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Analytical Methods

Electroanalytical tools for antioxidant evaluation of red fruits dry extracts

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ABSTRACT

Red fruits are rich sources of antioxidant compounds with recognized health benefits. Since they are perishable, dried extracts emerge as more durable products and their quality control must include antioxidant capacity assays. In this study, the redox behavior of commercial dried products obtained from camu-camu, açai, acerola and cranberry red fruits was evaluated by electroanalytical approaches. The antioxidant potential was determined by 2,2-diphenyl-1-picrylhydrazyl free radical assay and the electrochemical index concept. The total phenol content was estimated by using a laccase based biosensor. A significant correlation was found between all methods and literature data. The voltammetric profile (cyclic, differential and square wave) obtained for each type of dried extract showed distinguishable features that were correlated with their main major markers, being also useful for identification purposes. The electrochemical methods were cheaper and more practical for evaluation of antioxidant properties and total phenol content in dried powders obtained from different red fruits.

1. Introduction

The consumption of natural antioxidants is associated with a delay in aging processes, as well as with the prevention of degenerative diseases. Therefore, the search for sources of dietary antioxidants (Escarpa, 2012; Oliveira-Neto et al., 2016) has focused on red fruits that contain large amounts of polyphenols and vitamins with antioxidant activity (Jakobek, Šeruga, Novak, & Medvidović-Kosanović, 2007).

The fruits are usually processed in many ways, in order to improve their stability, because they rapidly spoil and their production is far from the target consumers (Zotareli, Porciuncula, & Laurindo, 2012). One of the most efficient methods to extend the stability of natural products is to remove their water content, since dehydration lessens deleterious chemical and biochemical processes (Lüle & Koyuncu, 2015; Zotareli et al., 2012). However, the heating based drying methods can also provoke decomposition, mainly in the case of thermally unstable compounds, which can include many antioxidants can be enrolled (Fujita, Borges, Correia, Franco, & Genovese, 2013).

In order to preserve the chemical and biological properties of these products, some analytical procedures must be performed. Considering that many biological properties of red fruits products are due to their antioxidants compounds with radical scavenging activity, a number of analytical protocols have been established with the aim to evaluate the antioxidant capacity of these substances, which include spectrometric methods such as DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2′-Azinobis-3-ethylbenzo thiazoline-6-sulfonic acid, diammonium salt), ORAC (Oxygen radical absorbance capacity) assays, measure of total phenols by Folin-Ciocalteu method (Mishra, Ojha, & Chaudhury, 2012; Oliveira-Neto et al., 2016), chromatographic determination (Huber & Rupasinghe, 2009; McDonald, Prenzler, Antolovich, & Robards, 2001), and evaluation of redox behavior by means of electrochemical approaches that provide an antioxidant profile (Blasco, González, & Escarpa, 2004; González, Vidal, & Tzanov, 2009; Kilmartin, Zou, & Waterhouse, 2002; Makhotkina & Kilmartin, 2009; Rebelo, Rego, Ferreira, & Oliveira, 2013). In addition, is possible to calculate the electrochemical index value, which is a reliable measure associated with the antioxidant potential (Escarpa, 2012; Lino et al., 2013; Oliveira-Neto et al., 2016).

Also, selective biosensors have been developed for the quantification of specific compounds or for determination of major compounds with similar structures. The laccase, superoxide dismutase (SOD) and DNA based biosensors have been used for total phenol content and antioxidant potential determinations (Barroso et al., 2011; Campanella, Bonanni, Finotti, & Tomassetti,

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2004; Escarpa, 2012; Mello, Hernandez, Marrazza, Mascini, & Kubota, 2006). The laccase performs the biochemical oxidation followed by electrochemical reduction in a biosensor, which is detected by amperometric means (Garcia et al., 2015). While the DNA-Biosensor is based in protection DNA against oxidative damage, the lower the decrease of former anodic peaks, the higher the protection (Campanella et al., 2004). In turn, SOD are based on the selective enzymatic dismutation of $O^{\cdot -}$ into O_2 and H_2O_2 followed by sensitive amperometric detection (Mourad, Barsan, Dridi, Ben Ali, & Brett, 2016).

This study was performed with the aim to evaluate the electrochemical approaches as a tool for quality control and redox characterization of natural products. The antioxidant and redox profile of red fruits crude extracts were characterized by means of cyclic voltammetry (CV), square wave voltammetry (SWV) and differential pulse voltammetry (DPV). The antioxidant potential expressed by electrochemical index (EI) was compared to results obtained with the DPPH method. Finally, a Laccase based biosensor was employed in order to determine the total phenol content of natural products here studied.

