Int. J. Electrochem. Sci., 13 **(2018)** 5433 – 5439, doi: 10.20964/2018.06.55

International Journal of ELECTROCHEMICAL SCIENCE
www.electrochemsci.org

Short Communication

Electrochemical Study of Commercial Black Tea Samples

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Received: 8 February 2018 / *Accepted:* 25 March 2018 / *Published:* 10 May 2018

Black tea (BT) is a widely consumed natural product whose myriad of therapeutic applications rely on its constituents of remarkable antioxidant power. The electroanalysis is unique to provide a precise redox characterization of samples presenting a single compound or even a pool of electroactive species. Hence, this work aims to study the BT redox profile through electroanalytical methods. Voltammetric techniques such as Ciclic Voltammetry, Square Wave Voltammetry and Differential Pulse Voltammetry were conducted in BT crude vegetal mater samples and pharmaceutical grade standardized dried extract. It was found for all BT samples two anodic processes, the first occurring at peak potentials bellow +0.5V, thus indicating a strong antioxidant activity. The results related to BT pool compounds voltammetric profile suggested that the first anodic process is reversible, whereas the second irreversible. In addition, such electron/proton transfer reactions undergo EC mechanisms, which are mostly diffusion controlled electrochemical processes. Since low anodic potential and reversible redox behavior are related to high reducing power and good regenerating ability, it can be inferred, that the recognized antioxidant activity of BT samples is undeniable.

Keywords: Free radical scavenging; black tea; redox characterization.

1. INTRODUCTION

Black tea (BT) is a *Camellia sinensis* based product whose myriad of therapeutic applications rely on its antioxidant activity. The main phytocomponents present in BT are polyphenols, whose electroactive moieties promote reactive oxygen species scavenging. These plant secondary metabolites set up a reductive cascade, which prevents lipid peroxidation leading up to a plethora of health benefits, being these features the main endorsers of BT nutraceutical use. Antioxidant activity in natural compounds such as BT is associated to phenolic phytochemical markers, whose presence is

mainstream in plant material. These compounds act in synergism to promote the therapeutic effect associated to this species, and their antioxidant activities are henceforth product of a complex pool of plant secondary metabolites [1-2].

Different tools are available to assess foodstuff antioxidant activity. Amongst them are methods such as spectrophotometry and electrochemical techniques, whose principles differ considerably. Although both methods display good reproducibility concerning pure samples, colorimetry based tests such as spectrophotometry are susceptible to a myriad of interferences when plant analysis is concerned. Since many plant secondary metabolites display chromophores, any colorimetric assay is unreliable to such assessment [3-4]. Therefore, electrochemical methods such as voltammetry are unique, as they allow precise sample antioxidant activity quantification without the drawbacks associated to spectrophotometry [5-6]. Moreover, voltammetry allows the inference on antioxidant activity quality, a parameter of upmost importance when foodstuff nutraceutical use is concerned [3-7].

Through voltammetry, the assessment of redox kinetics in a single component and/or components pool is feasible, leading to a better comprehension on sample antioxidant feature. Nonetheless, such methods can provide valuable information on redox processes occurrence and reversibility, whose data can be correlated to specific constituents, aiding overall chemical characterization [8-9]. Furthermore, voltammetric assessment of plant material allows quality and authenticity evaluation at a low cost, which in turn sheds light on the versatility displayed by electrochemistry towards natural products analysis [3-4].

Due to BTs importance in folk medicine and natural products trade, many literature reports were concerned on the assessment of its antioxidant activity instead of antioxidant quality. Therefore, this work focused on the electrochemical study of *C. sinensis* products in order to provide more information about BT redox kinetics and the quality of its antioxidant activity.

2. EXPERIMENTAL

2.1. Samples and Reagents

Two BT samples comprising crude vegetal mater from different distributors, namely BT1 and BT2 were collected from a local market in Goiânia, Goiás State, Brazil. The collected samples were assessed on their quality and found out to be in accordance to legislation. In order to compare the aforementioned samples, a *C. sinensis* pharmaceutical grade dried extract was purchased at a local pharmacy and also analyzed. All electrolyte salts, solvents and reagents were of analytical grade. Gallic acid and catechin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The standard solutions of gallic acid and catechin were prepared at the concentration of 1mM in Milli-Q water.

Electrolyte solutions were prepared with double distilled Milli-Q water (conductivity ≤ 0.1 μS cm^{-1}) (Millipore S. A., Molsheim, France). Alumina solution from Arotec S/A Ind. e Comércio was used to polish the glassy carbon electrode's surface between assays in order to further allow reproducibility.

