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RESEARCH ARTICLE

Evaluation of antioxidant capacity in different food matrices through differential pulse voltammetry and its correlation with spectrophotometric methods

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Abstract
Differential pulse voltammetry (DPV) and spectrophotometric methods were used to evaluate the antioxidant capacity (AC) in strawberry and orange juice, soluble coffee, rosemary extract, red wine, and sparkling white wine. The AC through spectrophotometric methods showed the following trend in solid foods, coffee > rosemary extract, and red wine > strawberry juice > orange juice > sparkling white wine in liquid samples. Charge measurements by DPV, presented in equivalent units of Trolox and gallic acid, showed the same trend in liquid samples; however, for solid samples, the AC of rosemary extract was greater than that of coffee. The charge expressed in equivalent units showed a high, positive, and significant correlation ($p < 0.001$) with total phenols (0.7919), FRAP (0.8875), and ABTS (0.8366). The proposed electrochemical technique proves to be a fast, reliable, and environmentally friendly alternative or complement to evaluate antioxidant capacity in food, which is not affected by the turbidity or color of the samples.

Graphical abstract
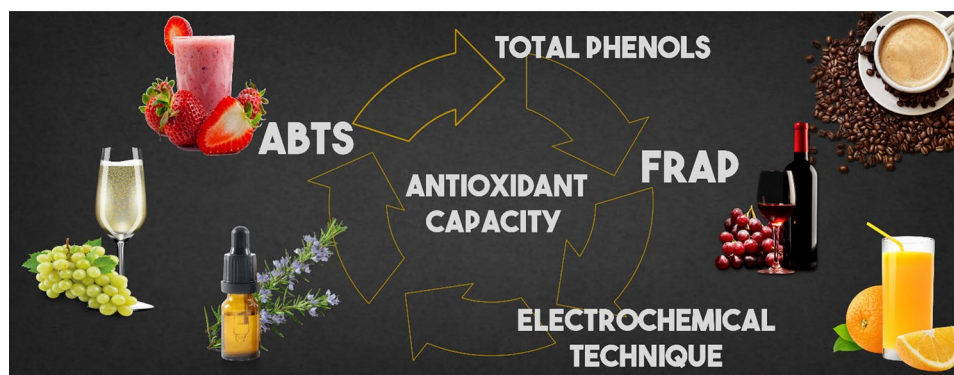
Keywords Antioxidant capacity · Foods · Beverages · Differential pulse voltammetry · Spectrophotometric methods · Voltammetric charge

1 Introduction

The consumption of antioxidants has been related to a positive influence on the human diet because it can prevent or reduce the risk of chronic and degenerative diseases, such as diabetes, obesity, cardiovascular problems, and even certain

Extended author information available on the last page of the article

types of cancer [1]. They are present in a wide variety of foods, including fruits, vegetables, drinks, such as tea, coffee, chocolate, and wine, as well as herbs and spices, and it is from their consumption that the antioxidants enter the body [2].

Antioxidants are compounds that interact with reactive oxygen species (ROS) and can end chain reactions before

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they affect vital molecules, such as lipids, proteins, and nucleic acids [3]. Antioxidant capacity (AC) refers to the ability to capture a radical or reduce an oxidizing agent [4]; however, a low antioxidant content does not imply a low antioxidant capacity since this is closely related to the structure and behavior of the antioxidant with other analogous compounds (synergism and antagonism) [2].

That is why different analytical methods are used not only to quantify antioxidants but also to measure antioxidant instead said capacity in different matrices; visible fluorescence spectroscopy and UV–Visible spectrophotometry are usually employed. The latter is applied frequently and is based on the monitoring of the increase/decrease of the spectrophotometric absorbance due to the coloration/discoloration of a synthetic radical, reduced by antioxidants, among which are DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)), ORAC (oxygen radical absorbing capacity), FRAP (ferric reducing antioxidant power), DCFH-DA (dichlorofluorescein-diacetate), and Folin–Ciocalteu Reagent [5]. In general, in the ABTS, FRAP, and Folin–Ciocalteu Reagent methods, the mechanism of action is the transfer of a single electron or capture of a synthetic radical according to the used method (SET); while the mechanism of action of the ORAC method is the transfer of hydrogen atoms (HAT).

However, although UV–Vis spectrophotometric methods are commonly applied, they are characterized by the use of costly and environmentally unfavorable reagents, undefined reaction times, pretreatment of the samples, expensive equipment, low sensitivity, errors of sub- or overestimation due to the presence of compounds that absorb in the same wavelength range affecting precision, and the interference of substances of a non-antioxidant nature that can reduce chromogenic compounds, which causes an overestimation of antioxidant capacity, among others [4, 5].

Given the above, it is necessary to search for new techniques for the determination of food properties, in this sense the electrochemical methods through the design of biosensors are a good alternative; the use of biosensors in the food industry is reported to determine: Formaldehyde in Fruit Juices [6], as well as the detection of Salmonella Typhimurium in milk [7], finally, is reported the developed sensor for the detection of phenolic compounds [8].

