*Int. J. Electrochem. Sci.*, 11 **(2016)** 2854 - 2876

**International Journal of ELECTROCHEMICAL SCIENCE**
www.electrochemsci.org

# Electrochemical Properties of Chlorogenic Acids and Determination of Their Content in Coffee Using Differential Pulse Voltammetry

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*Received:* 11 January 2016 / *Accepted:* 6 February 2016 / *Published:* 1 March 2016

The electrochemical properties of nine chlorogenic acids (CGAs) isomers: three caffeoylquinic acids, CQAs (5-CQA, 4-CQA, 3-CQA), three dicaffeoylquinic acids, diCQAs (3,4-diCQA, 3,5-diCQA, 4,5-CQA) and three feruloylquinic acids, FQAs (5-FQA, 4-FQA, 3-FQA) were studied by differential pulse voltammetry (DPV) method. The study has shown that electrochemical properties of CQAs and diCQAs are strongly dependent on their chemical structure and electronic properties, particularly on presence of electron-donating –OH and –CH=CH– groups, and a strong electron-withdrawing effect of ester (–COOR) group presented in their structures. The electrochemical properties of FQAs were additionally influenced by the presence of methoxy group ($–OCH_3$) attached on the aromatic ring. DPV measurements show that electrochemical oxidation of CQAs and diCQAs at a GCE is reversible, pH dependent, two- electron-two- proton process occured on catechol moiety in the structure of these molecules. Electrochemical oxidation of FQAs proceeded by an EC mechanism. In the first, electrochemical oxidation step, a phenoxy radical was formed by irreversible one-electron-one-proton process. In the second, chemical step, phenoxy radical undergoes hydrolysis generated a new more electroactive compound with methoxy-catechol moiety in the structure. This compound was reversible oxidised to methoxy-*ortho*-quionone structure by two-electron-two-proton process. The electrochemical properties of 5-CQA (main CGAs in coffee) were investigated more in detail. It was observed that anodic oxidation peak current of 5-CQA show its maximum in PBS solution of pH 7 and linear relationship within the concentration range of 5-50 μmol $L^{-1}$, with LOD of $1.2 \cdot 10^{-6}$ mol $L^{-1}$. DPVs of coffee show that electrochemical properties of coffee extracts were very similar to that of investigated CGAs. Therefore, DPV was used for characterization of CGAs in coffees. It was shown that DPV is a very sensitive and selective method for determination of total CGAs content in coffee.

**Keywords:** Electrochemical properties, Chlorogenic acids, DPV, Coffee, Total CGAs content

## 1. INTRODUCTION

Chlorogenic acids (CGAs) belong to a large group of natural polyphenolic compounds. Chemically, CGAs are a family of different esters (mono-, di-, tri- and mixed esters) formed between (-)-quinic acid and certain hydroxycinnamic acids, most commonly caffeic, ferulic and *p*-coumaric acids. They may be subdivided in more subgroups according to the type of ester substituent (e.g., caffeoylquinic acids, CQAs; feruloylquinic acids, FQAs; *p*-coumaroylquinic acids, *p*-CoQAs; different mixed esters, e.g. caffeoyl-feryloylquinic acids, CFQAs, etc.), the number of ester substituent (monoacylquinic acids, diacylquinic acids, or triacylquinic acids), and position of individual acyl residues (e.g. 5-caffeoylquinic acid, 5-CQA) [1]. CGAs occur widely in the different plant materials, especially in the coffee plant (green coffee beans), and many foods and beverages (including different type of coffee beverages) [2].

