Flavonoids electrochemical detection in fruit extracts and total antioxidant capacity evaluation

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Abstract
Phenolic compounds detection in fruit extracts from: açaí, bacuri, buriti, blackberry, black mulberry, blueberry, jujuba, physalis, raspberry, and tamarillo, have been performed by reverse-phase high performance liquid chromatography with electrochemical detection (RP-HPLC-EC), using two detectors in series: a wall-jet detector flow cell with a glassy carbon electrode, and a thin-layer flow cell detector with a boron doped diamond electrode. This methodology, in gradient elution mode, was successfully used to detect seventeen phenolic compounds in the fruit extracts. The total antioxidant capacity of the fruit extracts by the electrochemical quantitative index (EI) and the method of capture of diphenilpicrylhydrazil (DPPH) free radical “efficient concentration” (EC50), was evaluated. A very good correlation between EI and EC50 assays has been obtained, the fruit with the highest total antioxidant capacity being blackberry, while physalis exhibited the lowest antioxidant power.

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1. Introduction

Flavonoids are a large class of polyphenolic compounds of low molecular mass, which possess a fifteen carbon skeleton consisting of two benzene rings, linked via a heterocyclic pyrane ring [1,2]. They naturally occur in plants’ flowers and fruits, as well as in their leaves, stems and roots, where they are very important in many functions, including acting as antioxidants [3,4]. Flavonoids have been classified into six subgroups: flavones, flavonols, flavanones, flavan-3-ols, isoflavones, and anthocyanidins [5]. They are plant pigments, which are responsible for the red, blue and purple colours of many fruits, vegetables and flowers [6], making them more appealing to consumers. Apart from being tasty, the consume of some fruits with high antioxidants content is associated with the decrease in the risk of developing different diseases, such as anti-cancer, anti-inflammatory and vasoprotective properties, preventing coronary heart diseases and improving visual acuity [7–9].

Due to their perishable nature, fruits rapidly suffer deterioration, which difficult their commercialisation. Production of frozen fruit pulp or powder extracts is an alternative to take the benefits of fruits, facilitate trading and preserving their properties for a longer time. Antioxidant properties of frozen fruit pulp, as well as of dried fruit powders have been the subject of several studies [10–12]. The oxidation of flavonoids is of great relevance, since they act as antioxidants with the ability to scavenge radicals by an electron transfer process [13]. Electroanalysis, since it only detects the redox active compounds present, is an important methodology to detect flavonoids and evaluate their antioxidant capacity in food-stuffs. Differential pulse voltammetry (DP voltammetry) is a simple and sensitive technique that has the ability to provide relevant information even when used for coloured and turbid samples [14], and for these reasons electrochemical detection has become an alternative to traditional spectrophotometric methods.

The limits of detection and quantification of flavonoids were significantly lowered using the electrochemical detector (EC) because only the redox active compounds in the sample are detected, so the selectivity was increased. It was found that the flavonoids’ detection limit by EC was 20–90 pg/L, 1000 times lower when compared with photodiode array (PDA) detection limit of 12–55 ng/L [15].

The electrochemical behaviour and “antioxidant capacity” can be linked through a previously established relationship: the lower the oxidation potential the greater the antioxidant capacity [16]. Therefore the presence of DP voltammetric signals at low anodic potentials indicates the presence of polyphenolic compounds of high antioxidant capacity, while oxidation at high potentials denotes polyphenolic compounds of low antioxidant capacity [17].

The electrochemical index (EI) is defined as the total phenolics'...
concentration and can be obtained using electrochemical techniques, e.g., DP voltammetry, taking into account the compound peak potential ($E_p$) and peak current ($I_p$) using the following equation [18]:

$$E_I = \left( I_{p1}/E_{p1} \right) + \left( I_{p2}/E_{p2} \right) + \ldots + \left( I_{pn}/E_{pn} \right)$$

The spectrophotometric methods have used different compounds, such as DPPH$^*$ (1,1-diphenyl-2-picrylhydrazine), ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) and DMDP (N,N-dimethyl-p-phenylenediamine), for the determination of the total antioxidant capacity of phenolics [19].