Measuring the AC of food and beverages using electrochemical techniques has advantages over conventional spectrophotometric assays since the former are fast, inexpensive, environmentally friendly because they do not require a complex sample preparation using expensive chemical reagents, allows analyzing hydrophilic, lipophilic, colored, and cloudy samples (restricted in some conventional methods); also, they allow better sensitivity, precision, and reproducibility [4].

One of the most widely used electrochemical techniques for determining the antioxidant capacity in food matrices is cyclic voltammetry, which is the most used technique for characterizing redox systems, providing information on the number of redox states and qualitative information on the oxidation processes [9]. Other techniques such as: photoamperometry, linear sweep voltammetry, and polarization curves are also used as tools for determining antioxidant activity electrochemically [10]. On the other hand, differential pulse voltammetry (DPV) is a technique where a fixed magnitude pulse is superimposed on a linear potential ramp. The response current is recorded a moment before the application of the pulse and again at the end of the life of the pulse; the difference between the first current and the second is represented against the applied potential [4]. The results of this technique are plotted on a voltammogram, where the height of the peak is proportional to the concentration of the analyte, and the electric potential position (volts. vs a reference electrode) provides information about the redox capacity of a certain substance. Positive potential values give information about oxidation process and for antioxidant compounds, less positive potential implies a better antioxidant capacity (less energy for oxidation phenomena) [11]. In contrast to cyclic voltammetry, DPV does not present interference with residual current (capacitive).

A significant AC has been reported in coffee [12, 13], orange juice [14], white wine [15], red wine [16], rosemary [3], and strawberry [17], due to the presence of different groups of molecules among which are tannins, anthocyanins, phenolic acids, flavonoids, vitamin C, or carotenoids. AC can be affected by many factors, among which we can mention the state of the sample (solid or liquid), whether it is fresh or processed (roasting, drying, fermentation, etc.), by the presence of chromophoric compounds, among others.

One of the most used electrochemical tools for determining the AC in food matrices is the electrochemical index (EI) [13, 16, 18–21]. The peak potential (Epa) and peak current, the two primary voltammetric parameters, were considered when an electrochemical index (EI) was developed. The EI was computed using Eq. 1:

$$EI = \frac{i_{p,a1}}{E_{pa1}} + \frac{i_{p,a2}}{E_{pa2}} + \cdots + \frac{i_{p,an}}{E_{pan}}, \tag{1}$$

where i_{pa} corresponds to a current value and E_{pa} corresponds to a potential value for each anodic peak observed in the DPV voltammogram.

This index is useful in the determination of AC because the lower the potential (a thermodynamic parameter), the greater the capacity of electron donors and the higher the peak current (a kinetic parameter), the greater the quantity of electroactive species.

The method's lack of spectrophotometric readings makes it useful for colored materials. Although the EI does not account for the complete number of oxidizable species present in a sample because it uses a punctual value of current, all oxidizable species produce the voltammetric charge under the experimental conditions [22]. This represents a major disadvantage of this method because they might be ignoring important processes that could contribute to the total AC of the sample. It is crucial to keep in mind that the electrochemical approach entails analyzing the behavior of species that are electrochemically active under the experimental conditions, including pH, supporting electrolyte, and electrode material.

Although in a previous study the correlation of electrochemical and spectrophotometric methods was evaluated in extracts from different parts of the mango fruit [5], it has not been evaluated in different food matrices at the same time, which is relevant to validate the usefulness of electrochemical methods in the evaluation of antioxidant capacity against the different metabolites present in food, which have different reaction mechanisms. Therefore, the objective of this research was to determine the antioxidant capacity in different food matrices by DPV using the approach that is commonly in spectrophotometric methods that presents the AC of the sample in equivalents of a recognized standard, such as gallic acid or Trolox, and its correlation with spectrophotometric methods to evaluate if the electrochemical technique can be used as an alternative or complement to the spectrophotometric techniques.

2 Materials and methods

2.1 Chemical reagents

2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), and gallic acid were obtained from Sigma-Aldrich Chemical (Oakville-Ontario, Canada). Folin–Ciocalteu reagent (2.0 N) was obtained from Merck (Darmstadt, Germany). 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was purchased from MP Biomedicals (Illkirch-Graffenstaden, France). Potassium peroxodisulfate was obtained from PANREAC. Sodium carbonate, sodium chloride, potassium chloride, sodium acid phosphate, potassium diacid phosphate, hydrochloric acid, methanol, acetone, and other reagents used were analytical grade. All the aqueous solutions were prepared with Milli-Q water (18.2 MΩ cm).

2.2 Raw materials (fruit samples) and conditioning

Oranges (*Citrus sinensis Valencia*) and strawberries (*Fragaria ananassa*) at eating ripeness, red wine, sparkling white wine, rosemary (*Rosmarinus officinalis L.*), and soluble coffee (*Coffea arabica*) were acquired in a local market. The fruits were washed, manually squeezed, and filtered before analysis. The liquid samples were analyzed directly, after degassing the sparkling wine and dissolving the coffee (0.1 g·10 mL^{-1}), while for rosemary an extraction with solvents was carried out.