2. Materials and methods

2.1. Reagents

Ethanol, DPPH and the other HPLC solvents were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All electrolyte solutions were prepared by using analytical grade salts, which were diluted in double distilled Milli-Q water (conductivity $\leqslant 0.1\ \mu S\ cm^{-1}$) (Millipore S. A., Molsheim, France). The phenolic antioxidants, rutin, gallic acid, catechin, hesperidin, caffeic acid, peonidin-3-O-glucoside, quercetin were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Sample Stock Solution

The commercial dried products (powder) obtained from red fruits (RF) dried extracts (DEs), namely acerola (AC1, AC2 and AC2), açai (A1 e A2), camu-camu (CC1 and CC2) and cramberry (CR1 and CR2) were purchased from local pharmacies.

A suitable amount of each sample (50 mg) was weighed and then extracted with 5 mL of ethanol by sonication for 30 min, in order to reach 1% ethanol extract. The crude extracts were centrifuged at 1000 rpm, and 1 mL of the supernatant was diluted with 4 mL of a diluting solution prepared using 2 mL of ethanol and 2 mL of 0.1 M phosphate, pH 5.0, thus getting 0.2% RFDE solutions. The aliquots of 250 μL of this final solution were added to 2.5 mL of electrolyte, in order to reach 0.02% RF-DE for all voltammetric assays. In order to compare the antioxidant power of each class of RFDE a pool constituted by the same amount of each supplier (N = 2 or 4) was used.

2.3. Electroanalytical assays

Voltammetric experiments were carried out with a potentiostat/galvanostat μAutolab III® integrated to the GPES 4.9® software, Eco-Chemie, Utrecht, The Netherlands. The measurements were performed in a 5.0 mL one-compartment electrochemical cell, with a three-electrode system consisting of a carbon paste electrode, a Pt wire and the Ag/AgCl/KCl 3 M (both purchased from Lab solutions, São Paulo, Brazil), representing the working electrode, the counter electrode and the reference electrode, respectively. The experimental conditions for DPV were: pulse amplitude 50 mV, pulse width 0.5 s and scan rate 10 mV s^{-1}. The experimental conditions for SWV were: pulse amplitude 50 mV, were frequency 50 Hz and a potential increment of 2 mV, corresponding to an effective scan rate of 100 mV s^{-1}. The experimental conditions for cyclic voltammetry (CV) were: scan rate of 100 mV s^{-1} and scan range from 0 to 1 V. The DP voltammograms were background-subtracted and baseline-corrected, and then all data were analyzed and treated with the software Origin 8®.

All experiments were done at room temperature (21 ± 1 °C) in triplicate (n = 3) and the main electrolyte used was the 0.1 M phosphate buffer solution (PBS), pH 5.0.

2.3.1. Electrochemical index

The electrochemical index (*EI*) concept was first proposed by Escarpa group (Blasco, Rogerio, González, & Escarpa, 2005; Escarpa, 2012) taking into account to the main voltammetric parameters, peak potential (E_{pa}), and peak current (I_{pa}). Based on the fact that the lower the E_{pa} (thermodynamic parameter), the higher is the electron donor ability, and the higher the I_{pa} (kinetic parameter), the higher is the amount of electroactive species, *EI* was calculated using the following equation (Lino et al., 2013):

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

In which I_{pan} and E_{pan} correspond to current and potential value for each anodic peak, 1a, 2a ...na, observed in the DP voltammograms.

2.3.2. Laccase based biosensor (LBB)

A laccase crude extract, *Pycnoporus sanguineus* (CCT-4518), with 2019 $U.L^{-1}$ obtained as ascribed in previous work (Garcia et al., 2015) was used to prepare the optimized LBB. Briefly, 250 μL of enzymatic crude extract was mixed with 100 mg of graphite and left to dry at room temperature for 6 h. Then, 30 mg of mineral oil, the agglutinating agent was added and rigorously mixed in order to achieve a homogenous paste. This final paste was used to fill the cavity, that was 2 mm in diameter and 0.5 mm in depth within the electrode support, namely a Teflon cylinder with a central hole overpassed by brass wire, the electrical contact.

2.4. DPPH assay

The radical scavenging activity assays were performed using the stable DPPH· reagent, in accordance with very well established procedures (Oliveira-Neto et al., 2016). In summary, the blank control was composed by a mixture of and 2.7 mL of DPPH· ethanolic solution (0.1 mM) and 0.3 mL of ethanol, in which the final absorbance at = 517 nm was of *c.a.* A = 0.7. The ethanol was used in order to adjust the baseline (A = 0.000). Antioxidant activity was expressed as EC_{50}, representing the amount (L) of sample solution to produce 50% of decolorization of DPPH· relative to the blank control after five minutes of reaction.

The measurements were carried out by using a UV–vis spectrophotometer (Quimis, model Q-798U2VS, Brasil). All samples were analyzed in a 1 cm glassy cell length at room temperature.

2.5. Statistical analysis

Statistical analysis was performed using GraphPad Prism version 6.0 (GraphPad Software, San Diego, CA, USA). Comparisons among groups were made using ANOVA. Post hoc comparisons were performed using Tukey's comparison test. The significance level considered was 0.05.