The DPPH$^*$ free radical scavenging assay involves a stable and commercially available radical, is easy to perform, and leads to highly reproducible and accurate results. The decrease in absorbance is monitored at $\lambda$=516 nm and the DPPH$^*$ scavenging radical efficiency is calculated using the equation:

$$\text{DPPH}^* \text{ scavenging effect (ES)} = \left[ \frac{(A_0 - A_1)/A_0 \times 100}{A_0} \right]$$

where $A_0$ and $A_1$ correspond to the absorbance at $\lambda$ =516 nm of the DPPH$^*$ radical in the absence and in the presence of antioxidant, respectively. The total antioxidant capacity is expressed as “efficient concentration” or EC$_{50}$, representing the amount of extract to produce 50% of decolorization of DPPH$^*$ relative to the methanol blank control.

The antioxidant content and the antioxidant capacity of fruits has been evaluated by high performance liquid chromatography (HPLC) with photodiode array (PDA) associated with gas mass spectrometry (MS) detector [11,12,20–23], and electrochemical (EC) detection has been less used [15,24–27]. The voltammetric investigation of fruits is very scarce, and only studies concerning blackberry and raspberry were found [28,29].

The present work focuses on the investigation of the fruit extracts from Euterpe oleracea (açai), Euterpe edulis (juçara), Mauritia flexuosa (buriti), Platonia insignis (bacuri), Morus nigra (black mulberry), Physalis peruviana (physalis), Solanum betaceum (tamarillo), Rubus spp. (blackberry), Rubus idaeus (raspberry) and Vaccinium myrtillus (blueberry), by RP-HPLC-EC and the fruit samples will be referred by their common names.

The RP-HPLC-EC of phenolic compounds in fruit extracts was carried out using two detectors in series: a wall-jet flow cell detector, with a glassy carbon electrode, followed by a thin-layer flow cell detector, with a boron doped diamond electrode. Their electrochemical behaviour has been investigated by DP voltammetry, and the electrochemical index (EI) was determined and compared with the total antioxidant capacity expressed as “efficient concentration” or EC$_{50}$, evaluated by DPPH$^*$ free radical scavenging assay.

2. Experimental

2.1. Reagents and solutions

Delphinidin chloride, (-)-epigallocatechin gallate, ferrulic acid, hesperidin, hyperoside, kuromanin chloride, malvidin chloride, morin, quercetin dihydrate, quercetin-3-O-glucopyranoside, peonidin chloride, peonidin-3-O-rutinoside chloride, pelargonidin chloride, procyanidin A2, rutin and resveratrol were from Extrasynthese (Genay, France). Methanol, 99.8%, Catechin hydrate and 1,1-diphenyl-2-picrylhydrazine (DPPH$^*$) were obtained from Sigma-Aldrich (Steinheim, Germany). Formic acid 98-100% was from Merck (Darmstadt, Germany).

Two mobile phases were used in RP-HPLC-EC and in the DP voltammetry supporting electrolyte, with pH =2.2, in order to maintain the flavilum cation at a higher concentration. They contained Millipore Milli-Q (EMD Millipore, Massachusetts, USA) (Certificate ISO 9001 Quality systems standards) nanopure water, methanol and formic acid in different proportions, mobile phase A (83:16.1 v/v) and mobile phase B (68.5:30.15 v/v).

Stock standard solutions of ~ $10^{-4}$ M phenolic compounds were prepared in the HPLC mobile phase A. The stock solutions were protected from light with aluminium foil, kept in the freezer at ~ 20 °C, and remained stable for at least one month. The stock standard solutions were appropriately diluted to 5 $\mu$M and directly injected into the HPLC system. Working solutions were freshly prepared when needed.

All reagents were of analytical grade and were used without further purification. All solutions were prepared using Millipore Milli-Q nanopure water (resistivity > 18 MΩ cm) and the experiments were performed at room temperature, 25 ± 1 °C.

The volumes were measured using Pipetman pipettes, from Gilson S.A.S. (Villiers-le-Bel, France). The pH measurements were performed with a CRISON 2001 (CRISON Instruments, Barcelona, Spain) micro pH-meter with an Ingold-combined glass electrode. The ultrasound bath used was a Sonorex from Bandelin electronic (Berlin, Germany). The microwave was a Miele, M625 SEG (Gütersloh, Germany).