2.3 Rosemary extract preparation

The rosemary extract was obtained following the methodology described by [23] with some modifications. Fresh rosemary leaves were cut to a suitable size and 0.5 g of sample was placed in a falcon tube to which 8 mL of methanol–water (50:50 v/v at pH 2) was added, then it was taken to ultrasound (Ultrasound ELMA E30H) for 15 min at room temperature, and the supernatant was recovered by filtration; this procedure was repeated 3 times. Subsequently, 8 mL of acetone–water (70:30 v/v) were added to the residue of the filtrate and it was brought to ultrasound again under the same conditions, recovering the supernatant by filtration; this treatment was repeated 3 times. The supernatants were combined in a 50-mL volumetric flask and completed the volume with distilled water. The extracts were made in triplicate and kept at − 20 °C protected from light until analysis.

For the extraction of antioxidants from plant foods, at least two extraction cycles performed with aqueous organic solvents with different polarities should be combined to extract antioxidant compounds with different chemical structures. Another way to improve antioxidant extraction efficiency is to use acidified solvents [24].

2.4 ABTS assay

The assay was carried out as described by [25]. 100 μL of extract, previously diluted with distilled water in a ratio of 1:100 for strawberry, red wine, and coffee and 1:50 for sparkling white wine, orange, and rosemary, were mixed with 1 mL of ABTS$^{+·}$ and incubated at 30 °C for 30 min in the dark, after which the absorbance was measured at 730 nm (UV–Vis 1200 Mapada spectrophotometer). Trolox aqueous solutions at different concentrations (0 to 200 μM) were used for calibration. The results are expressed as micromoles of Trolox equivalents (TE) per gram or mL of sample depending on the nature of the sample (μmol of TE·g^{-1} or mL^{-1}).

2.5 FRAP assay

The assay was carried out following the methodology proposed by [12]. 30 μL of the sample, diluted appropriately with distilled water in the ratio 1:100 for strawberry, red wine, and coffee and 1:50 for sparkling white wine, orange, and rosemary, were mixed with 90 μL of distilled water and

900 µL of the FRAP reagent. The mixture was incubated at 37 °C for 30 min in the dark, and the absorbance was measured at 595 nm (Mapada UV–vis 1200 spectrophotometer). The calibration curve was prepared from aqueous Trolox solutions (concentrations between 0 and 1000 µM), and the results are expressed in µmol TE·g^{-1} or mL^{-1}.

2.6 Total phenols (TP)

The total phenol content was assessed by the Folin–Ciocalteu reaction as previously described in the literature [5]. 20 µL of a sample of each food matrix was combined with 1580 µL of distilled water, 100 µL of Folin–Ciocalteu reagent, and 300 µL of 20 % (m/v) sodium carbonate solution, stirred and left at room temperature and in darkness for an hour. The absorbance was measured at 725 nm (UV–Vis 1200 Mapada spectrophotometer). The calibration standards were generated from aqueous gallic acid solutions (concentrations between 0 and 2000 ppm). The results are expressed as mg of gallic acid equivalents per gram or mL of sample (mg GAE·g^{-1} or mL^{-1}).

2.7 Differential pulse voltammetry (DPV)

DPV measurements were carried out at room temperature with an Autolab PGSTAT 101 potentiostat controlled by Nova 1.11 Metrohm software, (The Netherlands). A typical three-electrode cell composed of glassy carbon (GCE), Ag/AgCl/KCl$_{sat}$, and a Pt wire were used as working, reference, and counter electrodes, respectively. Before every measurement, GCE was mechanically polished using powdered alumina of three particle sizes (1 µm, 0.3 µm, and 0.05 µm) for 1 min and rinsed with abundant distilled water to clean the working electrode's surface of organic molecules that can be adsorbed. The GCEs were activated by cyclic voltammetry in a potential window between −0.3 and 1.0 V (vs Ag/AgCl) using a scan rate of 100 mV s^{-1} until a constant response was obtained. The conditions for DPV were: potential window between −0.3 V and 1.0 V (vs Ag/AgCl), step potential (ΔE) 5 mV, pulse length 25 mV, pulse time 0.05 s, time interval between pulses 0.5 s. Previously, the samples were diluted in 10 mM phosphate-buffered saline (PBS) solution (in 0.1 M KCl) at pH 7 to resemble biological conditions in a 2:3 ratio (extract:buffer) [16].

The reference voltammograms for Trolox and Gallic Acid solutions were obtained by adding a solution of known concentration to a cell with 10 mM PBS (in 0.1 M KCl) at pH 7 and recalculating the cell concentration.