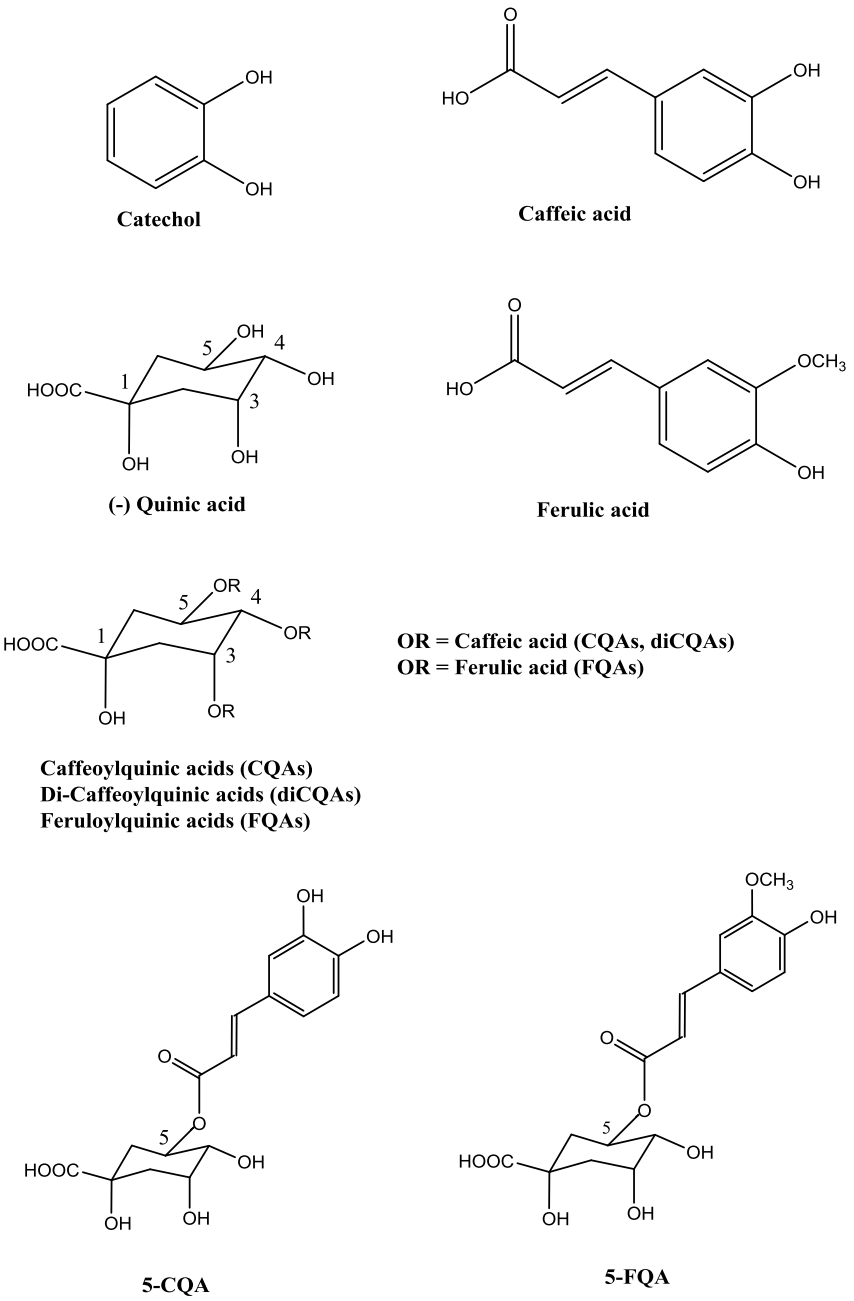
Numerous epidemiological, biological and pharmacokinetic studies have frequently linked the consumption of a diet rich in CGAs (especially through coffee beverage consumption) to many beneficial effect on human health. This include strong antioxidant activity of coffee constituents and their positive impacts on various diseases, like: type 2 diabetes, some neurodegenerative diseases (Parkinson's and Alzheimer's), some type of cancers (prostate, bladder, pancreas, breast, colon, etc.), cardiovascular and liver health, etc. [1,3,4].