2.2. Methods, instruments and cells

2.2.1. HPLC

Reverse phase HPLC (RP-HPLC) detection was performed using a Waters 2690 liquid chromatograph, with a Waters 996 photodiode array detector (PDA) and a Concorde wall-jet electrochemical detector (EC) from Waters (Milford, MA, USA). Data acquisition and remote control of the system were done by the Empower Workstation Chromatography Manager from Waters Corporation. The phenolic compounds chromatographic separation was carried out using a 150 mm x 4.6 mm i.d., 5 $\mu$m reversed-phase Inertsil DB ODS-C18 steel column with a guard column of the same type from GL Sciences Inc. (Tokyo, Japan).

The Concorde electrochemical wall-jet flow cell detector was a VT-03 flow cell from Antec Leyden (Zoetewoude, Netherlands), in a three-electrode configuration: a glassy carbon-working electrode (GCE) with 2 mm diameter, an in situ Ag/AgCl reference electrode and a stainless steel auxiliary electrode. The in situ Ag/AgCl reference electrode, referred as ISAAC (in situ Ag/AgCl) is in direct contact with the mobile phase that contains 2 mM KCl. There is a difference of +0.2 V between the potential of the Ag/AgCl (saturated KCl) reference electrode and the ISAAC reference electrode in contact with 2 mM KCl. All measurements were performed at the applied cell potential of $E_{ap} = +0.5$ V vs. ISAAC ($E_{ap} = +0.7$ V vs. Ag/AgCl (saturated KCl) reference electrode).

The electrochemical thin-layer flow cell detector, using a boron doped diamond electrode (BDDE), followed in series the wall-jet cell flow cell detector using a GCE. The amperometric experiments were independent, the thin-layer flow cell detector using an Ivium CompactStat potentiostat, Ivium Technologies B.V. (Eindhoven, Netherlands), in a three-electrode configuration: a BDDE working electrode with an exposed area of 0.64 cm$^2$, a miniaturized Ag/AgCl (3 M KCl) reference electrode, and a stainless-steel tube counter electrode [30]. The BDD film (1.2 cm x 1.2 cm) used as electrode have boron content of the order of 8000 ppm [31] and was prepared at the Centre Suisse de Electronique et de Microtechnique SA (CSEM), Neuchâtel, Switzerland [32]. The applied potential was $E_{ap} = +0.9$ V versus Ag/AgCl (3 M KCl).

Different mobile phases have been described for the analysis of anthocyanins using a reversed phase column [7,33–35]. In the present study, the organic solvent selected for the experiments was methanol due to the solubility of the anthocyanins [6] and formic acid was added in order to ensure the mobile phase low pH =2.2, necessary for flavonoids stability [15] and higher concentration of the flavilum cation.

The HPLC experimental conditions followed a programmed sequence of gradient elution between the mobile phases: solvent A
(water-methanol-formic acid 83:16:1 by volume) and solvent B (water-methanol-formic acid 68:5:30:1.5 by volume) [14,15]. The programmed sequence consisted in isocratic elution 100% solution A, for the first 11 min, followed by linear gradient elution from 100% solvent A to 100% solvent B, between 11 and 21 min, and finally an isocratic elution 100% solvent B, from 21 to 40 min, with the aim to accomplish the complete elution of the fruits’ extract mixture.

The HPLC conditions used were: range = 5 nA, 0.1 s, off = 10%. The mobile phase flow rate was 1.0 mL min⁻¹ and the injected volume was 25 μL. The guard column and the analytical column were kept in a Faraday cage with the thermostatic oven at 40 °C, together with the electrochemical wall-jet flow cell GCE detector.

2.2.2. Electrochemistry

The voltammetric experiments were carried out using the Ivium CompactStat potentiostat, in a one-compartment three-electrode system consisting of a glassy carbon electrode (GCE) or a boron doped diamond electrode (BDDE) as working electrode, a platinum wire as counter electrode and an Ag/AgCl (3 M KCl) as reference electrode.

