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# Electrochemical behaviour of polyphenol rich fruit juices using disposable screen-printed carbon electrodes: Towards a rapid sensor for antioxidant capacity and individual antioxidants

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ABSTRACT

The analysis of antioxidants in different foodstuffs and especially fruits has become an active area of research which has lead to numerous antioxidant-assays being recently developed. Many antioxidants exhibit inherent electroactivity, and hence employing electrochemical methods could be a viable approach for evaluating the overall antioxidant capacity of a fresh produce matrix without the need for added reactive species. This work shows the possibility of using square wave voltammetry (SWV) and other electrochemical methods with disposable screen-printed carbon electrodes, to quantify and assess antioxidant activity and abundance of specific antioxidants, mainly polyphenols in selected soft fruit juices. Freshly squeezed black currant and strawberry juices of different cultivars and maturity stages were chosen according to known differences in their antioxidant profile. As a result of the increasing applied potential (0–1000 mV vs. Ag/AgCl) the electroactive compounds present in the juices were oxidised leading to a characteristic voltammetric profile for each of the samples analysed. Generally, black currant juices had greater oxidation peaks at lower potentials (<400 mV) which were indicators of higher antioxidant capacities. The relationship between sensor cumulative responses at different applied potentials and total or individual antioxidants, as determined by conventional spectrophotometric methods (FRAP, Folin–Ciocalteu) and HPLC (individual anthocyanins and ascorbate), respectively, are discussed in the context of the development of a rapid sensor for antioxidants.



## 1. Introduction

The vast majority of biochemical reactions which ensure life are associated with the production of free radicals, which in turn lead to oxidative stress and associated damage to the living organism. Complex biochemical pathways within the human body are responsible for fighting oxidative stress by ensuring an appropriate balance between prooxidants (i.e. free radicals) and antioxidants. Epidemiological data have strongly suggested an inverse correlation between the intake of fruits and vegetables (FAV), naturally rich in antioxidants, and the incidence of certain diseases (viz. cancer, cardiovascular disorders, diabetes) [1–3]. Dietary antioxidants play some role in maintaining an optimum oxidative balance within the body [1–3], and hence, it is not surprising that the analysis of antioxidants or antioxidant capacity (AC) in different foodstuffs and beverages has become an expanding area of research [4].

Both black currant and strawberry fruits, despite their different composition, are amongst the richest sources of antioxidants, especially anthocyanins [5,6] and other phenolic compounds as well as ascorbic acid [7–9]. Recently, Wolfe et al. [10] and Haleem et al. [11] reported that strawberry fruits were amongst the top sources of antioxidants from FAV in the American and Scottish populations, respectively. Nevertheless, comparison of AC between different food sources still remains challenging due to the diversity of AC assays found in the literature [4]. Antioxidant capacity assays rely on two different reaction mechanisms: whether hydrogen atom transfer (HAT) or single electron transfer (SET) are responsible to deactivate radicals [4]. HAT-based assays monitor competitive reaction kinetics and these would include common assays such as oxygen radical scavenging capacity (ORAC) and the total radical-trapping antioxidant parameter assay (TRAP). In contrast, SET-based assays involve one redox reaction with the antioxidants and include standardised methods in the food arena such as Trolox equivalent antioxidant capacity assay (TEAC), ferric ion reducing antioxidant parameter assay (FRAP) and the ubiquitous Folin–Ciocalteu-based total phenolics assay [4,12]. Nevertheless, most of these aforementioned methods are based on spectrophotometric techniques which are relatively costly and not always

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appropriate to be routinely applied for screening large sample sets. In addition, many antioxidant assays suffer from interference when working with colourful or turbid samples [13] or when other compounds (i.e. vitamin C, sugars) rather than just the target analytes are abundantly present in the samples [13,19].

Many antioxidants exhibit inherent electroactivity, acting as reductants in solutions [14]. Therefore, employing electrochemical methods could be a viable approach for evaluating the overall reducing power of antioxidant compounds within a fresh produce matrix without the need for added reactive species [12]. In fact, over the past years, electrochemical techniques have been used, mainly as HPLC detection systems [15], flow injection measurements [16] and, to a lesser extent, for direct determination of antioxidants at inner electrodes [17,18]. No published studies, thus far, have investigated the potential application of disposable non-mediated screen-printed carbon electrodes for direct determination of antioxidants in berries or berry-derived products. Accordingly, the aims of this study were first to study the electrochemical behaviour of natural antioxidants and freshly squeezed berry juices (viz. black currant and strawberry) on screen-printed carbon electrodes and principally to assess the relationship between the electrochemical signals and the composition of the fruit juices with particular emphasis to their antioxidant capacity.

## 2. Materials and methods

### 2.1. Reagents

Anthocyanin standards (viz. cyanidin-3-glucoside, cyanidin-3-rutinoside, delphinidin-3-glucoside, malvidin-3-glucoside and pelargonidin-3-glucoside) were purchased from Extrasynthese (Genay, France). All other reagents and standards, including quercetin and myrecitin, were of HPLC-analytical grade and purchased from Sigma (Dorset, UK). Solvents were prepared immediately before analysis, unless otherwise stated.

### 2.2. Apparatus and electrochemical measurements

All electrochemical measurements were performed using a Palmsens potentiostat (Palm Instruments BV, The Netherlands) and results processed using Ivium Palmsens PC software (Palm Instruments BV, The Netherlands). Screen-printed electrodes (SPE) (Gwent Electronic Materials Ltd., GEM, Gwent, UK) were used for all measurements. The electrodes were screen-printed in a two electrode configuration comprising a generic carbon working electrode (28 $mm^2$) and a combined Ag/AgCl reference/counter electrodes onto a PVC substrate. A similar electrode configuration was described in an earlier work [19].

