Electrical bioimpedance spectroscopy measurement of polyphenols in three Colombian passifloras

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Abstract. Fruits and vegetables in the daily diet offer protective action against free radicals, inflammatory agents, and fungi. Polyphenols, found in these natural products, have been studied in order to understand their contribution to the prevention of multiple diseases. The identification and quantification of polyphenols have been evaluated using the Folin-Ciocalteu method. This procedure requires several chemical reagents and different electrical devices, generating chemical waste and at great expense. There is a need to develop polyphenol identification and quantification techniques that are less costly and generate minimum contamination. Electrical bioimpedance spectroscopy (EBS) is a promising alternative that could contribute to measuring total polyphenol content in fresh fruits and vegetables. This study focused on using EBS and characterizing electrical response in fruit pulp from three different Colombian passifloras. The study aimed to compare the electrical parameter values of an Equivalent Electrical Model (EEM) to the total polyphenol content quantified by the Folin-Ciocalteu method.

1. Introduction

Polyphenols are natural phytochemicals containing one or more benzene rings providing chemical strength and versatility to their molecules. These compounds are found in vegetables, fruits, tea, cocoa and wine [1]. Polyphenols are classified as flavanols, flavanones, hydroxycinnamates, flavonols, and anthocyanins. Scientific literature has shown multiple health benefits of these compounds in the fight against inflammatory, non-transmittable and chronic diseases such as obesity, diabetes, cancer, and cardiovascular disease [2]. There has been extensive research on the metabolism of polyphenols and flavonoids; it is well known that they can modulate the intestinal microbiota, contributing to a healthier digestive system and improved absorption processes [3].

There is evidence which recommend including, five or more portions of fruit and vegetables in daily diet in order to benefit from polyphenols. Concentrated polyphenol-rich extracts have been obtained from fruits and vegetables in an attempt to enhance healthy effects. To take full advantage of polyphenols, they need to interact with other dietary compounds, hence the reason for their inclusion in a varied diet [1]. It is necessary to obtain food extracts to identify and quantify polyphenols and to determine the dose required for their anti-inflammatory, antioxidant, and anticancer effects [4].
Fruits in the *Passiflora* family are widely cultivated in tropical countries. Some varieties are *edulis*, *alata*, and *ligularis* [5]. Polyphenols and flavonoids are secondary plant metabolites commonly found in *Passifloras* [2]. These compounds display several biological activities, being its antioxidant activity the one which has been evaluated the most. They have been reported to act as antihypertensive and anti-inflammatory agents. The total polyphenol content (TPC) is quantified based on chemical reagents using the Folin–Ciocalteu method.

TPC in foods and beverages can be calculated using several methods such as spectrophotometric [6], differential pulse voltammetry (DPV), Folin–Ciocalteu (FC), HPLC [7], and near-infrared spectroscopy (NIR) methods, among others [8]. Additionally, fruits and vegetables conduct electricity according to the amount and mobility of their ions. This property is interesting for food processing when electric fields, induction heating, radio frequency, pulse microwave heating or ohmic heating for industrial processes are used [9]. In this way, it is possible that the electrical bioimpedance method would be a useful tool to quantify total polyphenols in fruit extracts.

Electrical Bioimpedance Spectroscopy (EBS) measures the dielectric properties of a biological material as a function of frequency. An external electric field interacts with the material’s electric dipole moment giving results that can change when different frequencies are used. This phenomenon is dependent on the dielectric polarization of molecules emerging from the orientation with the electric field [10]. Various electrical properties of food have been examined to investigate physical conditions of fruit during ripening, extreme weather conditions or chill damage [11]. Although these external properties have been considered in the food industry in general, electric properties have been least explored in order to characterize, identify and quantify internal food components. EBS has the advantage that it offers Equivalent Electrical Models (EEM) that simulate the research matrix to prognosticate electrical signal responses at different amplitudes and frequencies [12].

This study aimed to use the EBS technique and to characterize the electrical response of fruit pulp of three Colombian *passifloras* dissolved in aqueous solutions. It is hypothesized that the total polyphenol content in the extracts could be qualitatively compared to the Folin–Ciocalteu chemical method commonly used for quantifying TPC.

2. Materials and Methods

2.1. Gathering, freeze-drying and storage of pulp

Fruits were obtained from the departments of Caldas, Tolima and Valle del Cauca (Colombia). Maturation stage 5, optimum for human consumption, was recommended for the fruit condition and the subsequent gathering and analysis [13]. The amount of each fruit was 1 Kg. Fruits were washed in a 50 ppm sodium hypochlorite solution and dried with absorbent paper [14].