In order to ensure reproducible results, the GCE was submitted to a cleaning procedure before every electrochemical assay, consisting: (1) polishing with diamond spray (particle size 1 μM, Kem-ent, Kent, UK); (2) rinsing thoroughly with Milli-Q water; and (3) placing into supporting electrolyte and perform DP voltammetry until a constant response was obtained.

The BDDE cleaning procedure has been replaced by an electrochemical treatment, since the BDDE surface cannot be mechanically polished. The cathodic electrochemical treatment consisted in: (1) placing into 1.0 M nitric acid and applying a potential of −3.0 V for 5 min in order to reduce all adsorbed species; (2) rinsing thoroughly with Milli-Q water; and (3) placing into supporting electrolyte and perform DP voltammetry until a constant response was obtained.

The experimental conditions for DP voltammetry were: pulse amplitude 50 mV, pulse time 100 ms, step potential 2 mV and scan rate ν = 5 mV s⁻¹.

2.2.3. Spectrophotometry

The absorbance measurements were recorded on a U-2810 Spectrophotometer Digilab® Hitachi (Tokyo, Japan) with UV Solutions Program. The experimental conditions for absorption spectra were: scan speed 400 nm/min, sampling interval 1.50 nm and path length 1 cm.

All UV-vis spectra were measured for 200 < λ < 800 nm.

2.3. Sample preparation

Some samples used were extracts from fresh fruits from different origins: blackberry (Mexico), blueberry (Chile), physalis, raspberry and tamarillo (Portugal). The black mulberry (Florien, Piracicaba, Brazil) sample was powder from leaves and stalk. All the other samples were powders from fruits.

The fruit powders were prepared using two different methods: 1) spray drying [36,37], in which moisture is removed from the food specimen by rapid evaporation on spray droplet under high temperature exposure; or 2) freeze-drying [37–39], which utilized the mechanism of ice sublimation under low pressure. The dried fruits prepared by spray drying were: açai (Florien, Piracicaba, Brazil) and black mulberry (Facial, Maranhão, Brazil). The dried fruits lyophilized using freeze-drying were: juçara, bacuri and buriti (Maranhão, Brazil).

All extracts from fresh and powders were prepared for analysis in a similar manner following a previously optimized extraction procedure [14,15], and after preparation they were kept in the freezer until analysed.

The weighted powders or fruits have been mixed in a mortar with 3 mL of the extraction solvent, containing hydrochloric acid and methanol 1:99 (v/v), a procedure previously optimized [40], then placed in a microwave, at 450 W during 30 s. The mixtures, placed in vials protected from light, were sonicated in an ultrasound bath at 25 °C, constant frequency of 35 kHz, during 15 min. After sonication, the samples were left at room temperature for 1 h, then centrifuged at 3500 rpm during 20 min and the extract collected in a laboratory flask and stored in the freezer.

The remaining solid sample was submitted to the same procedure, consisting in mixture with extraction solvent, microwave, sonication and centrifuge, for two more times, the only difference was that the sonication time was 30 min in the second procedure.

Finally, the three portions were mixed and the total volume measured. Before the HPLC analysis the extracts were filtered using the 0.45 μm syringe filter (Schleicher & Schuell, Dassel, Germany), and adequately diluted in the mobile phase. The standard extract concentration (mg/mL) has been calculated from the weighted amount of powders or fruits and considering the final filtrated volume.

3. Results and discussion

3.1. Flavonoids HPLC detection

The detection by RP-HPLC-EC using in series the wall-jet GCE and the thin-layer BDDE detectors was carried out (see chromatograms in Fig. 1). The electrochemical analysis allowed the detection of the phenolic standards at much lower concentrations than the photodiode array and without interferences [15]. The PDA detector was only used to confirm the identification of the phenolic standards, based on their UV–vis spectra recorded at λ = 280, 360 and 520 nm.

In order to identify as many phenolic compounds as possible two electrochemical detectors, GCE and BDDE, were used. The BDDE applied potential, +0.9 V vs. Ag/AgCl higher than the potential used at the GCE, +0.5 V vs. ISAA (± 0.7 V vs. Ag/AgCl), enabled the detection of phenolic compounds that were oxidized at a higher potential. The consequence was that more phenolic compound peaks could be detected with the BDDE, but the sensitivity was higher at the GCE. Hence the use of the two electrodes was complementary.