Sensor measurements were taken using either standards or juice samples after adjusting the pH to 4 or 7, which corresponded to moderated acid pH as commonly encountered in fruit juices or strawberry-based beverages, or neutral pH, respectively. The pH of the buffer/electrolyte solutions was determined using a JENWAY 3020 pH meter (Jenway, Essex, UK) and adjusted using NaOH 0.1 M. A 50 μl aliquot of diluted sample solution (1:5, v/v; see Section 2.3) or standard was deposited onto the surface of the electrode in order to complete the electrochemical cell. Immediately after sample deposition, square wave voltammograms (SWVs) were recorded by establishing the appropriate scan rate (50 mV $s^{-1}$) and scanning from 0 to 1.2 V. All experiments were carried out at 22 °C.

### 2.3. Plant materials and sample preparation

Black currant samples from three different cultivars (Ben Dorain, Ben Gairn and Ben Tirran) and harvested at different maturities (early ripe (ER), ripe (R) or fully ripe (FR)) were chosen due to known differences in their antioxidant profile [20]. Different maturities, within the range of commercial maturity, were selected when fruits presented different colourations (ER, FR and OR based on 90, 95 and 100% of black colouration, respectively) and total soluble solids (TSS). Similarly, strawberry samples from six different cultivars (viz. Christine, Elsanta, Florence, Jubilee, Sonata and Symphony) were grown under commercial practices and supplied by H.H. Duncalfe (Cambs., UK). A study on the composition of the strawberry samples described herein was detailed in an earlier paper [19]. Frozen berries (50 g) were diluted in phosphate buffer (500 mL), homogenized using a domestic blender for 1 min and the solution obtained (sample solution) was filtered through Whatman No. 2 filter paper prior to sensor analysis (Section 2.2). HPLC and antioxidant capacity analysis, except for the different antioxidant fractions, were performed on freeze-dried samples as described earlier [20,21].

### 2.4. Characterisation of berry antioxidants

The separation of anthocyanin and non-anthocyanin fractions was performed on a Waters C18 solid phase extraction (SPE) vacuum cartridges (Waters, Watford, UK) as described elsewhere [22], with some modifications. Briefly, cartridges were preconditioned by sequentially passing 10 mL of ethyl acetate, 10 mL of acidified aqueous methanol (MeOH:$H_2O$; 70:30, v/v) and 10 mL of 0.01 N aqueous HCl. Thereafter, 3 mL of black currant juice (fully ripe berries from cv. Ben Gairn; 20 g fruit and 180 mL Millipore $H_2O$) and strawberry juice (cv. Elsanta; 40 g fruit in 260 mL Millipore $H_2O$) sample were loaded into each cartridge. Cartridges were washed with 6 mL of 0.01 N aqueous HCl to remove sugars, organic acids and other water soluble compounds (fraction 1; F1). Following removal of water soluble compounds, cartridges were dried by continuously applying a pump generated vacuum for 5 min. Non-anthocyanin polyphenolic compounds were eluted from the cartridge by using 20 mL of ethyl acetate (fraction 2; F2). Finally, absorbed anthocyanins (fraction 3; F3) were eluted from the cartridges with 6 mL of 70:29.5:0.5 (v/v/v; MeOH:$H_2O$:HCl). All fractions (F1, F2 and F3) were separately collected and 3 mL of each sample was placed in amber glass vials and stored briefly at −40 °C. Solvents were removed overnight in an Edwards Modulyo freeze-drier and resuspended in 0.1 M PBS for FRAP and sensor measurements.

The HPLC system comprised an Agilent 1200 series HPLC system (Agilent, Berks., UK), equipped with an Agilent 1200 DA G1315B/G1365B photodiode array with multiple wavelength detector (DAD). Individual anthocyanins were extracted by mixing freeze-dried samples (150 mg) with 3 mL of HPLC grade methanol:HCl:water (70:0.5:29.5; v/v/v) and the anthocyanin profile determined as described recently [21] by means of HPLC-DAD (520 nm). Similarly, ascorbic acid concentrations in strawberry and black currant fruits were measured after a simple aqueous extraction of the freeze-dried material [6,23] and analysed by HPLC-DAD (210 nm).

Total phenolic concentrations and antioxidant capacity of the fruits, and different polyphenolic fractions, were quantified from freeze-dried or fresh-frozen material, respectively, as described earlier [6]. Briefly, total phenolic concentrations were measured by means of the Folin–Ciocalteu method (FCM) and total antioxidant capacity measured by the FRAP assay as described in recent works [6].

### 2.5. Data analysis

All statistical analyses were carried out using Genstat for Windows Version 12 (VSN International Ltd., Herts., UK). Data presented corresponds to the mean of minimum triplicate measurements. Tests for correlations between mean values were made

using Spearman's Rank correlation. Hence, correlations are given with the Spearman's correlation coefficient ($r$) based on a two-tailed test only when $P < 0.01$. Regression analysis was performed in order to explain the possible relationship between the cumulative sensor responses ($Q$) at different formal potentials (300, 500 and 1000 mV) and antioxidant capacity (FRAP), total phenolics (TP), ascorbic acid and anthocyanin content in different berry juices.