The total content of fruit, in portions of 40 grams, was homogenized with 100 ml of distilled water in Ultra-Turrax. The homogenized product was centrifuged at 4000 rpm and room temperature for 10 minutes. The supernatant was placed in amber glass vials and stored, as the working solution, at 4°C [15]. The total content of fruit pulp was lyophilized at 0.05 mbar. The product was stored in sealed bags at -20°C [14].

2.2. Total polyphenol content (TPC)

Samples of 4.0 g of lyophilized fruit were dissolved in 30 ml of 80% ethanol, agitated at 500 rpm and 37 °C for 15 minutes, left to stand for 24 hours in the dark at –4°C and centrifuged at 3500 rpm for 10 minutes. The supernatants were recovered and evaluated for TPC using the Folin–Ciocalteu method [16]. Samples of 0.5 ml were mixed with 0.5 ml of distilled water, 1.0 ml of the Folin-Ciocalteu reagent, and 1.0 ml of 3.5% sodium bicarbonate. Samples were incubated at room temperature in the dark for 90 minutes. All reactions were done in triplicate. A standard gallic acid curve was prepared to calculate TPC in each fruit extract. Results of TPC were expressed as mg gallic acid equivalent (GAE) per gram of lyophilized fruit [16]. Samples were read on a UV/VIS spectrometer at 760 nm.
2.3. Electrical Bioimpedance Spectroscopy Measurements

An enhanced version of the CoreBioZ system was used for EBS measurements [17]. This enhanced system performed a frequency sweep in the bandwidth between 500 Hz and 10 kHz, using a tetrapolar arrangement of electrodes [18]. For EBS measurements of aqueous solutions, a Printed Circuit Board (PCB) and a 3D-printed chamber for four reusable wells were utilized. For each of these wells a tetrapolar array of gold-coated electrodes was printed on the top layer of the PCB as shown in Figure 1(a). This PCB was used as a platen, and the 3D-printed chamber was coupled onto it with intermediate seals to build the reusable wells as shown in figure 1(b) [19,20].

The following experimental protocol to perform EBS measurements in each Passiflora extract was implemented. One millilitre of each fruit extract was added separately into each measurement well. A blank solution, or ethanol 80%, was also measured for EBS response in five trials. This assembly was kept in a controlled environment (25 °C and 60% Relative Humidity) for 30 minutes to achieve the thermodynamic equilibrium of the electrode-solution interface. Ten bioimpedance spectra were taken to determine data repeatability.

3. Results

3.1. Total polyphenol content

Total polyphenol content, quantified by the Folin-Ciocalteu method for granadilla (Passiflora ligularis Juss) from the State of Caldas yielded 30.61 mg GAE/g of lyophilized fruit. Gulupa (Passiflora edulis Sims) from Tolima registered 29.95 mg GAE/g, and maracuyá (Passiflora edulis Flavicarpa) (passion fruit) from the State of Valle del Cauca yielded 26.48 mg GAE/g of lyophilized fruit.

3.2. EBS output for standard solution and lyophilized passifloras

The range of measurements, for this resistive electrical model study, was from 500 Hz to 10 kHz. The magnitude of the impedance response in each Passiflora extract was registered and plotted in Figure 2. Electrical response for the solvent, ethanol 80%, was registered at an average of 160 kΩ for impedance magnitude with a mean standard deviation ($\sigma_{mag}[\Omega]$) of 14.5 kΩ. With respect to repeatability a $\sigma_{mag}[\Omega]$ of 4%, with respect to the mean value for the solvent, was considered acceptable for the polyphenol measurements in the three different fruits. Once the fruit extracts were dissolved, all differences in electrical responses were registered for numerical and graphical analysis.

Figure 2 shows EBS measurements for each fruit extract. These experimental results display the impedance magnitude of the extracts as a function of frequency. Lines shown here follow a similar pattern; however, the average of each line is different. Results prove that granadilla records the highest
and maracuyá the lowest impedance magnitude values. Bioimpedance magnitude $[\Omega]$ for the fruit extracts were 6700 $\Omega$ for granadilla, 6400 $\Omega$ for gulupa and 4200 $\Omega$ for maracuyá.