The phenolic standards were tested in order to check their presence in the fruit extracts and to determine their elution order, e.g. their retention times (tᵣ), which are given in Table 1. The delay in the retention time at the BDDE compared with the GCE is due to the time lag as the sample needs to pass through the small plastic tube from the exit of the GCE wall-jet flow cell to the entry of the BDDE thin-layer flow cell.

Twelve flavonoids could be detected using the GCE, and five more were detected using the BDDE (Table 1). The reverse phase column used enabled the separation of the most polar compounds, antocyanidins (delphinidin, malvidin, peonidin) and flavanols (catechin, epigallocatechin gallate) in less than 10 min, followed by antocyanins (kuromanin), and the slowest were the flavonols (rutin, morin, quercitin), in more than 25 min.

The most abundant flavonoid was peonidin-3-O-glucoside, found in 8 extracts from: açai, bacuri, buriti, blackberry, blueberry (Fig. 1A), tamarillo (Fig. 1B), raspberry, and juçara (Fig. 1C). The next predominant flavonoid was peonidin detected in extracts from: buriti, blueberry (Fig. 1A), tamarillo (Fig. 1B), juçara (Fig. 1C), physalis, raspberry, as well as, in black mulberry from leaves and stalk (Fig. 1D).

Catechin was found in 7 extracts from: açai, bacuri, blackberry, blueberry (Fig. 1A), tamarillo (Fig. 1B), raspberry, and juçara (Fig. 1C). The less frequent flavonoids were pelargonidin detected only in physalis and hesperidin found only in açai.
These results showed that HPLC with electrochemical detection presented excellent suitability for the detection and quantification of low levels of electroactive phenolic compounds in fruit extracts. Other methods, such as mass spectrometry (MS) may bring additional information for the identification of some peaks, however this is controversy. It was reported that in açai [11], due to partial elimination of non-anthocyanic flavonoids, a higher number of anthocyanin peaks was detected in the HPLC-PDA-MS/MS system compared with only HPLC-PDA, however there were peaks that were not detected by MS, and hence could not be identified. In another study, for berries extracts, MS only confirmed the antocyanins found by HPLC-PDA and also left some peaks unidentified [23].

3.2. Electrochemical study of fruit extracts

The electrochemical behaviour of all extracts has been investigated by DP voltammetry, in pH = 2.2 supporting electrolyte. This pH value was chosen in order to ensure flavonoids stability [15], higher concentration of the flavilum cation, and to have similar pH conditions to those used for the RP-HPLC-EC detection.
Since by HPLC was found that catechin is one of the most common flavonoids in the extracts, the electrochemical results will be discussed and compared with those of the catechin standard.

Other standards have been also studied by DP voltammetry, such as: procyanidin A2, delphinidin, kuromanin and resveratrol at the same concentration as catechin, 5 mM. However among them, catechin showed the less positive potential and the highest oxidation peak current, which is in agreement with the highest antioxidant capacity. For this reason catechin was chosen for comparison, and the results are shown in Table 2.

The electrochemical behaviour of catechin has been studied [13]. The differential pulse voltammograms (DP voltammograms) in pH = 2.2 (Fig. 2) showed two peaks related to the groups catechol (Ep~ +0.4 V), and resorcinol (Ep~ +0.7 V).

The fruit extracts showed different behaviours. Well defined DP voltammograms oxidation peaks were observed for blackberry (Fig. 2A), blueberry (Fig. 2A), raspberry (Fig. 2A), bacuri (Fig. 2B) and black mulberry fruit (Fig. 2C).