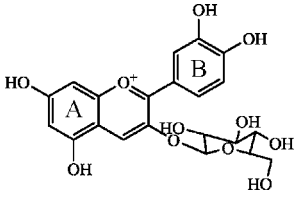
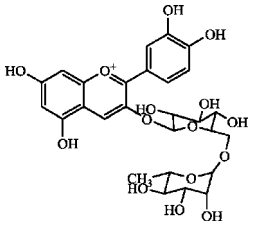
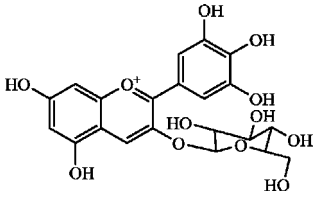
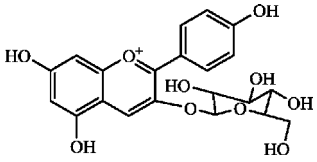
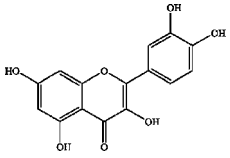
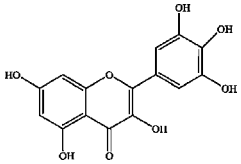
## 3. Results and discussion

### 3.1. Electrochemistry of standard antioxidants on screen-printed carbon electrodes

Considering the reducing properties, which indeed are somehow related to their antioxidant capacities, different berry antioxidants (viz. cyanidin-3-rutinoside, delphinidin-3-glucoside, pelargonidin-3-glucoside, myricetin and quercetin; Table 1) were analysed by means of square wave voltammetry (SWV) using disposable screen printed electrodes. The reducing properties of the different antioxidants were assessed by the potential at which the compounds were oxidised (Table 1), whereas the antioxidant capacity of the same compounds was directly related to the area under the peak obtained when using SWV. Disposable sensors are of particular interest for the analysis of antioxidants because during the oxidation process of these compounds (i.e. phenolics) coupling between two radicals may lead to the formation of a polymeric film which in turn inactivates the electrode [24].

All the compounds measured acted as powerful antioxidants and were easily oxidised on screen-printed carbon electrodes. Even though different electrochemical techniques and electrode configurations were used, the electrochemical behaviour of the different compounds analysed agreed with those found in the literature [17,18,25–27]. Cyclic voltammetry (CV) has been, so far, the method of choice when analysing antioxidants in wines [28], fruit juices [18]

**Table 1**
Voltammetric behaviour (viz. number of peaks and peak potential (mV)) on disposable screen printed carbon electrodes, and chemical structure of berry antioxidants studied under different pH conditions.

| Compound[a] | Phenolic class | Chemical structure | pH studied | Peaks | Oxidation potential (mV) |
|---|---|---|---|---|---|
| Cyanidin-3-glucoside | Anthocyanin |  | 4 | I | 300 |
| | | | 7 | I | 258 |
| | | | 4 | II | 820 |
| | | | 7 | II | 763 |
| Cyanidin-3-rutinoside | Anthocyanin |  | 4 | I | 296 |
| | | | 7 | I | – |
| | | | 4 | II | 818 |
| | | | 7 | II | – |
| Delphinidin-3-glucoside | Anthocyanin |  | 4 | I | 275 |
| | | | 7 | I | 249 |
| | | | 4 | II | 800 |
| | | | 7 | II | 747 |
| Pelargonidin-3-glucoside | Anthocyanin |  | 4 | I | 318 |
| | | | 7 | I | 299 |
| | | | 4 | II | 828 |
| | | | 7 | II | 790 |
| Quercetin | Flavonol |  | 4 | I | 207 |
| | | | 7 | I | – |
| Myricetin | Flavonol |  | 4 | I | 168 |
| | | | 7 | I | – |

(–) Analysis for these compounds was not performed at pH 7.
[a] Anthocyanins and flavonol concentration were 0.05 and 0.1 mg mL$^{-1}$, respectively.