**Table 1.** Structural pattern of investigated **c**hlorogenic acids (CGAs)

| No. | Name | Abbreviation | $OR^3$ | $OR^4$ | $OR^5$ |
|---|---|---|---|---|---|
| 1 | 3-*O*-Caffeoylquinic acid | 3-CQA | CFA | H | H |
| 2 | 4-*O*-Caffeoylquinic acid | 4-CQA | H | CFA | H |
| 3 | 5-*O*-Caffeoylquinic acid | 5-CQA | H | H | CFA |
| 4 | 3,4-Di-*O*-caffeoylquinic acid | 3,4-diCQA | CFA | CFA | H |
| 5 | 3,5-Di-*O*-caffeoylquinic acid | 3,5-diCQA | CFA | H | CFA |
| 6 | 4,5-Di-*O*-caffeoylquinic acid | 4,5-diCQA | H | CFA | CFA |
| 7 | 3-*O*-Feruloylquinic acid | 3-FQA | FA | H | H |
| 8 | 4-*O*-Feruloylquinic acid | 4-FQA | H | FA | H |
| 9 | 5-*O*-Feruloylquinic acid | 5-FQA | H | H | FA |

CGAs are one of the most important dietary polyphenols in the human diet and coffee is the major source of dietary CGAs intake in humans. Coffee contained a number of different CGAs, for example in green coffee bean about 70 isomers were detected, while in the roasted bean increases to over 200 derivates [1]. But, according to many HPLC analyses [5-8] nine CGAs prevailed in all investigated coffee samples: three isomers of caffeolquinic acids (CQAs), three isomers of dicaffeolquinic acids (diCQAs) and three isomers of feruloylquinic acids (FQAs). CQAs were the most

abundant CGAs in coffee. Thus, in the green coffee beans CQA represented ca. 80-83 % of total CGAs content, diCQAs represented ca. 13-15 % of total CGAs content, while FQAs are minor CGAs from quantitative point of view (ca. 4 % of total CQAs content). 5-*O*-caffeolquinic acid (5-CQA) always dominated in the content (ca. 60 % of total CGAs content) [7]. All other CGAs isomers, excepting above mentioned nine CGAs, are presented in minor amount in coffee.




**Scheme 1.**

**Scheme 1.** Chemical structures of CQAs, diCQAs, FQAs, caffeic acid, ferulic acid, quinic acid, catechol, 5-CQA, and 5-FQA

The general chemical structures of CQAs, diCQAs, FQAs and full structure of 5-CQA and 5-FQA were shown in Scheme 1. In addition, the structure of their structural moieties: caffeic acid, ferulic acid, quinic acid and catechol, were also shown. The nomenclature of investigated CQAs, according to IUPAC nomenclature, was shown in Table 1.

Many studies have reported that biological activities of polyphenolic compounds are in very close connection with their electrochemical properties. Thus, e.g. compounds with lower oxidation potentials showed higher antioxidant activity [9-11]. Also, it seems that the processes which proceeded during the antiradical/antioxidant activity of polyphenols are very similar or the same, as the processes observed during the electrochemical oxidation/reduction of these compounds. Therefore, to understand better the mechanism of antioxidant/antiradical activity of CGAs their electrochemical properties must be investigated.

Electrochemical properties of CGAs were only rarely investigated. Thus, Namazian and Zare [12], Yardim [13], and Ziyatdinova et al. [14] investigated the electrochemical properties of chlorogenic acid (probably 5-CQA -name for chlorogenic acid according to the IUPAC nomenclature [1], Scheme 1.). Electrochemical behaviour of other important CGAs contained in coffee (Scheme 1.) was not investigated until we recently published paper on this subject [15]. In this paper, the electrochemical behaviour of six main CGAs contained in coffee: three isomers of CQAs (5-CQA, 4-CQA, 3-CQA) and three isomers of diCQAs (3,4-diCQA, 3,5-diCQA, 4,5-diCQA), was investigated by square-wave voltammetry (SWV) method. Electrochemical properties of other three important isomers of CGAs contained in coffee, i.e. three isomers of FQAs (5-FQA, 4-FQA and 3-FQA) until now have not been investigated according to the literature data.