Physalis exhibited very poorly defined four DP voltammograms oxidation peaks between +0.6 and +1.0 V (not shown). The extracts of juçara (Fig. 2B), açai (Fig. 2C) and black mulberry from leaves and stalk (Fig. 2C) showed very similar characteristics: three

<table>
<thead>
<tr>
<th>Extract from</th>
<th>Compound (concentration)</th>
<th>EC50 mg/mL</th>
<th>ARP (mg/mL)</th>
<th>EI nA/mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>Catechin (1.45 μg/mL)</td>
<td>0.006</td>
<td>166.7</td>
<td>0.520</td>
</tr>
<tr>
<td>Freeze-dry powders</td>
<td>Juçara (444.4 mg/mL)</td>
<td>3.6</td>
<td>0.277</td>
<td>0.443</td>
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<tr>
<td></td>
<td>Buriti (672.0 mg/mL)</td>
<td>6.4</td>
<td>0.156</td>
<td>0.481</td>
</tr>
<tr>
<td></td>
<td>Bacuri (364.5 mg/mL)</td>
<td>59.0</td>
<td>0.016</td>
<td>0.172</td>
</tr>
<tr>
<td>Spray-dry powders</td>
<td>Açai (444.5 mg/mL)</td>
<td>37.3</td>
<td>0.026</td>
<td>0.168</td>
</tr>
<tr>
<td></td>
<td>Black mulberry (fruit)</td>
<td>45.0</td>
<td>0.022</td>
<td>0.136</td>
</tr>
<tr>
<td></td>
<td>Black mulberry (leaves)</td>
<td>70.4</td>
<td>0.014</td>
<td>0.093</td>
</tr>
<tr>
<td>Fresh fruits</td>
<td>Blackberry (387.1 mg/mL)</td>
<td>2.45</td>
<td>0.408</td>
<td>0.738</td>
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<td>Blueberry (220.2 mg/mL)</td>
<td>8.91</td>
<td>0.112</td>
<td>0.474</td>
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<td>Raspberry (266.2 mg/mL)</td>
<td>19.9</td>
<td>0.050</td>
<td>0.474</td>
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<tr>
<td></td>
<td>Tamarillo (457.7 mg/mL)</td>
<td>35.4</td>
<td>0.028</td>
<td>0.035</td>
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<td></td>
<td>Physalis (765.4 mg/mL)</td>
<td>81.5</td>
<td>0.001</td>
<td>0.003</td>
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</tbody>
</table>

Fig. 2. DP voltammograms in pH = 2.2 of (A) blackberry, blueberry and raspberry extracts at GCE, diluted 10 times, (•) (B) juçara, bacuri and Buriti extracts at GCE, diluted 2 times, (C) açai, black mulberry fruit and black mulberry leaves and stalk extracts at GCE, not diluted and (D) 5 μM catechin standard at GCE and BDDE. Scan rate 5 mV s⁻¹.
oxidation peaks, the better defined peak at $\sim +0.8$ V.

Two DP voltammogram oxidation peaks occurred for bacuri (Fig. 2B), buriti (Fig. 2B) and tamarillo (not shown). At least five extracts: blackberry, black mulberry, blueberry, raspberry and tamarillo, exhibited an oxidation peak $\sim +0.45$ V, which can be ascribed to the catechol group. In all cases, a better response was obtained at GCE comparing with BDDE, similar with that illustrated for catechin (Fig. 2D). This behaviour can be explained taking into account that the fruit extracts are rich in polyphenolic compounds that strongly adsorb on GCE surface, while the adsorption at the BDDE surface was much less.

The electrochemical quantitative index (EI) values obtained for the fruit extracts are shown in Table 2, together with those for the catechin standard. The electrochemical quantitative index (EI) values were calculated using the DP voltammograms obtained at GCE. The highest antioxidant capacity, higher than catechin, was obtained for blackberry, followed by buriti, then blueberry, and raspberry. The lowest antioxidant capacity was obtained for physalis, tamarillo and black mulberry, from leaves and stalk.

Although the highest antioxidant capacity was exhibited from a blackberry fresh fruit extract, other extracts such as buriti and juçara also showed high antioxidant capacity, indicating that dried fruits can also maintain elevated antioxidant capacity. The electroanalytical methods were very useful tools for the evaluation of low concentrations of polyphenols and their antioxidant capacity of fresh and dried fruits during storage or transport.