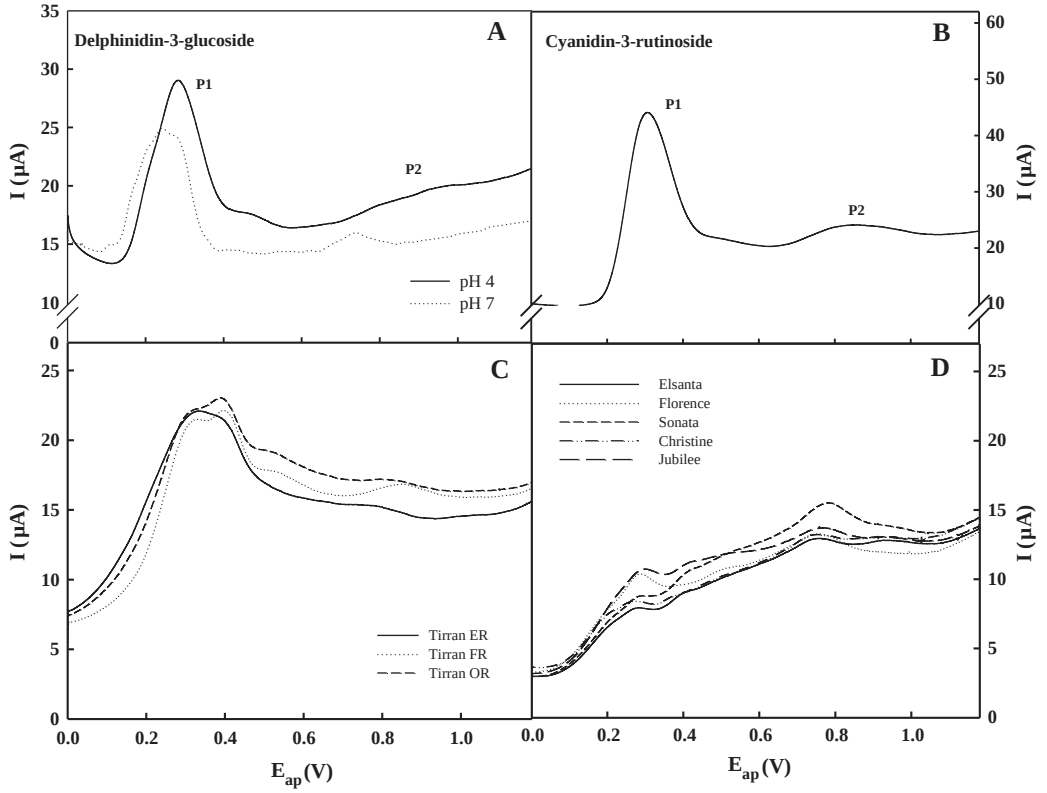



**Fig. 1.** Square wave voltammograms of: (A) delphinidin-3-glucoside and (B) cyanidin-3-rutinoside standards diluted in 0.1 M PBS (pH 4 and/or 7; P1 and P2 represent the first and second oxidation peaks, respectively); (C) different black currant and (D) strawberry juice samples diluted in 0.1 M PBS at pH 4. Samples in (C) and (D) corresponded to juices of black currant cultivars (cv. Ben Tirran) harvested at three different maturities (early ripe (ER), fully ripe (FR) and over-ripe (OR)) and strawberry samples from different cultivars (viz. Christine, Elsanta, Florence, Jubilee and Sonata), respectively.

and even plant extracts [29]. In the present study, SWV was chosen rather than CV. Besides sharing most of the advantages described for CV [18], SWV allows for easy interpretation of the results, rejects background current 'noise' and can have very low detection limits. In addition, SWV enables, while performing the scan, one to observe whether an oxidation reaction is reversible or not, since the current is sampled in both positive and negative pulses. Accordingly, oxidation and reduction peaks can easily be obtained in the same experiment [25] allowing for direct determination of specific compounds.

The square wave voltammogram for pelargonidin-3-glucoside showed two peaks, the first one (P1), reversible at 318 mV and the second one (P2), irreversible at 828 mV (Table 1). Similar results were obtained for cyanidin-3-rutinoside (Fig. 1) which at pH 4 showed two peaks at 296 and 820 mV. No significant changes were observed in P1 of either cyanidin-3-glucoside or cyanidin-3-rutinoside, as was also highlighted by Janeiro and Oliveira-Brett [30] indicating that differences in the sugar moiety attached to the anthocyanidins (Table 1) did not change the first oxidation peak (P1) (Table 1). In this context, the first oxidation peak for each anthocyanin corresponded to the oxidation of the OH groups in the B ring of the molecule whereas the peak at greater potentials was most probably related to the 5,7-dihydroxyl moiety of the A ring [27,30]. Besides anthocyanins, both quercetin and myricetin were also investigated. Their voltammetric profile differed significantly from that observed for anthocyanin molecules, since the first oxidation peak was observed for both compounds at lower potentials (150–200 mV) (Table 1). In contrast, using a glass carbon electrode, quercetin led to two oxidation peaks [31]. Lower oxidation potentials for these compounds, but in particular for quercetin, were also reported by others [31,32] when investigating different natural antioxidants using CV. It is well accepted that adjacent hydroxy groups, as in the catechol (1,2-dihydroxy; cyanidin) and pyrogallol (1,2,3-trihydroxy; delphinidin), stabilise the phenoxy radical leading to a lower oxidation potential of the compound and hence accounting for the potential shift observed herein and elsewhere [15,30]. Lower oxidation potential has been referred as high antioxidant power [16] and consequently, the results presented herein point to the following sequence: myricetin > quercetin > delphinidin > cyanidin > pelargonidin.

In all the compounds measured, the voltammetric profile depended on the pH of the solution (Table 1). It has been accepted that oxidation of polyphenols in phosphate buffers mimics physiological conditions [31]. A neutral pH resulted, for all the compounds measured, in a shift of all oxidation peaks towards lower potentials and agreed well with the observations made by others [17]. Structural changes in anthocyanins in solutions of different pH are well documented [27]. The pH dependency observed herein and elsewhere [12,31] demonstrates that not only $e^-$ but also $H^+$ may be involved in the oxidation of polyphenols at the inert electrodes. Nevertheless, further experiments should try to elucidate the mechanisms of these reactions.

When SWV is applied to samples containing antioxidants, anodic peaks may refer to specific compounds with their concentration being proportional to the intensity of the peak. Accordingly, a very good correlation coefficient was obtained when the sensor was challenged with increasing concentrations of standard solutions of cyanidin-3-rutinoside ($r > 0.988$; data not shown) or when standard additions of pelargonidin-3-glucoside, the main anthocyanin found in strawberry fruits [21], were mixed with freshly extracted strawberry juice and the area under P1 was recorded ($r > 0.996$) (Fig. 2). It is worth-mentioning that different screen-printed electrodes were used for each measurement and hence the variability, as shown by the standard deviation in Fig. 2, depicts

