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Antioxidant capacity of mango fruit (*Mangifera indica*). An electrochemical study as an approach to the spectrophotometric methods

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ABSTRACT

The antioxidant capacity in mango (pulp, peel, and seed) was measured using spectrophotometric and electrochemical methods in order to make traditional methods comparable with electrochemical ones, using reference standards Gallic Acid and Trolox. ABTS, DPPH, Total polyphenols, and electrochemical index were evaluated. In order to present the electrochemical results in a more comparable way, the voltammetric charge (using Differential pulse voltammetry) was used. Spectrophotometric methods allowed to determine the difference in contents of metabolites with antioxidant capacity, except in peel and seed, while the electrochemical method separated the three extracts and is not affected by interferences. Spectrophotometric methods present a good correlation with electrochemical methods, using the same reference standards, therefore, a better comparison between methods is possible.

1. Introduction

The determination of the antioxidant capacity in fruits, vegetables, and beverages for their potential application as functional foods that complement the normal diet is an analysis of interest for the food industry (Bigliardi & Galati, 2013; Pradeep & Guha, 2011). Antioxidant capacity is usually determined using methods based on synthetic radical capture and spectrophotometric monitoring (UV–Visible) (Alam, Bristi, & Rafiquzzaman, 2013; Huang, Boxin, & Prior, 2005; Oroian & Escriche, 2015). These methods are characterized by the use of unfriendly reagents with the environment, the need to perform a pretreatment to the samples, and long reaction times.

Another disadvantage that must be taken into account, is the interference of substances that can reduce the chromogenic compounds, which leads to an overestimation of the antioxidant capacity that can affect the results (Escarpa, 2012; Oliveira-Neto et al., 2016; Singleton, Orthofer, & Lamuela-Raventós, 1999). Likewise, in food matrices there may be substances that absorb in the same wavelength range at which the spectrophotometric experiment is carried out, affecting the accuracy of methods such as total phenols (Escarpa, 2012; Lino et al., 2014; Souza, Calegari, Zarbin, Marcolino-Júnior, & Bergamini, 2011). In contrast, the use of electrochemical techniques as an indication of the total reducing power of the sample, allows for quicker, simpler measurements without the use of expensive reagents and with reduced sample pretreatment (Hoyos-Arbeláez, Vázquez, & Contreras-Calderón, 2017; Lino et al., 2014).

The electrochemical index (EI) concept was first proposed by the Escarpa group (Blasco, González, & Escarpa, 2004; Escarpa, 2012), by taking into account the voltammetric parameters: the anodic peak potential (E_{pa}) and the anodic peak current (I_{pa}) independently. Thus, since the lower is the potential (thermodynamic parameter), the higher is the electron donor ability; likewise, the higher is the peak current (kinetic and concentration parameter), the higher is the electron transfer rate and/or the number of electroactive species (Makhotkina & Kilmartin, 2009). The determination of the EI is based on measuring the current and potential of each anodic peak in a differential pulse voltammetry, and perform a summation of the i/E ratio using the following equation:

$$EI = \frac{I_{pa1}}{E_{pa1}} + \frac{I_{pa2}}{E_{pa2}} + \cdots + \frac{I_{pan}}{E_{pan}} \quad (1)$$

Where I_{pa1} is the anodic current of peak 1, and E_{pa1} is the anodic potential of the same peak. Results are expressed as μA/mV.

The method is advantageous in colored samples by not involving spectrophotometric measurements. However, considering that the EI requires the use of a punctual value of current, it does not contemplate the total amount of oxidizable species that are present in a sample, nevertheless, the voltammetric charge is produced by all the oxidizable

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species at the experimental conditions (Bard & Faulkner, 2001). It is also important to consider that the electrochemical method involves the analysis of the response of species that are electrochemically active under the experimental conditions: electrode material, supporting electrolyte, pH.

The EI is usually used as a comparison tool between large amounts of samples, e.g., wines (Lino et al., 2014), coffee (Oliveira-Neto et al., 2016), and red fruits extracts (de Macêdo et al., 2017), but there is no a direct comparison between the samples and selected standards like is done in the spectrophotometric assays.

As the subject of study, mango fruit (*Mangifera indica* L. cv. Hilacha) was chosen. This fruit is recognized as a source of vitamins (Ascorbic acid, thiamine, riboflavin, niacin, and beta-carotene), and for its antioxidant properties, related to the presence of polyphenols in different parts of the fruit (pulp, peel, seed) and tree (Ajila & Prasada Rao, 2013; Asif et al., 2016; Ribeiro & Schieber, 2010; Serna-Cock, García-Gonzales, & Torres-León, 2016; Shah, Patel, Patel, & Parmar, 2010; Torres-León et al., 2016). In Colombia, mango is an important consumption fruit. In 2016, 262,493 tonnes of mango were produced, used for human consumption and industrial processing (Agriculture, 2016), a process in which only the pulp (45%–50% of the fruit) is used, generating around 131,247 tonnes of waste including shell, seed, and pulp attached to both (Mejía Giraldo, Martínez Correa, Betancourt Gutiérrez, & Castrillón Castaño, 2007). Taking into account the great biodiversity that Colombia presents, the availability of edible fruits is very broad. In this case, mango was selected as a study model for the available information on its antioxidant properties.