The obtained voltammograms were corrected for the baseline using the "moving average" algorithm (Nova 1.11 software) to better identify the oxidation peaks. Also, the voltammetric charge (Q) (defined as the area under the curve for the amperometric signal obtained in the voltammograms,

i vs E, where i is the obtained current and E is the applied potential) was determined and converted to equivalent units of Trolox and gallic acid, facilitating the comparison of the results with the spectrophotometric methods used.

2.8 Statistical analysis

The analyses performed by the DPV and by the spectrophotometric methods were done in triplicate for each matrix. The results are expressed as the mean ± standard deviation. To determine the existence of significant positive or negative correlations between the antioxidant capacity values of the ABTS, FRAP, total polyphenols assays, and the electrochemical method, a non-parametric Spearman method was used with a significance level of 95%. This indicates that the values found did not show a behavior of normal or Gaussian distribution [26]. The data were analyzed and processed using the STATGRAPHICS Centurion XVI software (Statistical Graphics Corporation, Version 16.0.07, Rockville, USA).

3 Results and discussion

3.1 Determination of antioxidant capacity through spectrophotometric

Table 1 shows the antioxidant capacity values obtained by spectrophotometric methods. In general, coffee showed the highest values ($p < 0.05$) of antioxidant capacity by all methods, followed by rosemary, red wine, strawberry juice, orange juice, and white wine. Similar trend for these analyses in which coffee is in the first place, followed by red wine and orange is reported by [27].

Previous studies have widely reported on high AC in coffee [28, 29], which is derived from its natural phenolic compounds and the products of the Maillard reaction (PMR), such as melanoidins, predominant in roasted coffee. It has been reported that the greatest contribution of melanoidins is given by low-molecular weight structures and, also, it has been found that the mechanisms associated with PMRs include both the transfer of hydrogen atoms (HAT) and the transfer of a single electron (SET) [30].

Polyphenols are compounds that also exhibit a high AC that is related to the capacity to capture radicals, among the most important polyphenols in coffee are chlorogenic acids, caffeic acid, ferulic acid, and coumaric acid (Niseteo et al., 2012). Vignoli, Bassoli, & Benassi, (2011) report for soluble coffee with a light roast and two types of extraction, AC values for ABTS between 750 and 930 µmol TE·g^{-1}, FRAP 770–1000 µmol TE·g^{-1}, and TP between 121 and 150 mg GAE·g^{-1}; in turn, CA results are also reported for soluble coffee of variable characteristics (freeze-dried, decaffeinated,

Table 1 Antioxidant capacity determined by spectrophotometric methods for all the samples

Sample	ABTS (μmol TEs g^{-1})	FRAP (μmol TEs g^{-1})	Total phenols (mg GAEs g^{-1})
Solid samples			
Coffee	629.3 ± 21.9[a]	993.1 ± 110.2[a]	123.0 ± 0.7[a]
Rosemary	335.7 ± 16.4[b]	686.4 ± 15.0[b]	57.6 ± 2.6[b]
Sample	ABTS (μmol TEs mL^{-1})	FRAP (μmol TEs mL^{-1})	Total phenols (mg GAEs mL^{-1})
Liquid samples			
Red wine	16.3 ± 0.3[c]	23.7 ± 1.0[c]	2.12 ± 0.08[c]
White wine	3.3 ± 1.2[d]	4.4 ± 0.5[d]	0.30 ± 0.01[d]
Orange juice	3.0 ± 0.1[d]	5.0 ± 0.5[d]	0.7 ± 0.1[e]
Strawberry juice	6.4 ± 0.2[e]	9.6 ± 0.2[e]	1.32 ± 0.08[f]

Mean value $\pm$ standard deviation of dry weight; n = 3. a, b, c, d, e, and f mean there are statistically significant differences between values in the same column

seasoned) measured by ABTS of 442–1000 μmol TE·g^{-1}, FRAP 352.2—885.9 μmol TE·g^{-1}, and TP 36.8–127 mg GAE·g^{-1} values that are around and within the range of those reported in the present study [12].

Among the different presentations, soluble or instant coffee has the highest AC compared to coffee beans, even with different degrees of roasting; this is attributed to its processing conditions since soluble coffee is produced by extraction with high-temperature water that solubilizes active compounds, including polyphenols, and later, they are concentrated in the dehydration stage [31].

Regarding the rosemary extract, Vallverdú-Queralt et al. (2014) [32] describe more than 20 compounds with radical scavenging capacity in this matrix as gallic acid, caffeic acid, chlorogenic acid, coumaric acid, ferulic acid, sinapic acid, hesperidin, naringenin, rutin, and quercetin, among others, which are also found in some of the matrices analyzed in this work; rosmarinic acid, carnosol, and rosmanol stand out as the most powerful; these latter are derivatives of carnosic acid which has a high AC. This author also reports values of TP 5.02 mg GAE·g^{-1} and ABTS 2390 μmol TE·g^{-1} for hydroalcoholic extracts of rosemary, lower than our results for phenolic compounds and higher than the AC values obtained by ABTS assay. The variation may be due to differences in varieties, climate, ripeness, extraction method, etc. [23].