Nevertheless, flavonoids are modified after metabolism [41,42], hence the fruit extract antioxidant capacity determined here cannot be directly transposed to human health benefits. A more exact comparison can be made and a correct order can be established analysing all the extracts prepared in the same initial manner, e.g. all spray dried, all freeze dried or all fresh.

### 3.3. Spectrophotometric determination of antioxidant activity

The total antioxidant capacity of the different extracts has been determined using the DPPH* free radical scavenging assay at different incubation times from 0 to 30 min, as well as different extracts concentrations, ranging from 3 to 130 mg mL$^{-1}$. Most extracts, like açai, bacuri, black mulberry from leaves and stalk, buriti and physalis, showed an increase in the reaction rate with increasing extract concentration (not shown). A similar behaviour was also observed for juçara, raspberry and black mulberry; however for these the differences were less obvious between different concentrations. The extracts of blueberry, blackberry and tamarillo tended to reach reaction equilibrium for a lower concentration.

Regarding the reaction time different behaviours were observed. All extracts showed a biphasic reaction with DPPH*: a fast decay in absorbance in the first minutes (Fig. 3A), followed by a slower step, involving degradation products, until an equilibrium was reached [39] (Fig. 3B). Some differences were observed for the extracts. All the spray-dry powders and one of the freeze-dried samples, bacuri, exhibited a very fast reaction in the first 5 min and then tended to reach equilibrium. One fresh fruit, physalis, exhibited similar kinetics, a fast reaction with DPPH* was observed with the highest slope in the first 10 min. The extracts from the other fresh fruits exhibited a slow reaction in both phases, although blackberry and tamarillo reacted less slow.

The amount of antioxidant necessary to decrease the absorbance of DPPH* by 50% of the initial absorbance, “efficient concentration” or EC$_{50}$, as well as antiradical power, ARP = 1/EC$_{50}$, in order to evaluate the total antioxidant capacity of the different fruit extracts (Table 2), were determined.

The results were divided and discussed by groups of extracts because was observed a general trend if the initial method of preparation before extraction was taken into account. In this way for the freeze-dried powders the lowest EC$_{50}$, corresponding to highest ARP value, was obtained for juçara, followed by buriti and bacuri. For spray-dried powders, the highest antioxidant power was found for açai and the lowest for black mulberry. For the fresh fruit extracts, the most antioxidant compound was blackberry and the less was physalis.

Generally, much higher EC$_{50}$ values were obtained for the fruit extracts compared with the catechin standard; blackberry has the lowest EC$_{50}$ value from all extracts, which means that it has the highest antioxidant capacity and physalis the poorest antioxidant capacity.

A good correlation with the results obtained from the DP voltammograms was achieved, since the EC$_{50}$ and EI are inversely proportional [14]. In the same group, the compound with the lowest EC$_{50}$ exhibited the highest EI, showing the accuracy of both methods for the total antioxidant capacity evaluation.

Therefore, determination of EI and/or EC$_{50}$ is a good alternative for evaluating the antioxidant capacity of fresh or dried fruits and...
shows that dried fruits can maintain high antioxidant capacity, which is crucial during storage and transportation, important steps for fruits industry and commercialisation.

4. Conclusions

The proposed RP-HPLC-EC method enabled the EC detection in series, using two different detectors: wall-jet flow cell with GCE and thin-layer flow cell with BDDE, of seventeen phenolic compounds present in dried and fresh fruit extracts. The EI and EC<sub>aq</sub> assays determination of fruit extracts total antioxidant capacity showed a good agreement and allowed to order the total antioxidant capacity, from blackberry, with the highest antioxidant capacity, to physalis, with the lowest antioxidant capacity, of the fruit extracts investigated. A more exact order can be established considering the initial preparation method. Extracts, from dried fruits exhibited high antioxidant capacity, such as jujura and buriti, showing that this form of fruit storage is a good alternative for fresh fruit. These results can contribute for increasing the commercialisation of some dried fruits, which in the fresh form would never reach destination.

The electrochemical detection allowed the determination of much lower concentrations of the analyte and without interferences comparing with the spectrophotometric one. These results showed the excellent sensitivity of electrochemical detection, and its suitability for the detection of low levels of electroactive phenolic compounds in dried and fresh fruit extracts.

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