![Figure 2. Experimental frequency response of passiflora extracts with a resistive model.](image)

Results obtained from the EBS measurements of polyphenol in the fruit extracts in ethanol 80% are comparable to determine and proportional to the chemically quantified method. Folin-Ciocalteu values showed that maracuyá has the lowest polyphenol content (26.48 mg GAE/g of lyophilized fruit) while granadilla revealed the highest content (30.61 mg GAE/g of lyophilized fruit). With respect to bioimpedance magnitude values, both fruits displayed the same trend. The average impedance amplitude value in maracuyá is 4085 $\Omega$ and in granadilla is 6668 $\Omega$. Percentage differences in the electrical response of maracuyá and granadilla (13.4%) are in concordance with Folin-Ciocalteu values. This relation is similar, for TPC with Folin-Ciocalteu, when a smaller difference is present such as in the case of gulupa and granadilla (2.2%). Similar proportions are registered based on the electrical response values. Comparative results for maracuyá and granadilla yielded a 38.4% value. With respect to the difference between gulupa and granadilla, only a 6.2% difference was observed.

Table 1 shows TPC values and the average impedance $|Z|$ magnitude obtained from each *Passiflora* extract. From these values, we can produce a graph with a linear trend, as shown in Figure 3, therefore suggesting a linear equation (1). This represents a relationship between the $|Z|$ value of a fruit extract solution and its polyphenol content expressed in mg-GAE/g. Through knowing the $|Z|$ value of the fruit extract solution and using the equation (1) it is possible to calculate TPC in the solution.

| Extract      | TPC [mg GAE/g] | Average of $|Z|$ [Ω] |
|--------------|----------------|---------------------|
| Granadilla   | 30.61          | 6668                |
| Gulupa       | 29.95          | 6270                |
| Maracuyá     | 26.48          | 4085                |

$$TPC = K \cdot |Z| + b$$
In this case, to obtain the value of the K and b parameters of the equation (1), we perform a linear regression using the data presented in Table 1. The calculated values for these parameters are: 

\[ K = 0.0016 \, \text{mg} \cdot \frac{GAE}{Ω \cdot g} \quad \text{and} \quad b = 19.963 \, \text{mg} \cdot \frac{GAE}{g}. \]

Figure 3. Linear trend between the values of the TPCs and the averages of \(|Z|\).

4. Conclusions and discussion

The use of the Folin-Ciocalteu reagent generates phosphoric and hydrochloric acids, tungsten and lithium salts, as well as sodium bicarbonate waste. Polyphenol quantification, based on this method, requires the use of a lyophilizer, a magnetic stirrer, ultrasonic devices and a spectrophotometer. Costs, laboratory work time, and complexity are also negative factors in this situation. Total polyphenol content has been long quantified by this technique. Current evaluation of food components and their physicochemical properties call for non-destructive, economically efficient, and environmentally friendly techniques [21].

With the current work, electrical bioimpedance spectroscopy could distinguish differences or amounts of compounds dissolved, such as polyphenols, normally detected by chemical reagents in the Folin-Ciocalteu reaction. The experimental results obtained in this work highlight the different electrical responses that each Passiflora extract displays, related or based on the total polyphenol content. In this case, we find that the changes in the impedance magnitude of the passiflora extracts are sensitive to small variations in the content of polyphenols and that this magnitude is proportional to the content of polyphenols in the bandwidth of the EBS measurements. Concerning these findings, we propose a simple linear equation that allows us to relate the magnitude of the impedance \(|Z|\) of a passiflora extract and its TPC value. That is, by knowing the value of \(|Z|\) obtained from EBS measurements it is possible to calculate the TPC value without carrying out the Folin-Ciocalteu reaction.

Impedance magnitude values, for the solvent showed that electrical response is clearly different when polyphenols are dissolved in it. The value for ethanol was 160 kΩ. With Passiflora extract in ethanol impedance magnitude decreased significantly. These changes provided a clue for the differentiation between polyphenolic compounds with respect to the electrical responses.

Solutions with Passiflora extracts display different bioimpedance magnitude responses that could be compared to those of a normal spectrophotometric study. EBS measurements provide bioimpedance magnitude values for evaluating the amount of total polyphenol content in three Passiflora of similar characteristics suggesting that this is a promising quantitative alternative to a chemical reagent-based analysis. A concordance was registered between the measured differences of polyphenols extracted from each fruit and the traditionally quantifying chemical method of Folin-Ciocalteu. There was an
equivalent relation between bioimpedance magnitude values and mg of GAE/g in each polyphenol extract.

On the other hand, since the electrical bioimpedance technique is environmentally friendly, cheap, simple to use and reliable, the results obtained show that this technique could be an alternative to reduce the adverse effects relating to the traditional measurement methods for the quantification of total polyphenol content in food products. The previous facts are relevant considering that food production and its evaluation throughout different ripening stages is fundamental in food safety [22]. Current challenges in the food industry call for the implementation of new politics focused on sustainability, as well as harvesting yields and quality. It is, therefore, a current need in agricultural practices to implement technology that contributes to industrial, social, and environmental factors.

Conflict of interest
No conflicts of interest are declared by authors.

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