The literature reports the content of total phenolic compounds as 18.1 mg GAE·g^{-1} in fresh rosemary extract and 38.2 mg GAE·g^{-1} in lyophilized rosemary extract [33]. These values are still below ours and maybe because the extractions are carried out only with ethanol and water, leaving part of the non-polar components with AC in the solid residue. Some authors explain that in the lipid systems, rosemary extracts with a higher content of phenolic diterpenes

show great antioxidant capacity; while in aqueous systems, the AC is due to the rosmarinic acid [34]. The catechol group is the most outstanding structure. In this sense, phenolic diterpenes such as carnosic acid and carnosol exhibit a catechol group in its structure, while rosmarinic acid has two catechol groups in its structure translated into four hydroxyl groups.

On the other hand, Shan et al. (2005) [35] studied 26 spices from different families, finding that the AC of the methanolic extract of rosemary was 378 μmol ET·g^{-1} for ABTS and 50.7 mg EAG·g^{-1} for TP, which are similar to those found in the present investigation.

Comparing the liquid samples, we found that red wine has the highest values for AC and TP, with significant differences ($p < 0.05$) between all samples except between orange juice and sparkling white wine in the analysis of ABTS and FRAP.

The values for red wine (Table 1) agree with those reported by Lino et al. (2014) [16]. The participation of polyphenols in the antioxidant capacity has been outstanding, finding mainly gallic acid and catechin [36]. The contribution to the antioxidant capacity of wines is presented thanks to tannic acids, anthocyanins, phenolic acids, and other complex compounds that have as precursors basic molecules found in the seeds and tissues of the grape skin, which explains that red wine is 4–5 times greater than white wine [37]. Tourtoglou, Nenadis, & Paraskevopoulou, (2014) [15] report that the TP in white wine and ABTS ranged between 0.241 and 0.336 mg GAE·mL^{-1} of wine and 2.7 to 4.0 μmol TE·mL^{-1} of wine, respectively, ranges that agree with those obtained in the present study (Table 1).

In the case of strawberry, Hartmann et al. (2008) [38] reported for juices without treatment, values for TP of 2.3 mg GAE·mL^{-1}, FRAP 9.7 μmol TE·mL^{-1}, and ABTS 23.3 μmol TE·mL^{-1}, the latter showed a big difference to

our results, which may be due to environmental factors and the variety of the crop, which affect the expression of metabolites with antioxidant capacity. Its high antioxidant activity has also been linked to phenolic compounds. Cyanidin glucoside, pelargonidin (glucoside and rutinoside), kaempferol (glucoside), quercetin, catechin, coumaric acid glucose ester, and ellagic acid (glucoside) have been the main antioxidants reported [39]. It has also been found that in strawberry extracts that cyanidin glucoside, pelargonidin, and their conjugated glycoside structures were the most powerful antioxidants [40].
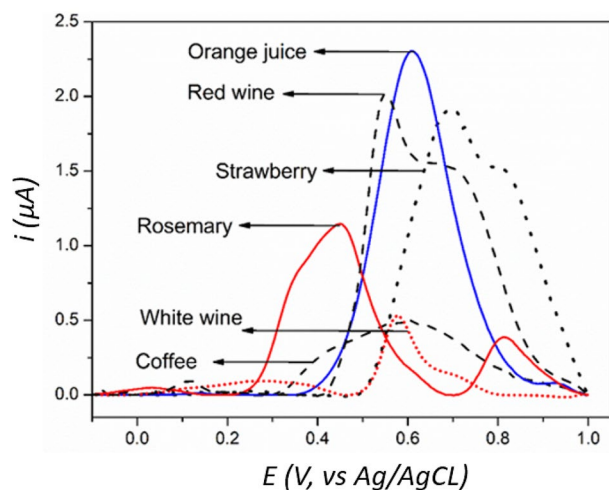
Fig. 1 Differential pulse voltammograms for the different evaluated extracts in PBS pH 7 and conditions, potential range from − 0.2 to 1.0 V, $\Delta Es = 5$ mV, modulation amplitude = 25 mV, modulation time = 50 ms, and interval time = 0.5 s

Arena et al. (2001) [41] reported ABTS values between 2.38 and 5.18 µmol TE·mL^{-1} that are close to our results for freshly squeezed juice. The higher values obtained in their work may be due to the orange variety used for their study, orange blood, which has a darker color and, therefore, another kind of bioactive compounds with AC. Hesperidin, naringin, narirutin, and ferulic acid have been the most abundant polyphenols reported in orange juice. Also, the presence of gallic acid, vanillic acid, chlorogenic acid, coumaric acid, quercetin, rutin, ascorbic acid, and sinapic acid has been reported [38].