2.2. BT Sample preparation

1.8g of BT plant material was macerated in 10 mL analytical grade ethanol, the macerated was extracted for 15 minutes by sonication at room temperature (25^{o}C) and then filtered. 100µL of this solution was mixed with 2 mL of each buffer solution (pH 4.0, 5.5, 7.0, 8.5, 10.0) for voltammetric assays.

2.3. Extract Preparation

A suitable amount of *C. sinensis* dried extract (60-80 mesh) was weighed (1,0 g) then solubilized in 10 mL of ethanol. The crude extract was centrifuged at 1000 rpm. Then 1 mL (supernatant) of the sample was diluted in 9 mL of water to reach out to 1% ethanolic extract. 100µL of this final solution was thereby used for voltammetric analysis.

2.4. Voltammetric assay

Voltammetric experiments were carried out in a potentiostat/galvanostat Autolab III® integrated to the GPES 4.9® software, Eco-Chemie, Utrecht, Netherlands. The measurements were performed in a 2.1 mL one-compartment/three-electrode system electrochemical cell consisting of glassy carbon electrode (GCE) 1.0 mm^2 area, a Pt wire and Ag/AgCl/KCl$_{sat}$ electrode (Lab solutions, São Paulo, Brazil), representing the working electrode, the counter electrode and the reference electrode, respectively.

The experimental conditions for cyclic voltammetry (CV) were: scan rate of 100 mVs^{-1} and scan range from 0 to 1.25 V. The experimental conditions for Square Wave Voltammetry (SWV) were: pulse amplitude 50 mV were frequency (f) 50 Hz and a potential increment of 2 mV, corresponding to an effective scan rate (v) of 100 mVs^{-1}. The experimental conditions for Differential Pulse Voltammetry (DPV) were: pulse amplitude 50 mV, pulse width 0.5 s and scan rate 10 mVs^{-1}. The voltammetric assays were performed in 0.1 M phosphate buffer solution (PBS), at different pH (4.0, 5.5, 7.0, 8.5, 10.0).

All experiments were performed in triplicates, DP voltammograms were background-subtracted and baseline-corrected to provide better data visualization, and all data was analyzed and treated with Origin 6® software.

3. RESULTS AND DISCUSSION

3.1. CV Results

BTs main phytochemical markers are phenolic compounds, which are therefore electroactive. This feature turns voltammetric techniques optimal for their analysis, however such natural products are complex samples, whose myriad of plant secondary metabolites present unique voltammetric

profiles. Therefore, CV was employed in order to preliminary assess BT redox profiles. The results can be seen in Figure 1.

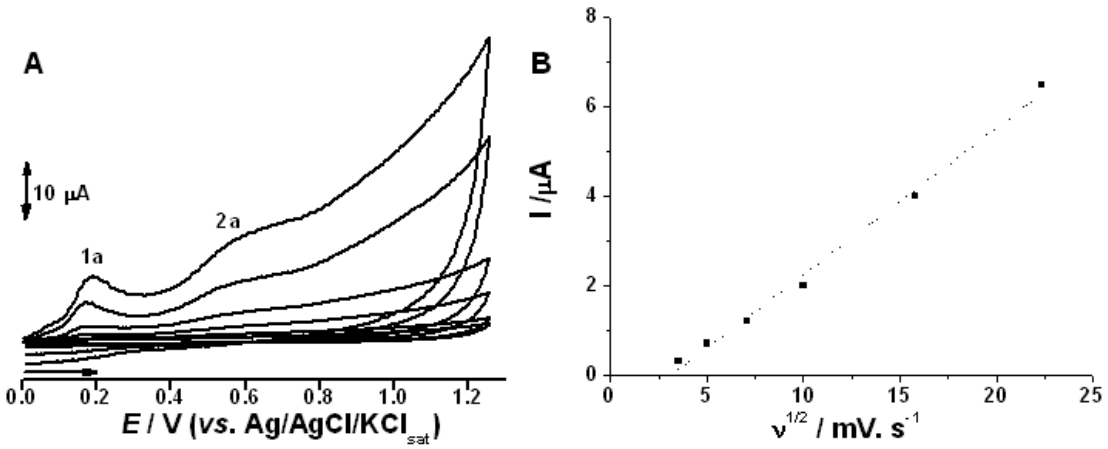

Figure 1. A. CV voltammograms of BT at different scan rates (500, 250, 100, 50, 25, 12.5 mVs^{-1}) scan range from 0 to 1.3 V. **B.** Plot of peak current (*I*) vs scan rate square root ($v^{1/2}$) obtained from first anodic process (1a), determination coefficient (r^2) = 0.983. All analysis carried out in 0.1 M PBS, pH 7.0 at GCE.

