data collection and analysis. The results were obtained in triplicate and analyzed by variance analysis ($p \leq 0.05$). All data were processed by using the program Microcal Origin® 7.5.

3 Results and discussion

The preliminary characterization of the systems was carried out by cyclic voltammetry on the BDD electrode in a H_2SO_4 0.5 mol.L^{-1} aqueous solution. All the experimental results are shown in Figure 1 where curve 1 is the response of the pure oxidant (1 × 10^{-3} mol.L^{-1} Ce^{4+} in H_2SO_4

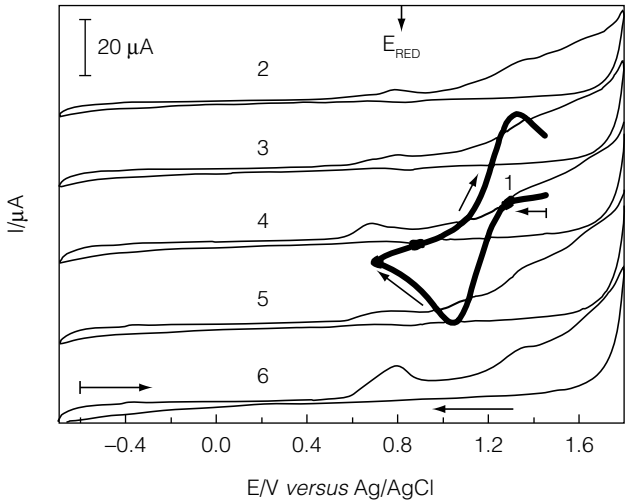

Figure 1. Cyclic voltammograms recorded at 100 mV.s^{-1} on BDD for 1) the oxidant (1 × 10^{-3} mol.L^{-1} Ce^{4+} in H_2SO_4 0.5 mol.L^{-1}) and for 50 μL of wood extracts: 2) pequi, 3) imbuia, 4) cabreúva, 5) cabreúva-vermelha and 6) oak.

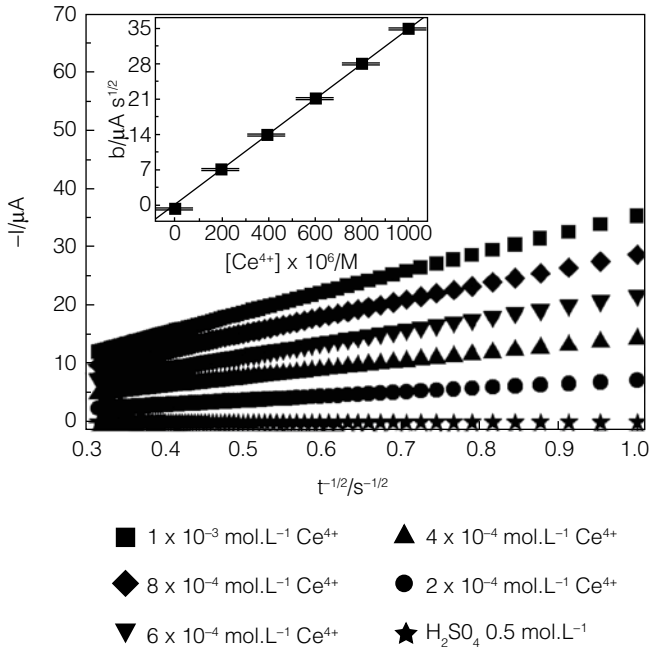

Figure 2. Data plotted as I *versus* t$^{-1/2}$ (Cottrell lines) recorded for the supporting electrolyte (H_2SO_4 0.5 M) and for Ce^{4+} solutions at different concentrations. Inset: values of the slopes (b) for the Cottrell lines plotted against Ce^{4+} concentration.

0.5 mol.L^{-1}) displaying both the reversibility of the system as well as the potential region (E_{RED} = 0.8 V) used for the chronoamperometric determination of the remaining Ce^{4+} ions after reaction with the different antioxidant samples. Figure 1 also shows the irreversible voltammetric response for the five samples of wood extracts under investigation, namely, pequi, imbuia, cabreúva, cabreúva-vermelha and oak (curves 2 – 6, respectively). Note from this figure that the reduction process of the oxidant taking place at 0.8 V, after the reaction between the oxidant and each extract sample, will not be affected by the oxidation products from the extract samples.

The mathematical dependence of the Cottrell slope (b) with the Ce^{4+} concentration is shown in Figure 2. This figure display the actual Cottrell lines obtained from the current decays recorded for Ce^{4+} solutions at different concentrations while the inset of Figure 2 exhibit the values of the slopes plotted against Ce^{4+} concentration.

The mathematical expression obtained by regression analysis for the values of the slopes acquired from the inset of Figure 2 for b in μA.s$^{1/2}$ and [Ce^{4+}] in molarity (mol.L^{-1}) is (Equation 2):

$$b_{exp} = (0.19 \pm 0.10)\ \mu A.s^{1/2} + (3.46 \times 10^4 \pm 1.11 \times 10^2)\ \mu A.s^{1/2} / mol.L^{-1} \times [Ce^{4+}]\ mol.L^{-1} \quad (2)$$

where b_{exp} is the experimental value of Cottrell slope and [Ce^{4+}] is the concentrations of Ce^{4+} species in solution.