3.2 Differential pulse voltammetry (DPV)

All the measurements carried out in this research were taken using a potential window between 0 and 1 V (vs Ag|AgCl|AgClsat), considering that all the molecules responsible for the AC in the studied matrices are known to be polyphenols, anthocyanins, etc.) and such family of molecules have electrochemical activity within the range of 0–1 V (vs Ag|AgCl|AgClsat) [4, 5]. Figure 1 shows the electrochemical response for the different extracts in which the electrochemical extracted data were interpolated in the curves constructed from the data in Fig. 2. Table 2 shows the value of the charge expressed in equivalent units (µmol TE and in mg GAE) for solid and liquid matrices and values calculated from the calibration curves (Fig. 2), which relate the voltammetric charge (Q) with the concentration of the GA (Fig. 2A) and Trolox (Fig. 2B) solutions, from which an R^2 of 0.9990 and 0.9905 were obtained, respectively. According to literature, the oxidation of the catechol group to quinone corresponding to gallic acid
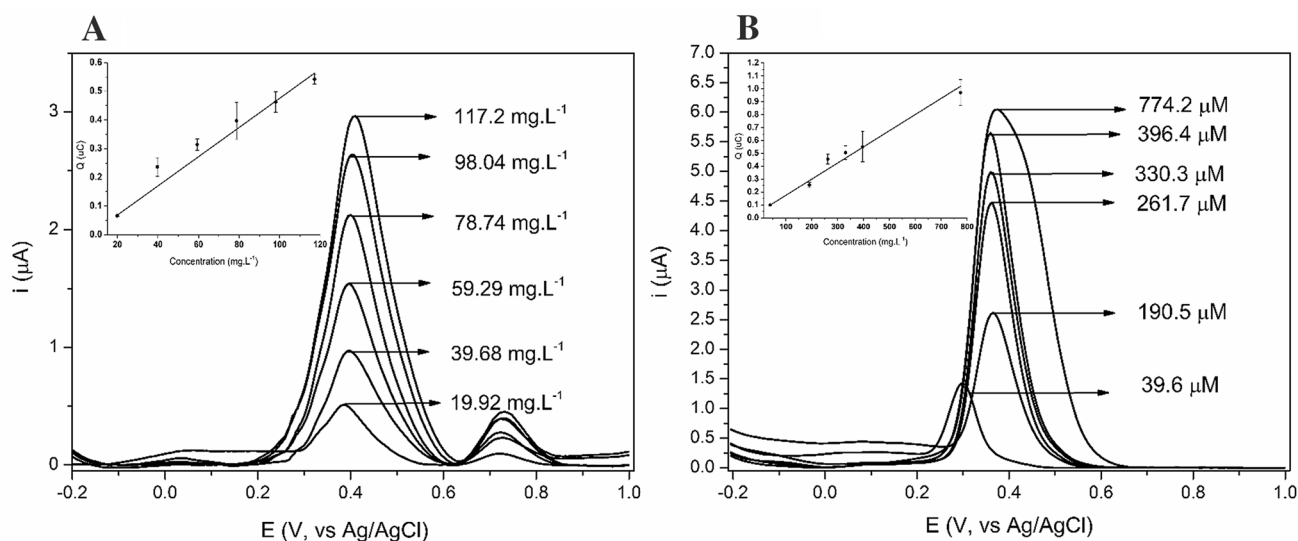

Fig. 2 **A** DPV for Gallic Acid solutions of different concentrations. Inset: calibration curve (Charge vs concentration). **B** DPV for Trolox solutions of different concentrations. Inset: calibration curve (Charge vs concentration)

Table 2 Voltammetric charge expressed in equivalent units for all the samples

Sample	Voltammetric charge Q (μC)	Voltammetric charge (Q) (μmol TEs g^{-1})	Voltammetric charge (Q) (mg GAEs g^{-1})
Solid samples			
Coffee	0.175 ± 0.003[a]	9.02 ± 0.26[a]	3.89 ± 0.05[a]
Rosemary	0.270 ± 0.02[b]	18.75 ± 2.08[b]	5.88 ± 0.40[b]
Sample	Voltammetric charge Q (μC)	Voltammetric charge (Q) (μmol TEs mL^{-1})	Voltammetric charge (Q) (mg GAEs mL^{-1})
Liquid samples			
Red wine	0.56 ± 0.02[c]	0.48 ± 0.02[c]	0.116 ± 0.003[c,d]
White wine	0.07 ± 0.02[d]	0.05 ± 0.03[d]	0.030 ± 0.008[c]
Orange juice	0.48 ± 0.03[e]	0.40 ± 0.03[d]	0.101 ± 0.006[d]
Strawberry juice	0.534 ± 0.005[c]	0.454 ± 0.004[e]	0.1114 ± 0.0009[d]

Mean value $\pm$ standard deviation of dry weight; $n = 3$. a, b, c, d, and e mean there are statistically significant differences between values in the same column

occurs at 0.4 V vs Ag/AgCl, having shifted to less positive values due to the effect of the pH of the buffer solution [5]. The Trolox signal (as shown in the calibration curve) describes the oxidation of the phenol group present in its structure [5].