Determination of total CGAs content in coffee was performed using different electroanalytical methods, mainly by different electrochemical sensors or biosensors using SWV [16-18], DPV [19] and chronoamperometry [20] as methods of analysis. Adsorptive transfer stripping square-wave voltammetry (AdTSSWV) on boron-doped diamond electrode [13, 21], differential pulse voltammetry (DPV) on multi-walled carbon nanotube-modified glassy carbon electrode (MWCNT-GCE) [14], and DPV on carbon paste electrode [22] were also used for determination of total CGAs content in coffee. All these methods were shown as very good for quantification of total CGAs content in coffee, but in all these papers [13-14,16-22] was not quite correct assignation of oxidation peak(s) observed in voltammetry of coffee samples. Most of the authors [13,16-18,21] analysed CGAs content in coffees by SWV and observed only one oxidation peak assigned to the oxidation of chlorogenic acid (probably 5-CQA). Ziyatdinova et al. [14] and Oliveira-Neto et al. [22] used DPV and ascribed this oxidation peak to the oxidation of mixture of caffeic acid and chlorogenic acid. According to our previously published investigation of coffee samples by SWV [15] such assignation of this first oxidation peak in coffee is not correct. Coffee contained different CGAs and therefore this oxidation peak in coffee samples is composed of electrochemical oxidation of different CGAs, mostly from oxidation of CQAs and diCQAs (the main CGAs in coffee). In addition, Ziayatdinova et al. [14] using DPV, observed in coffee samples a second oxidation peak, assigned to the oxidation of ferulic acid. Oliveira-Neto et al. [22] observed a second and third oxidation peak and ascribed these peaks to oxidation of other distinct phenolic species that occur in minor proportion in coffee, without specification regarding the chemical composition of these species. Such assignation of the second and especially third oxidation peak of

coffees is also not correct. The coffee do not contained free ferulic acid, but contained their esters with quinic acid, i.e feruloylquinic acids (FQAs), as were reported by many HPLC analyses [5-8]. As we will show later in this paper, from the results of DPV measurements, the second oxidation peak observed in coffees could be assigned to the electrochemical oxidation of different FQAs. Third oxidation peak in coffee samples in our DPV measurements was not observed.

Therefore, the aims of this study were: (i) to investigate the electrochemical properties of nine chlorogenic acids contained in coffee (3-CQA, 4-CQA, 5-CQA, 3,4-diCQ, 3,5-diCQA, 4,5-diCQA, 3-FQA, 4-FQA, 5-FQA); (ii) to investigate the influence of chemical structure of these compounds on their electrochemical properties; (iii) to investigate the oxidation mechanism of CQAs, diCQAs and FQAs, (iv) to use the results of electrochemical behaviour of all investigated CGAs (including 5-CQA as a standard) in order to developed DPV as a sensitive and selective electrochemical method for determination of total CGAs content in different brands of coffee samples.

## 2. EXPERIMENTAL

### *2.1. Chemicals*

Caffeoylquinic acids: 3-CQA (neochlorogenic acid), 4-CQA (cryptochlorogenic acid) and 5-CQA (chlorogenic acid), were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dicaffeolyquinic acids: 3,5-diCQA (isochlorogenic acid A), 3,4-diCQA (isochlorogenic acid B) and 4,5-diCQA (isochlorogenic acid C), were obtained from Chengdu Biopurify Phytochemicals Ltd. (Chengdu Sichuan, China). Feruloylquinic acids (3-FQA, 4-FQA and 5-FQA) were donated from Dr. I. Dokli and Dr. Z. Hameršak, Ruđer Bošković Institute, Zagreb, Croatia. They synthesize these three isomers of FQAs, and characterised and confirmed their structure by $^{1}H$ and $^{13}C$ NMR spectroscopy and High Resolution Mass Spectrometry, HRMS (MALDI) [23]. Caffeic acid (CFA), ferulic acid (FA) and 5-hydroxyferulic acid (5-HFA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol (HPLC grade) was purchased from Merck (Darmstadt, Germany). Sodium dihydrogen phosphate, disodium hydrogen phosphate and phosphoric acid (for preparation of the supporting electrolyte, 0.1 mol $L^{-1}$ phosphate buffer solutions, PBS, of pH 8 to 3) were obtained from Kemika (Zagreb, Croatia). Alumina powder of 0.05 μm for polishing of glassy carbon electrode (GCE) was purchased from Buehler (USA).