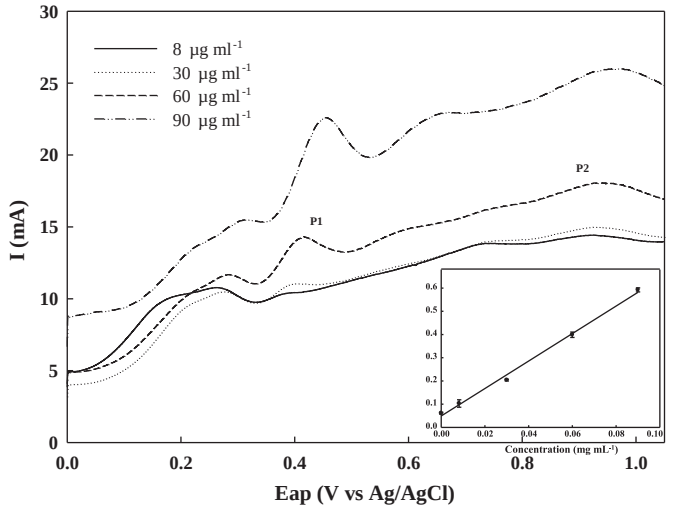



**Fig. 2.** Square wave voltammograms of strawberry juice (cv. Symphony) diluted in 0.1 M PBS and adjusted to pH 4, after standard additions of pelargonidin-3-glucoside solutions at different concentrations (P1 and P2 represent the first and second oxidation peaks, respectively). Insert shows calibration curve after standard additions of increasing concentrations of pelargonidin-3-glucoside.

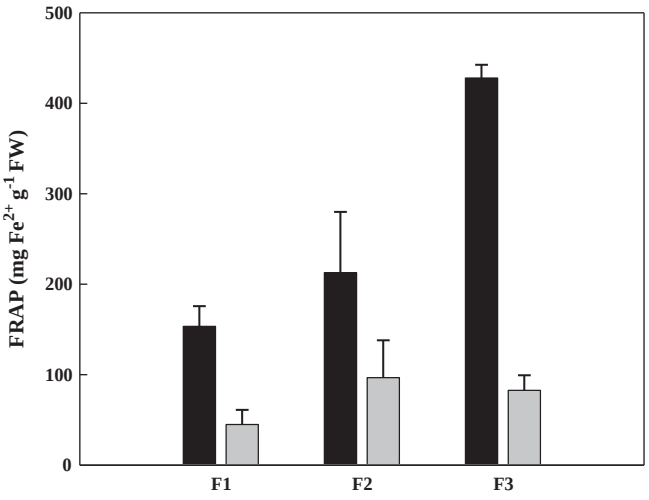



**Fig. 3.** Antioxidant capacity measured by the FRAP assay from different strawberry (▬, cv. Elsanta) and black currant (–, cv. Ben Tirran) fractions. F1, organic acids and sugars; F2, non-anthocyanins polyphenolic fraction and F3, anthocyanin fraction. Error bars indicate standard deviation for $n = 3$.

the reproducibility when considering different sensors and measurement conditions. When comparing the voltammetric profile of pelargonidin-3-glucoside standard solutions added in either PBS (Table 1) or strawberry juice (cv. Symphony; Fig. 2), it was noticed that both P1 and P2 were shifted towards more positive potentials when the standard solutions were added in strawberry juice. Similar findings were observed by [27] who highlighted the importance of the solvent matrix to stabilise the phenolic groups present in the anthocyanins investigated in this study. From these findings, it is therefore plausible to speculate that other compounds, commonly present in strawberry juice, may lead to a stabilisation of the antioxidants and hence account for the greater potentials required for their oxidation.

### 3.2. Electrochemical characterisation of black currant and strawberry juices

As a result of the increasing applied potential (0–1200 mV vs. Ag/AgCl) the electroactive compounds present in either black currant or strawberry juices were oxidised leading to a characteristic voltammetric profile for each of the samples analysed (Fig. 1). Generally, black currant juices had greater oxidation peaks at lower potentials (<500 mV) which indicated the greater antioxidant capacity (Fig. 1) of these berries as compared to strawberry fruits (Fig. 3).

In both berry-based juices, the first oxidation wave (W1) occurred at ~300 mV, followed by a second one (W2) at ~500 mV and the last (W3) around 800 mV (Table 2). The area underneath the peak current, corresponding to cumulative response at specific potentials, has earlier been proposed as an indicator of antioxidant capacity of complex mixtures of antioxidants [14,29]. In the present study, cumulative responses at the end of these oxidation waves are shown in Table 2. If compared to standard compounds, the broadness of the peaks in either black currant or strawberry fruits was greater than that obtained when working with standard compounds (Fig. 1) which, in agreement with previous works [33], may be related to a combined effect between different antioxidant compounds with similar formal oxidation potentials.

To further understand the electrochemical behaviour of the samples analysed, anthocyanins were separated from non-anthocyanin polyphenols and other antioxidant compounds by solid-phase extraction [22]. AC from the different fractions was then measured electrochemically and verified using the standard FRAP assay. Regardless of the quantification method used, anthocyanins represented most of the antioxidants present in black currant fruits, with AC values 2-fold greater than that of other polyphenolic compounds (F1) and/or water soluble compounds (F2), including ascorbate. In strawberry fruits both anthocyanin and other phenolic compounds (F3 and F2, respectively) were the fractions showing greater AC followed closely by ascorbate and other water soluble compounds (Fig. 3). Several authors [6,21] have reported the poor correlation between anthocyanin concentration in strawberry fruits and AC, which is not surprising given that these compounds accounted for no more than $38 \pm 5\%$ of the fruit AC (Fig. 3). In contrast, the black currant–anthocyanin fraction accounted for >55% of the total fruit AC, and hence anthocyanin concentrations measured by HPLC correlated well with FRAP values (Fig. 4) and agreed with that found by others [34].