The electrochemical technique has been slightly used in fruits (Baldeón et al., 2015; Piovesan, Jost, & Spinelli, 2015) and little or nothing has been done in mango, which facilitates the analysis of this type of raw materials that are being more analyzed due to the tendency of healthy eating (Bigliardi & Galati, 2013; Vasilescu & Marty, 2016), and therefore it can provide important information that allows for the valorization of the by-products of fruit processing (a possible use a raw material for food, cosmetic, or pharmaceutical industries). The main contribution of this work lies in the extension of spectrophotometric assays' methodology into the electrochemical method using the voltammetric charge instead of the i/E ratio.

It is possible to apply the same analytical approach used in the spectrophotometric determination of antioxidant capacity in the electrochemical methods with the intention of homogenizing the data presentation to facilitate the parallelization or correlation between different methods.

In this work, the objective was to determine EI of mango pulp, peel, and seed extract in order to evaluate if the results obtained by this technique are comparable to those obtained with the usual spectrophotometrical methods.

2. Material and methods

2.1. Chemical reagents

2.2′-Azino-bis(3-ethylbenzenothiazoline6-sulfonic acid) (ABTS), 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich. Folin-Ciocalteu reagent was purchased from Merck, Gallic Acid (GA) and potassium peroxodisulphate were PANREAC, sodium carbonate, acetic acid, boric acid, phosphoric acid, methanol, acetone, and other reagents used were analytical grade.

2.2. Fruit samples

Mango fruits (*Mangifera indica* L. cv. Hilacha) at eating ripeness were purchased in a local market, rinsed with tap water. 50 mango units were randomly taken and the pulp, the peel, and the seed were separated for each one. The resulting fractions for each part of the mango were manually combined and homogenized and subjected to drying processes. Pulp and peel samples were lyophilized (LABCONCO, FreeZone 6 Plus, at 0.140 mBar, −85 °C for 72 h) for conservation purposes. Seed samples (endocarp and kernel) were dried at 65 °C overnight and triturated using an Oster blender. The dry samples were stored in a desiccator protected from light until extraction was carried out.

2.3. Extract preparation

The different extracts (pulp, peel, and seed) were obtained using the method described by Contreras-Calderón, Calderón-Jaimes, Guerra-Hernández, and García-Villanova (2011) with slight modifications. 1 g of each sample was placed in a capped centrifuge tube and 8 mL of acidic methanol–water (50:50, v/v pH 2) were added, after which the tube was vortexed for 1 min at normal atmosphere (Vortex V1 plus, BOECO) and sonicated (Ultrasound ELMA E30H) for 15 min at room temperature. The tube was then centrifuged at 6000 RPM (Hettchin Zentrifugen, EBA 20) for 15 min and the supernatant was recovered. This procedure was repeated 3 times. After that, 8 mL of acetone-water (70:30) was added to the residue, followed by stirring, sonication, and centrifugation. This procedure was also repeated 3 times. The supernatants were combined and transferred to a 50 mL volumetric flask, and milli-Q water was added to make the final volume 50 mL. The extracts were stored at −20 °C.

2.4. ABTS assay

The ABTS assay was performed following Re et al. (1999). 100 μL of the test sample, diluted 1:10, 1:60, and 1:90 for pulp, peel, and seed extract respectively with milli-Q water, or Trolox standard was mixed with 1 mL of $ABTS^{+\cdot}$ solution and incubated at 30 °C/30 min. Absorbance readings at 730 nm (UV/VIS-Genesys 10S). Aqueous solutions of Trolox concentrations (between 0 and 200 μM) were used for calibration. Results were expressed as micromoles of Trolox equivalents (TEs) per gram of dry weight (μmol of TEs/g DW).

2.5. DPPH assay

The DPPH assay was performed following Alam et al. (2013) with a slight modification. 100 μL of the test sample was mixed with 500 μL of methanol and 200 μL of DPPH solution (0.5 mM) and kept for 30 min at room temperature in the dark. Absorbance readings at 517 nm were taken (UV/VIS-Genesys 10S). Aqueous solutions of Trolox concentrations (between 0 and 500 μM) were used for calibration. Results were expressed as μmol of TEs/g DW.

2.6. Total phenolics (TP)

The total phenolic content was determined using the Folin–Ciocalteau assay (Contreras-Calderón et al., 2016) with a slight modification. 20 μL of the test sample, diluted 1:2 for peel and seed extract with milli-Q water, or Gallic Acid (GA) standard was mixed with 1580 μL of water, 100 μL Folin–Ciocalteu reagent and 300 μL of 20% sodium carbonate solution. The mixture was stirred and kept for 60 min at room temperature in the dark. The absorbance was measured at 725 nm against the blank (UV/VIS-Genesys 10S). Aqueous solutions of GA (between 0 and 500 ppm) were used for calibration. Results were expressed as mg of GA equivalents (GAEs) per g of dry weight (mg of GAEs/g DW).

2.7. Electrochemical method

The electrochemical experiments were carried out using an Autolab PGSTAT 101 potentiostat controlled by Nova 1.11 software (Metrohm, The Netherlands). A conventional three-electrode configuration cell

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