Stock solutions of all CGAs were prepared in methanol (HPLC grade) and stored at 4 $^{o}C$. Working solutions of CGAs of different concentration and pH for electrochemical measurements were prepared by dilution of appropriate quantity of stock solution in supporting electrolyte 0.1 mol $L^{-1}$ PBS. Supporting electrolyte solutions (PBS of different pH) were prepared using the analytical grade chemicals and purified water obtained from Milipore purification system (conductivity ≤ 0.1 μS $cm^{-1}$).

### *2.2. Coffee samples*

Ten different brands of coffee, widely consumed in Croatia, were chosen for investigation as follows: (i) two brands of green coffee beans (*Coffea Arabica* cv. Rio Minas harvested in Brazil and

*Coffea Robusta* cv. Cherry harvested in India); (ii) two brands of roasted coffee beans produced by roasting of above mentioned *C. Arabica* and *C. Robusta* samples at 150-155 $^{o}$C for 18 min; (iii) two brands of ground coffees (Franck Quatemala and Flatscher Olimpia, declared as 100 % Arabica coffee); (iv) four famous instant coffees brands (Nescafé Classic, Nescafé Espresso, Jacobs Monarch and Jacobs Intense). Green and roasted coffee beans were obtained by one of the leading coffee manufacturer company in Croatia, while ground and instant coffees samples were purchased from local supermarkets.

The coffee samples for DPV investigations and determination of total CGAs content by HPLC analysis, were prepared according to the extraction (brewing) procedure used in our previously published paper [15].

### *2.3. Differential pulse voltammetry (DPV)*

DPV measurements were performed using an μAutolab potentiostat/galvanostat running with GPES software, version 4.9 (Eco Chemie, Utrecht, Netherlands). Measurements were carried out in a standard three-electrode electrochemical cell (Metrohm, Switzerland). Glassy carbon electrode (GCE) of 3 mm diameter (model MF-2012, Bioanalytical Systems, USA) was the working electrode, Pt-wire electrode was the counter electrode and Ag/AgCl (3 mol $L^{-1}$ KCl) electrode was reference electrode (both electrodes made by Metrohm, Switzerland).

The experimental conditions for DPV measurements were: pulse amplitude 50 mV, pulse width 70 ms, potential increment 2 mV, interval time 0.4 s and scan rate of 5 mV $s^{-1}$.

Prior to each electrochemical measurement, the surface of GC working electrode was carefully hand-polished to a mirror-like finish with 0.05 μm alumina slurry using a polishing cloth and then thoroughly rinsed with Milli-Q water. After mechanical polishing, the GC electrode was cleaned electrochemically by cyclic voltammetry (in potential range -0.2 to 1.0 V, with scan rate of 50 mV $s^{-1}$) in supporting electrolyte (0.1 mol $L^{-1}$ PBS of pH at which further DPV measurements were done) until a steady-state cyclic voltammograms were obtained. Very reproducible DPVs were ensured through this procedure. All measurements were done (at least) in triplicate, at room temperature (298 K).

All DP voltammograms were analysed using two different types of software: GPES 4.9 software (Eco Chemie, Utrecht, Netherlands) and ECDSOFT software [24], to get the electrochemical parameters. There was not significantly difference in the results obtained using these two software. Drawing of all Figures and statistical analysis of the numerical results presented in Tables were done with Origin® 2015 software (OriginLab Corporation, Northampton, USA). Chemical structures were drawn using ChemDraw® Professional 15.0 software (PerkinElmer Informatics, USA).

## 3. RESULTS AND DISCUSSION

### *3.1. DPV of chlorogenic acids (CGAs)*

The electrochemical properties of nine CGAs isomers contained in coffee: three CQAs (5-CQA, 4-CQA, 3-CQA), three diCQAs (3,4-diCQA, 3,5-diCQA, 4,5-diCQA) and three FQAs (5-FQA,

4-FQA, 3-FQA), were studied at a glassy carbon electrode (GCE) in 0.1 mol $L^{-1}$ phosphate buffer solutions (PBS), using DPV method. The electrochemical properties of caffeic acid (CFA) and ferulic acid (FA) were also studied in order to identify the redox centres of CGAs, due the fact that CFA is structural moiety of CQAs and diCQAs, while FA is structural moiety of FQAs (see Sheme 1).