The voltammograms for each fraction differed considerably between black currant and strawberry fruits. F1, which compromises a variety of water-soluble compounds, including ascorbate, showed for both samples a first oxidation wave at ~200 mV followed by a second oxidation at ~900 mV (data not shown). The greater intensity of the peak maxima at ~200 mV in black currants as compared to strawberry fruits, clearly reflected differences of up to 6-fold in ascorbate concentrations between these fruits as found by others [6,8,9,35]. Even though ascorbate standard solutions per se were not assessed in the present study, it is known that oxidation of the catecholic structure of ascorbic acid occurs at low potentials [33]. In diluted black currant juice, the oxidation wave for F2 overlapped that from F1 (~200 mV), whereas for the same fraction in strawberry juice the oxidation wave occurred at potentials as low as ~110 mV. Differences in the polyphenolic composition, besides anthocyanin, of black currant and strawberry fruits are well known [5,35,36]. For instance, strawberry fruits are rich sources of ellagic acid and ellagitannins which contain several pyrogallol groups and hence may be oxidised at very low potentials [5]. In fact, under the conditions described by Aaby et al. [15], ellagic acid and ellagic acid glycosides had dominant oxidation potentials at ~300 mV with no further oxidation, whereas ellagitannins started to be oxidised at 100 mV but had dominant oxidation potential at the end of 800 mV. Conversely, no studies so far, have reported high concentration of ellagic acid or ellagitannins in black

**Table 2**
Oxidation waves[a] and cumulative responses at three oxidation potentials of black currant and strawberry juices and different juice fractions diluted in 0.1 M PBS (pH 7).

| Sample | Cultivar | Max. peak[c] | Cumulative peak areas[b] | | |
|---|---|---|---|---|---|
| | | | 300 | 500 | 1000 |
| Strawberry | Christine | 790 | 2.057 ± 0.09 | 3.870 ± 0.20 | 9.780 ± 0.31 |
| | Elsanta | 790 | 2.268 ± 0.12 | 4.537 ± 0.03 | 11.602 ± 0.45 |
| | Florence | 790 | 2.313 ± 0.21 | 4.618 ± 0.50 | 11.192 ± 0.28 |
| | Jubilee | 790 | 2.161 ± 0.14 | 4.788 ± 0.41 | 12.016 ± 0.15 |
| | Sonata | 790 | 2.683 ± 0.05 | 5.350 ± 0.11 | 13.459 ± 0.32 |
| Black currant | Ben Tirran (ER)[d] | 310 | 4.006 ± 0.22 | 8.01 ± 0.16 | 15.743 ± 0.27 |
| | Ben Tirran (FR) | 400 | 3.365 ± 0.41 | 7.505 ± 0.28 | 15.78 ± 0.33 |
| | Ben Tirran (OR) | 400 | 3.781 ± 0.26 | 8.11 ± 0.19 | 16.802 ± 0.51 |
| | Ben Dorain | 320 | 3.865 ± 0.09 | 7.646 ± 0.10 | 17.798 ± 0.17 |
| | Ben Gairn | 310 | 3.543 ± 0.36 | 8.326 ± 0.29 | 15.004 ± 0.21 |
| Strawberry (cv. Elsanta) | F1 | >900 | 0.318 ± 0.03 | 0.562 ± 0.06 | 2.574 ± 0.10 |
| | F2 | 110 | 0.760 ± 0.03 | 1.273 ± 0.04 | 3.105 ± 0.08 |
| | F3 | 210 | 1.162 ± 0.02 | 2.174 ± 0.13 | 5.776 ± 0.14 |
| Black currant (cv. Ben Tirran) | F1 | >900 | 0.779 ± 0.08 | 1.283 ± 0.04 | 4.817 ± 0.12 |
| | F2 | 190 | 2.256 ± 0.06 | 3.528 ± 0.11 | 7.475 ± 0.10 |
| | F3 | 300 | 2.038 ± 0.02 | 3.775 ± 0.03 | 8.168 ± 0.09 |

[a] Oxidation waves rather than peaks are considered given that different antioxidant compounds may have similar formal potentials and contribute for the same oxidation wave.
[b] Cumulative peak areas were calculated considering the response across several potentials and results expressed as μC mmol sample$^{-1}$ (analysis were performed in triplicate and expressed as means ± standard deviation).
[c] Potential at which the peak with the greatest intensity was recorded.
[d] ER, early ripe, FR, fully ripe; OR, over-ripe berries.