Comparing all the samples, it is observed that red wine, orange juice, and strawberry juice have higher charge values than solid matrices; however, the trend is again similar to the spectrophotometric results when the value has been converted to TE and GAE. This highlights the practical effect of representing values with equivalent standards between electrochemical techniques and spectrophotometric methods.

Coffee and rosemary show significant differences between both methods, for the case of coffee in the spectrophotometric method its value is higher since it shows colored substances, such as melanoidins, which may have contributed in a greater proportion to the absorbance.

In contrast to the results obtained by spectrophotometry, in DPV, rosemary had a higher antioxidant capacity than coffee (Table 1). In Fig. 1, it can be seen that the first oxidation peak for the rosemary extract was found between 0.3 and 0.55 V (vs Ag/AgCl), while the coffee signal extends from 0.4 to 0.8 V (vs Ag/AgCl); this means that the electroactive molecules of rosemary have a higher AC since they react at a lower potential; meanwhile, the current accounts for the concentration of electroactive species and the number of electrons transferred [42].

The spectrophotometric methods used for the analysis only evaluate the capture of radicals by the SET mechanism, while the DPV measures the antioxidant capacity generated by the action of the molecules by both SET and HAT mechanisms. As mentioned above, PMRs act by both mechanisms; however, no studies were found that alluded to rosemary compounds in that sense. This would also explain the differences obtained between the spectrophotometric methods and

the electrochemical techniques, confirming the reliability of the latter.

The redox behavior of rosemary extracts has been little studied by electrochemical analysis. The first pronounced anodic peak compared to the other food matrices, before a potential of 0.5 V, suggests the presence of species with high antioxidant capacity. Only Leite et al. (2018) [43] studied by voltammetry an ethanolic extract of rosemary that showed two peaks at 0.37 V and 0.97 V (*vs* Ag|AgCl|KCl$_{sat}$) at pH 6, assigning the first to the oxidation of rosmarinic acid, carnosic acid, and their derivatives carnosol and rosmanol and the second to rosmadial. The first group of molecules presents in its structure the catechol group, which is associated with low oxidation potentials, as well as the oxidation of gallic acid. In this sense, it should be noted that the presence of flavonoids in rosemary extracts and carnosic acid in the presence of significant amounts of carnosol has a synergistic antioxidant behavior [34].

The coffee has a particularly flat and wide peak. Among the main polyphenols coffee possesses are hydroxycinnamic acids, caffeic acid, and chlorogenic acid; the latter being two main markers that show high antioxidant capacity [13]. In general, it is possible for caffeic acid to be oxidized in a potential range between 0.55 and 0.60 V (vs Ag|AgCl|KCl$_{sat}$) at a pH of 1.4 [44]. On the other hand, Oliveira-Neto et al. (2016) [13] report that caffeic acid, chlorogenic acid, and a mixture of these with caffeine show a peak in a potential of 0.18 V, 0.22 V, and 0.23 V (vs Ag|AgCl), respectively, at pH 5.0, accounting for the change in behaviors that electroactive molecules can have both when they are isolated, as well as the synergy mechanisms that they present in a complex matrix and the dependence on pH. Furthermore, these authors report the appearance of a second peak in samples of roasted coffee beans between 0.4 and 0.5 V, and a third peak around 0.6 V (vs Ag|AgCl) that is attributed to

different phenolic species which are not found related to the oxidation products of the main markers. It is possible that the peak's width (0.4 V to 0.8 V (vs Ag/AgCl) in our sample) is due to a synergistic effect between components, with a slight difference in oxidation potential, that contribute to the antioxidant capacity of coffee being outstanding, among them, ferulic acid and coumaric acid. The latter together with sinapic acid are not found in green coffee but rather in roasted coffee [45]. To this must be added the products of the Maillard reaction, which are formed during coffee roasting and contribute to the total antioxidant capacity [12]. It is necessary to highlight that antioxidants depend on the variety of coffee, the degree of roasting, and type of preparation, among others [13].

Regarding the wine, it has been reported that red wine has a high content of polyphenols and AC and commented that when comparing foods, the qualitative differences of the bioactive molecules must be taken into account, in addition to the synergistic and antagonistic effects that can be present with other components [46]. Red wine and strawberry juice did not show significant differences ($p < 0.05$), obtaining the highest values in the liquid samples as seen in Fig. 2; these samples share oxidation signals at similar potentials with white wine, orange, and coffee, suggesting that they may have similar compounds with AC.

Red wine shows its maximum peak near 0.5 V (vs Ag/AgCl), this can be attributed to phenolic compounds in wine with high AC that contain in their structure the catechol group in the B ring, such as catechin, epicatechin, quercetin, and phenolic acids, such as gallic and caffeic [36]. The difference between flavonoids with high AC as those mentioned has been determined by the number of hydroxyl groups that the structure contains, for example, myricetin has six hydroxyl groups and a greater capacity to capture radicals than kaempferol with four hydroxyl groups [47]. On the other hand, phenolic compounds with *ortho-* and *para*-diphenol groups are characterized by having lower oxidation potentials, that is, higher AC than structures with meta-diphenol groups or isolated phenols [48].