The relative CRAC value for each antioxidant sample corresponds to the concentration of Ce^{3+} formed after a fixed reaction time between pre-established amounts of the oxidant (Ce^{4+}) and the antioxidants present in the samples followed by comparison with a standard material (e.g. Trolox). This can be achieved using the experimental value of the Cottrell slope for a given antioxidant sample (b_{AO}) together with Equation 2 and the initial value of the oxidant (Ce^{4+}) concentration, namely, 1 × 10^{-3} mol.L^{-1}. All this information can be collected in the Equation 3 shown below:

$$CRAC\ value([Ce^{3+}]/mol.L^{-1}) = 1 \times 10^{-3} - \frac{b_{AO} - 0.19}{3.46 \times 10^4} \quad (3)$$

The Trolox equivalent (TE) is obtained from a simple relationship between the CRAC value determined for a given antioxidant sample and that measured for Trolox under the same experimental conditions (Equation 4):

$$TE = \frac{CRAC\ Value_{AO}}{CRAC\ Value_{Trolox}} \quad (4)$$

The CRAC value for each sample was calculated from the Cottrell slope (b_{AO}) obtained from the experiments, where the pure solution of Ce^{4+} reacted for 4 min with an aliquot of 50 μL of the wood extracts. The set of Cottrell lines obtained experimentally are shown in Figure 3 together with that corresponding to the Ce^{4+} alone.

The CRAC value for each extract as well as the corresponding Trolox equivalent (TE) were calculated using the slope values measured from Figure 3 with Equations 3 and 4 and are listed in Table 1. For the pure solution of Ce^{4+}, the slope calculated was $b_{Ce(IV)}$ = 34.78 ± 0.03 μA.s$^{1/2}$.

The TE values presented in Table 1 can be used to make a comparison among the samples. From these

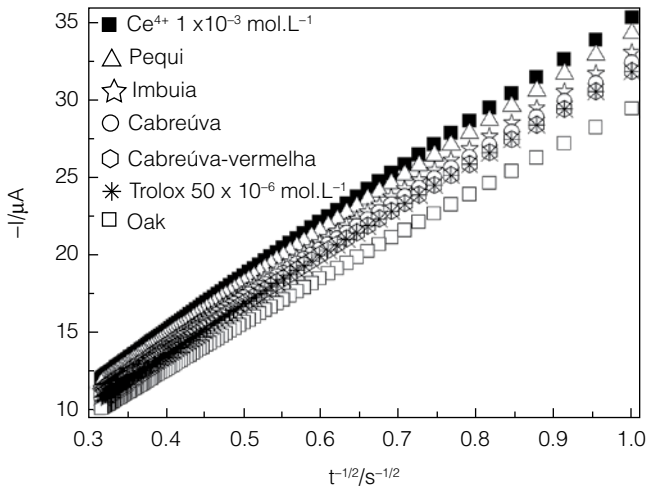

Figure 3. Dependence of I with $t^{-1/2}$ (Cottrell lines) before and after the addition of the different antioxidant extract samples.

Table 1. Cottrell slopes, CRAC values and TE values for the addition of 50 μL of wood extracts and 50 × 10^{-6} mol.L^{-1} of the standard antioxidant (Trolox).

Sample	b_{AO} (μA.$s^{1/2}$)	CRAC value × 10^6 ([Ce^{3+}]/mol.L^{-1})	TE
Trolox	31.36 ± 0.26	99.23 ± 7.61	1.00 ± 0.08
Pequi	33.72 ± 0.06	30.82 ± 1.59	0.31 ± 0.02
Imbuia	32.35 ± 0.12	70.61 ± 3.45	0.71 ± 0.04
Cabreúva	31.69 ± 0.19	89.69 ± 5.55	0.90 ± 0.05
Cabreúva-vermelha	31.19 ± 0.02	104.14 ± 0.44	1.05 ± 0.01
Oak	28.83 ± 0.18	172.25 ± 5.07	1.73 ± 0.05

results it is possible to observe that, from all the Brazilian wood tested, cabreúva-vermelha presents the higher antioxidant capacity which was somewhat closer to that of the oak extract. This effect is mainly due to the high phenolic content present in this species (OLIVEIRA et al., 1978). In contrast, the pequi extract presents an antioxidant capacity almost six times lower than the obtained for the oak extract. At the end of the assays the following classification was obtained for the antioxidant capacity of the wood extract samples analyzed: oak > cabreúva-vermelha > cabreúva > imbuia > pequi.

4 Conclusions

The chronoamperometric assays have shown that among the Brazilian wood analyzed, the cabreúva-vermelha specie stands out with an antioxidant capacity closer to the oak. This suggests that, for Brazil, this wood could be a good substitute for the already spread oak barrels at least in relation to its phenolic content, as revealed by its antioxidant capacity.

On the other hand, previous studies (FERREIRA and AVACA, 2008b) and the results of this investigation have proved that the CRAC assay is a powerful tool for the determination of the antioxidant capacity of real samples, mainly due to the fact that the chronoamperometric assays do not suffer interference by turbidity and/or the color of the samples. In addition, the CRAC assay is a simple, fast, and cheap method for the determination of antioxidant capacity.

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