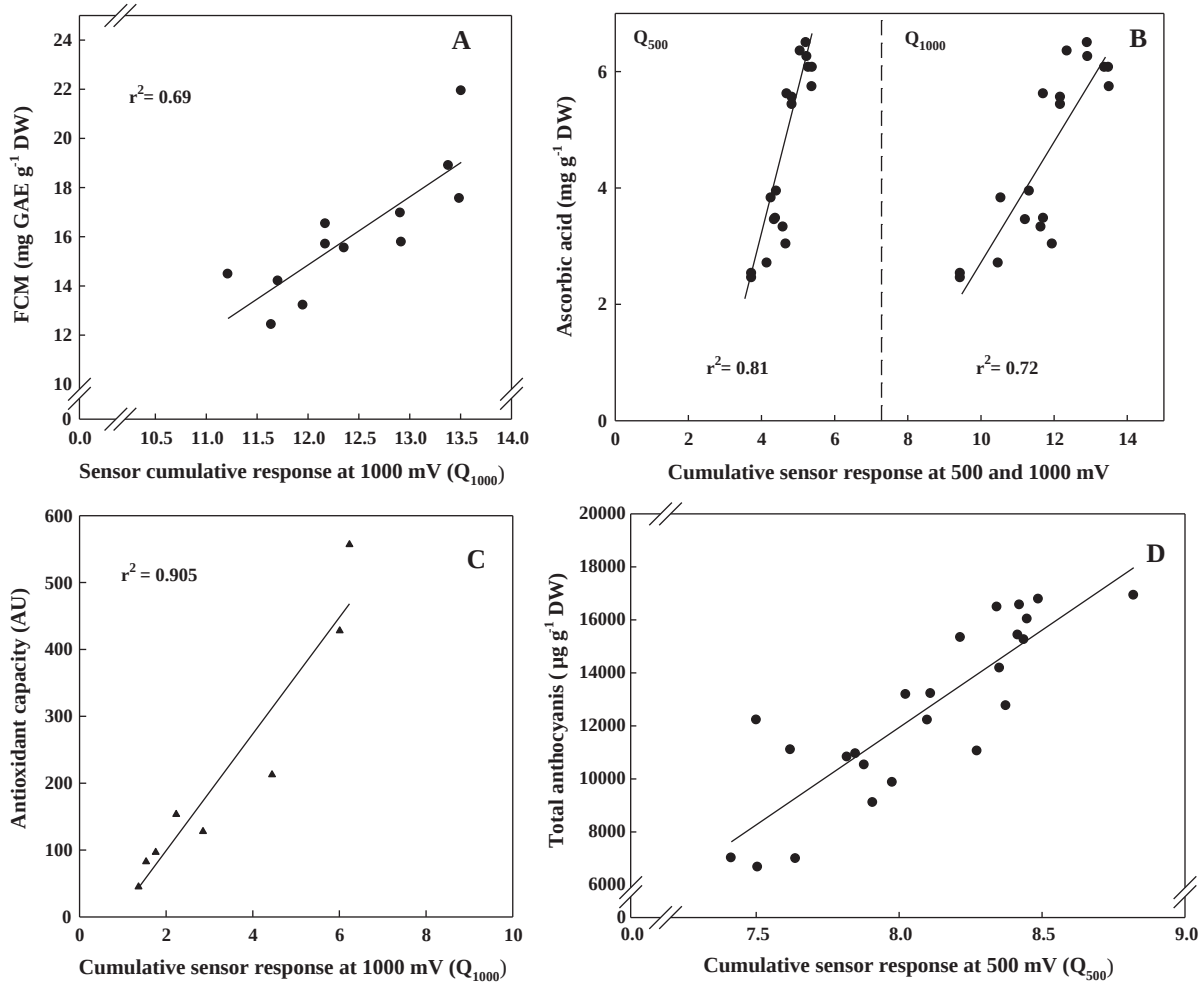



**Fig. 4.** Correlation between: (A) cumulative response at 1000 mV ($Q_{1000}$) and total phenolics concentrations in strawberry fruits; (B) cumulative response at both 500 mV ($Q_{500}$) and 1000 mV ($Q_{1000}$) with AsA concentrations in strawberry fruits from different cultivars (viz. Christine, Elsanta, Florence, Jubilee, Sonata and Symphony); (C) cumulative response at 1000 mV ($Q_{1000}$) and antioxidant capacity (arbitrary units; AU), measured by the FRAP assay, of different black currant and strawberry fractions; (D) cumulative response at 500 mV ($Q_{500}$) and total anthocyanin concentrations, as determined by HPLC, from different black currant cultivars harvested at different maturity stages.

currant berries and therefore it is reasonable to hypothesize that the observed oxidation wave at ~100 mV in F2 of strawberry juices was associated with ellagitannins. Nevertheless, black currants contain a pool of phenolic compounds [37] which may account for the observed oxidation wave in the non-anthocyanin polyphenolic fraction (F2; Table 2). As expected, the anthocyanin fraction (F3) for black currant berries was characterised by several oxidation waves at ~200, ~250, ~400 and ~700 mV which generally overlapped each other. The complexity observed in this study may be related not only to the four major anthocyanins commonly found in black currants (viz. cyanidin-3-glucoside, cyanidin-3-rutinoside, delphindin-3-glucoside and delphinidin-3-rutinoside) but also to other anthocyanins present in lesser amounts [35,36]. Further sample separation steps or more complex procedures would be required to simplify these voltammograms and draw relationships with individual anthocyanin concentrations. Overall, variations in the AC of fruits from different cultivars or maturity stages were in agreement with that reported in previous studies [19,20].

### 3.3. Antioxidant sensor performance versus standardised antioxidant capacity assays

Contradictions between the values given by different antioxidant assays or between antioxidant assays and sample composition have been extensively reported [21,38] and may be attributed to different reaction kinetics between the diverse antioxidants present, especially in complex samples containing an array of antioxidant compounds or a range of interfering compounds [38]. Both FRAP and Folin–Ciocalteu assays are ET-based methods involving a redox reaction with the antioxidant [4]. To overcome the limitations of the current antioxidant assays, electrochemistry is now amongst the most important approaches in the analysis of antioxidants in food and other biological samples [12], given that electrochemical methods have the same conceptual principle as those exhibited by antioxidants in real in vitro systems. Previous studies [14,17,18] showed that AC is generally well correlated with electrochemical response, especially if considering cumulative responses up to potentials of 800 or 1000 mV [12]. Likewise, $Q_{500}$ and $Q_{1000}$ values agreed well ($r > 0.69$) with the results obtained by either the Folin–Ciocalteu or the FRAP assay, respectively, when assessing AC of black currant or strawberry fruits (Fig. 4).