In the potential range around 0.5 to 0.6 V (vs Ag/AgCl), the coffee, white wine, red wine, and orange samples share signals with a current in which for the first two it is ~ 0.50 µA, and for the remaining samples, it has a value between 2.00 and 2.25 µA (Fig. 1). The signals may be due to the oxidation of some phenolic acids and anthocyanins, among them ferulic acid and flavonoids, such as malvidin (responsible for the pigmentation of red wine) and trans-resveratrol, present in a lower proportion in white wine. A peak shoulder for red wine is observed between 0.7 and 0.8 V; high potentials that are attributed to the second oxidation of the hydroxyl group in position 3 in the C ring of the structure of flavonoids, such as catechin and epicatechin, and phenolic acids (coumaric and vanillic acid) [44].

The above also describes some of the signals produced by strawberry juice. No other studies on strawberry juice using DPV were found; however, Giné Bordonaba and Terry, (2012) [49] studied the electrochemical behavior of pelargonidin, quercetin, myricetin, and cyanidin in strawberry juice more extensively through square wave voltammetry measured with a screen-printed carbon electrode.

Regarding orange juice, it presents a well-defined peak at 0.6 V (vs Ag/AgCl), with the highest peak current over all the other matrices. In addition to the previously mentioned compounds, such as ferulic and coumaric acid, which are oxidized at this potential, the contribution of flavonoids with a large number of hydroxyl groups such as hesperidin, naringin, and narirutin could be expected [50].

3.3 Correlation between spectrophotometric methods and DPV

Table 3 shows the values of the Spearman correlation coefficients between the spectrophotometric methods and the electrochemical technique for all the samples analyzed. The correlation between the charge expressed in equivalent units (Trolox and GA) and the spectrophotometric methods was high, positive, and statistically significant ($p < 0.001$). This indicates that charge values expressed in equivalent units are a reliable tool to analyze the behavior and antioxidant capacity of different food matrices in comparative terms compared to conventional methods, based on a common standard, even though the analytical principles present significant differences.

The correlation between the charge obtained by DPV expressed in equivalent units and the TP analysis is higher (0.7919) compared to the other spectrophotometric methods (0.8366 with ABTS and 0.8875 with FRAP). This suggests that electroanalysis may have a good estimate in the detection of antioxidant compounds, especially phenolic in different food matrices. Some authors have reported a high correlation of DPV measurements with the phenol content

Table 3 Pearson's correlation coefficients between spectrophotometric and electrochemical methods

	Q TE	Q GAE	ABTS	FRAP	T. Phenols
Q	− 0.3789[c]	− 0.4357[c]	− 0.4848[c]	− 0.4836[c]	− 0.5037[c]
Q TE		0.9861[a]	0.7267[a]	0.7935[a]	0.6672[b]
Q GAE			0.8366[a]	0.8875[a]	0.7919[a]
ABTS				0.9885[a]	0.9953[a]
FRAP					0.9756[a]

[a]Significant at $p < 0.001$

[b]Significant at $p < 0.002$

[c]$p > 0.05$

determined by the Folin–Ciocalteu method; $r = 0.98$, using catechin and gallic acid as a standard [36].

On the other hand, we observe that the correlation of all the methods to the charge (Q) is not significant, which leads us to think that this parameter does not allow to predict, by itself, reliably the antioxidant capacity in food matrices of different nature, contrary to what was observed in a previous study in which a positive correlation between Q and spectrophotometric methods can be observed in different parts of the same matrix (mango) [5]. Data obtained just using Q has a limitation related with some other electroactive compounds that could be present in the samples but do not have any antioxidant activity [51]. These compounds can react in the potential window studied and affect the values obtained. This justifies the need to reference the values, either current or charge, with a chemical standard, such as Trolox or gallic acid.

4 Conclusion

The spectrophotometric methods used show a highly significant correlation with the voltammetric charge expressed in equivalent units of Trolox and gallic acid in the different types of matrices analyzed. The correlation between the charge obtained by DPV expressed in equivalent units and the TP analysis is higher (0.7919) compared to the other spectrophotometric methods (0.8366 with ABTS and 0.8875 with FRAP).

Electrochemical techniques are shown as a fast, inexpensive, versatile, and reliable alternative to evaluate antioxidant activity in various foods, with the advantage of generating less waste compared to conventional methods. Additionally, DPV allows a quantitative evaluation and a qualitative approach to electroactive molecules, it is not affected by the interferences that the color of the sample can generate, and it measures the transfer mechanisms of a single electron and hydrogen atom.

Also, it was concluded that the voltammetric charge (without conversion to equivalent units) does not allow inferring the behavior of other variables if it is measured in foods of different nature.

Declarations

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