*3.1.1. Effect of chemical structure*

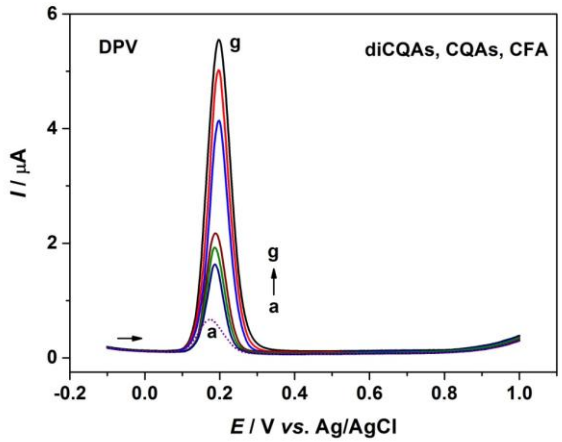



**Figure 1.** DPVs of $3 \cdot 10^{-5}$ mol $L^{-1}$ solution of: CFA (a), 5-CQA (b), 3-CQA (c), 4-CQA (d), 3,4-diCQA (e), 3,5-diCQA (f), and 4,5-diCQA (g), in 0.1 mol $L^{-1}$ PBS pH 7. The experimental conditions for DPV measurements were: pulse amplitude 50 mV, pulse width 70 ms, potential increment 2 mV, interval time 0.4 s, scan rate 5 mV $s^{-1}$.

Differential pulse voltamograms (DPVs) of $3 \cdot 10^{-5}$ mol $L^{-1}$ solutions of CQAs (5-CQA, 4-CQA, 3-CQA), diCQAs (3,4-diCQA, 3,5-diCQA, 4,5-diCQA) and CFA in 0.1 mol $L^{-1}$ PBS of pH 7 were shown in Fig. 1. All DPVs showed one well expressed anodic oxidation peak, at oxidation potentials ($E_p$) of 0.175 V for CFA, 0.187 V for CQAs and 0.200 V for diCQAs. The peak current ($I_p$) values increases from CFA up to 4,5-diCQAs (see Fig. 1). The observed width at half height of anodic oxidation peaks of all DPVs, $W_{1/2}$ ~ 60 mV, indicated the transfer of two electrons during the electrochemical anodic oxidation of CFA, CQAs and diCQAs [25].

The shift of $E_p$ to the more positive values and the increase of $I_p$ from CFA to diCQAs, observed in DPV measurements, clearly suggest that esterification of CFA with quinic acid (QA) significantly change the electrochemical properties of CQAs and diCQAs isomers. The difference in the electrochemical reactivity of these molecules is result of different chemical structure (Scheme 1).

In our previously published paper [15] we investigated by SWV the same CQAs and diCQAs, as in presented paper and explained in the detail the influence of chemical structure on the electrochemical behaviour of these CGAs. DPV measurements confirm the conclusion from SWV measurements [15] that electrochemical properties of investigated CQAs and diCQAs are strongly dependent on their chemical structure (first of all on the presence of catechol moiety) and the different electronic properties due to different chemical structure, particularly due to electron-donating effect of

–OH and –CH=CH– groups, and a strong electron-withdrawing effect of ester (–COOR) group presented in their structure.

In the solutions of CQAs (see Scheme 1.) it seems that a strong electron-withdrawing effect of ester group –COOR and influence of R substituent (quinoyl moiety) [32, 33]), prevailed over the moderately electron donating effect of –CH=CH– group.

In the case of diCQAs, with two CFA moieties and two esters groups in the structure (Scheme 1.), the above mentioned strong electron-withdrawing effect of the two ester groups is more pronounced and therefore oxidation potential of diCQAs is moved to the more positive value in comparison to that of CQAs.