All BT samples presented almost identical features through analysis, thus this case study is herein represented by a single/pool sample named as BT. Through CV analysis two anodic peaks (I_{pa}) can be seen at anodic potentials (E_{pa}) of approximately +0.19V (1a) and +0.58V (2a) (Figure 1.A). These peaks are correlated to the oxidation of compounds present in the sample. The first I_{pa} (1a) is possibly correlated to the presence of phenolic compounds among sample constituents, this is further corroborated by literature, which attributes to polyphenols the oxidation peak values bellow to near +0.5V [11-12]. The graphical depiction of *I* vs $v^{1/2}$ presented linear dependence (r^2 = 0.983), therefore indicating that the electrochemical processes in sample are mostly diffusion controlled (Figure 1.B) [8].

It is noteworthy to mention that E_{pa} bellow +0.5V are correlated to the thermodynamic feasibility of the reducing process, while high amplitude I_{pa} implicates higher concentration of electroactive species or higher electron transfer kinetics. Due to endogenous antioxidants undergoing oxidation at potentials above +0.5V, the presence of low E_{pa} values and high amplitude I_{pa} implicate that the analyte or compounds pool could be able to reduce endogenous antioxidants, henceforth enabling them to once again exert their free radical scavenging properties [9-13].

3.2. SWV and DPV Results

SWV and DPV were conducted in order to identify possible reversibility in the oxidation peaks observed through CV and better evaluate pH influence in the detection of compounds present in sample. Results are displayed in Figure 2.

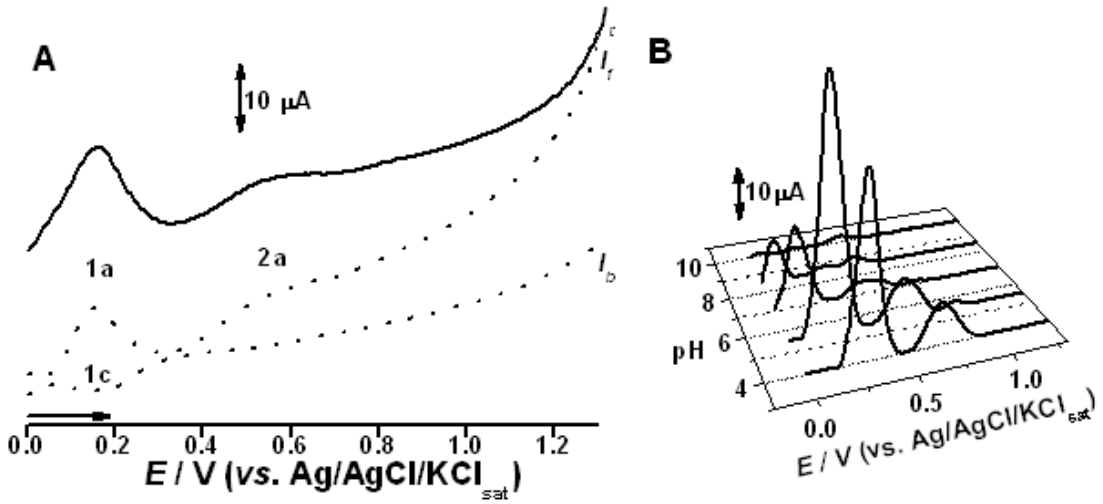

Figure 2. **A.** SW voltammograms of BT1. Pulse amplitude 50 mV, frequency of 50 Hz and a potential increment of 2 mV, corresponding to an effective scan rate (v) of 100 mVs^{-1}. Analysis carried out in 0.1 M PBS, pH 7.0. **B.** DP voltammogram of BT samples at different pH (4.0, 5.5, 7.0, 8.5, 10.0). Pulse amplitude 50 mV, pulse width 0.5 s and scan rate 10 mVs^{-1}. Analysis carried out in 0.1 M PBS, at aforementioned pH at GCE.