In contrast, $Q_{300}$ values were poorly correlated with AC values (data not shown) as was also observed by Kilmartin and Hsu [28] when assessing AC in different tea extracts by CV on carbon electrodes. In the former study, the authors [28] suggested that the poor correlation between both parameters was due to the relatively high concentrations of other phenolics, commonly present in complex samples like berries [36], with may possess low AC and which will not contribute to $Q_{300}$ values. Indeed, partitioning of different antioxidant fractions in both berry-based juices, thereby simplifying the samples, considerably improved the correlation between the values obtained by SWV and those given by the standard FRAP assay ($r > 0.91$; $P < 0.01$) (Fig. 4).

Unlike for commonly used spectrophotometric methods, the proposed antioxidant sensor was, however, not only able to discriminate between samples differing in their AC but also able to qualitatively determine specific antioxidants (viz. ascorbate and major anthocyanins). It has been described that ascorbic acid in strawberry fruits (cv. Senga Sengana) accounted for ca. 24% of the cumulative area at 300 mV when measured by HPLC coupled to coulometric detection [15]. The relatively poor contribution of ascorbic acid observed by Aaby et al. [15] may explain the low correlation between $Q_{300}$ and ascorbic acid (AsA) concentrations detailed herein and strongly suggests that other antioxidants, most probably phenolic acids are responsible for most of the reducing ability of strawberry juices at potentials below 300 mV. Even though the irreversible oxidation of ascorbate on inert electrodes occurs generally at low potentials, the results showed that ascorbic acid concentrations in both strawberry and black currant fruits could be accurately predicted by $Q_{500}$ and $Q_{1000}$ values (Fig. 4). This said, using SWV in combination with solid electrodes may lead to an underestimation of the AC of ascorbate, if compared to other compounds containing catechol groups, given the irreversible nature of the oxidation peak of the former compound. Nonetheless, the observed correlation was even improved when considering $Q_{500}$ values and AsA concentrations of both black currant and strawberry juices together ($r^2 = 0.88$; AsA (mg g$^{-1}$ DW) $= -7.4 + 2 \times Q_{500}$ (μC); data not shown). Accordingly, it was recently proven that the ascorbic peak at glassy carbon electrodes shifted to greater oxidation potentials when added alongside polyphenols present in diluted white wines [39] and hence it may further explain the positive high correlation between this compound and $Q_{500}$ observed in this study. In the same way, individual or total anthocyanin concentrations in black currant berries from different cultivars and maturity stages were correctly estimated using the signal given by the sensor, especially if considering cumulative responses up to 500 mV (Fig. 4).

In summary, the results presented herein have shown that both black currant and strawberry fruits contain a characteristic pool of antioxidants which when investigated electrochemically led to a specific voltammetric profile for each of the samples attested. Regardless of the cultivar or degree of maturity, black currant juices were richer sources of antioxidants than strawberry juices and generally presented a more complex electrochemical profile. Partitioning of the different samples revealed that anthocyanins were for both berry-based juices the major contributor to the antioxidant capacity of the fruits, followed by other phenolic-type compounds. In this context, and as pointed out earlier, the suitability of this type of sensors combined with SWV is of special interest for samples containing an array of antioxidants compounds showing a similar electrochemical behaviour (reversible or not) at solid electrodes. In some cases, comparison of the results provided by this approach should be taken with some caution since, for instance, samples containing high concentrations of ascorbate with no polyphenols may provide irreversible peaks at lower potentials than samples containing only little amounts of other polyphenols (reversible at greater oxidation potentials) but no ascorbate suggesting erroneously lower AC.

By, using screen-printed electrodes combined with SWV, the mechanisms of electron transfer of several anthocyanin standards were examined. It was demonstrated that multiple hydroxyl groups on the B ring like in the pyrogallol group (i.e. delphinidin) were more easily oxidised than two (i.e. catechol; cyanidin) or single hydroxyl substituents (i.e. pelargonidin). Hydroxyl groups in the B ring did not vary their oxidation peak due to the glycosylation of the molecule in the A ring but seemed to be stabilised when other antioxidants were present in solution.

## 4. Conclusions

A new electrochemical approach has been described and tested in the context of developing a disposable sensor to rapidly measure antioxidant capacity and individual antioxidants in polyphenol-rich berry juices which are known to be of paramount importance for their possible impact on human health [40]. The proposed sensor allowed for fast and easy discrimination of the main classes of antioxidants present in both polyphenol-rich berry types but more importantly was able to discriminate between samples based on their AC as verified by standardised assays (viz. FRAP and Folin–Ciocalteu). Sensor cumulative responses at formal potentials

of 500 ($Q_{500}$) and 1000 mV ($Q_{1000}$) correlated well with AC as well as with anthocyanin and ascorbate concentrations in the juices. The proposed methodology would accomplish most of the criteria recommended by Prior et al. [4] for the development of standardised AC assays, and would be of biological relevance since electrochemical techniques use the same basic principle to that exhibited by antioxidants in real biological systems. Besides, the use of disposable screen-printed sensors would overcome the main drawback of electrochemical methods which centre around the deactivation of the electrode, after single measurements, due to the formation of a polymeric film produced by the coupling of electrogenerated phenoxy radical. In this context, the use of other electrochemical techniques in combination with disposable screen-printed electrodes should be further explored in the context of developing sensors for rapid determination of AC in different food samples.

Given the importance of the sample matrix (viz. pH, presence of other electrochemically active compounds), further standard compounds should be investigated, preferably after standard additions in berry-based juices and hence allow a better identification of these compounds in real samples. In order to have a better understanding of the antioxidant profile of berry-based juices, phenolic compounds rather than anthocyanins, with formal oxidation peaks at low potentials, should be quantified and values correlated with sensor cumulative responses at 300 mV ($Q_{300}$).

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