The figure 2A shows a cathodic peak at peak potential akin to anodic peak 1a. On the other hand, the peak current ratio (I_{pa}/I_{pc}) was superior to 1, which can be attributed to the complex nature of such crude samples (Figure 2.A). In fact, some phenolic compounds present variable tendency to undergo electrochemical oxidation followed by chemical reactions, namely electrochemical polymerization, leading to insulating film at electrode surface [14]. In turn, the 2a peak is irreversible hence no associated cathodic peak can be seen. Since redox processes of organic species are often associated to proton transfer, a pH study was undertaken in order to evaluate voltammetric profile. Different buffers were used, namely at pH 4.0, 5.5, 7.0, 8.5 and 10.0. Results showed that media pH influences the potential associated to oxidation peaks (Figure 2.B), this indicates gradual deprotonation of the compounds pool [15]. Furthermore, I_{pa} amplitude is also influenced by pH, wherein pH 5.5 presented highest amplitude, hence was this value used in further DPV analysis.

3.3. Standards and Samples Comparison

The redox profile of the pool of chemical species in BT agrees with the antioxidant appeal of such dietary natural product. Thus, the DP voltammograms of some phenolic constituents, namely catechin and gallic acid, as well as, of *C. sinensis* dried extract were performed in order to establish further correlations (Figure 3).

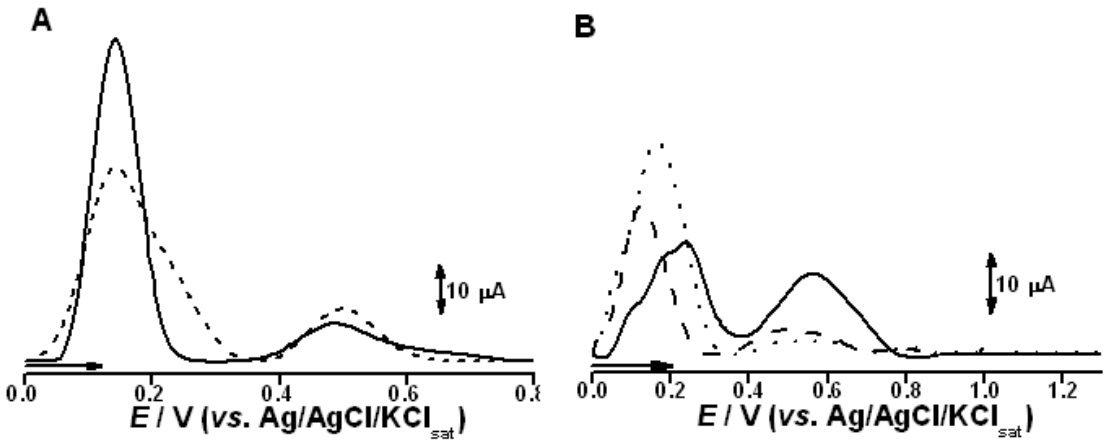

Figure 3. A. DP voltammogram of polyphenol Standards: catechin (▬); gallic acid (▪▪▪). Pulse amplitude 50 mV, pulse width 0.5 s and scan rate 10 mVs^{-1}. Analysis carried out in 0.1 M PBS, pH 5.5 at GCE. **B.** DP voltammogram of dried *C. sinensis* extract and BT samples: *C. sinensis* extract (data normalized to 0.8) (▬); BT1 (▪▪▪) and BT2 (−−). Pulse amplitude 50 mV, pulse width 0.5 s and scan rate 10 mVs^{-1}. Analysis carried out in 0.1 M PBS, pH 5.5 at GCE.

The voltammetric profiles of the selected standards were quite similar to those exhibited by the *C. sinensis* samples (Figure 3.A). This result is in consonance with literature, which reports catechin and gallic acid as BT phytocomponents. Nonetheless, the presence of such compounds may also shed light on BT antioxidant power, since these compounds are strong free radical scavengers. Furthermore, BT samples presented almost identical voltammetric profiles, whereas the dried extract presented small shoulder peaks on the first I_{pa} (Figure 3.B). These shoulder peaks can be attributed to a different phytochemical profile present in the extract, which can be moreover correlated to its botanical origin or extract production. The results hint a potential use of voltammetry as a cheap tool to preliminary assess BT samples authenticity, however, more studies must be undertaken in order to evaluate reproducibility [16-17].

4. CONCLUSION

This work reported the redox processes assessment in *C. sinensis* samples. The crude vegetal samples tested, namely BT1 and BT2 presented almost identical voltammetric profiles, and their analysis suggested that the first anodic process is reversible, whereas the second irreversible. In addition, an electrochemical catalytic mechanism can be attributed to the first redox pair, which therefore indicates that electron transfer is coupled with preceding or follow-up chemical reactions during sample analysis. The low E_{pa}, high I_{pa} amplitude and reversible redox behavior correlates to high reducing power and good regenerating ability, which justifies BT known antioxidant activity.

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