Differential pulse voltamograms (DPVs) of $3 \cdot 10^{-5}$ mol $L^{-1}$ solutions of FQAs (5-FQA, 4-FQA, 3-FQA) and FA in 0.1 mol $L^{-1}$ PBS of pH 7 have been shown in Fig. 2.

DPVs of FQAs show one well defined anodic oxidation peak, at oxidation potentials ($E_p$) of 0.419 V for 5-FQA, 0.425 V for 3-FQA and 0.430 V for 4-FQA. The peak current ($I_p$) values increase from 4-FQA up to 5-FQA (see Fig. 2). The observed width at half height of anodic oxidation peaks of all FQAs, $W_{1/2}$ ~ 100 mV, indicates the transfer of one electron during the electrochemical anodic oxidation of FQAs [25].

The DP voltamogram of ferulic acid (FA) shows two convolved anodic oxidation peaks, first peak at 0.363 V and the second peak at 0.466 V. The observed value of $W_{1/2}$ ~ 80 mV for the first and second anodic peak suggest the transfer of one electron during the anodic oxidation of FA [25].

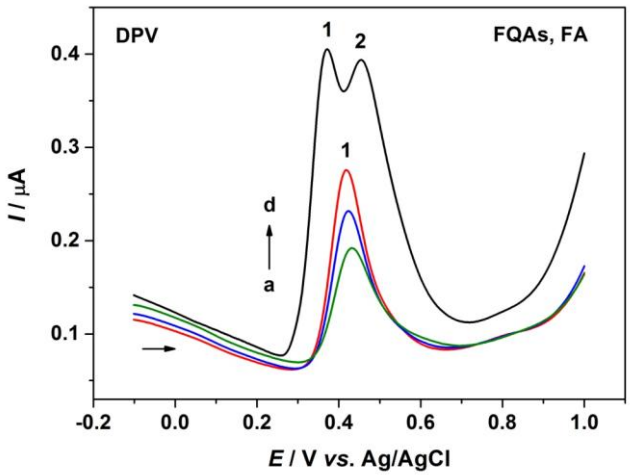



**Figure 2.** DPVs of $3 \cdot 10^{-5}$ mol $L^{-1}$ solution of: 4-FQA(a), 3-FQA (b), 5-FQA (c), FA (d) in 0.1 mol $L^{-1}$ PBS pH 7. The experimental conditions for DPV are as in Figure 1.

There is a great disagreement in the literature regarding the number of anodic oxidation peaks, their assignation, and especially regarding the oxidation mechanism of ferulic acid. Some authors [34-35] found only one anodic oxidation peak which they ascribed to the one-electron, one-proton oxidation of FA to the phenoxy radical, which then dimerises by a radical-radical coupling mechanism, forming a polymeric film on the electrode surface. Other authors observed two anodic oxidation peaks of FA [36, 37], but differed in assignation of these peaks and mechanism of their formation. Trabelsi et

al. [36] proposed three different mechanisms of oxidation of FA, with caffeic acid, methoxyhydroquinone and 5-hydroxyferulic acid (5-HFA) as a final oxidation product. They ascribed the first oxidation peak to discharge of FA molecules in its free form and the second anodic peak to the oxidation of adsorbed form of FA molecules. Manaia et al. [37] assigned the first irreversible oxidation step of FA (peak 1) to the formation of a catechol moiety, and the second reversible step (peak 2) to the oxidation on double bond position of the side chain in the structure of FA (see Scheme 1.).

The only consensus that has been accepted in the literature is the fact that ferulic acid has more positive redox potential than caffeic acid, which is well documented in a many papers [38-40]. The substitution of the 3-hydroxyl group of caffeic acid by a methoxy group in the structure of ferulic acid (see Scheme 1), considerably shifts the redox potential toward the more positive values. The possible explanation is that the methoxy group is less strong electron donating group than the hydroxyl group, what lowering the electron density on aromatic ring. Methoxy group disturb the stability of the phenoxy radical formed as well as its stabilization through the intramolecular interactions (e.g. hydrogen bonds). In other words, the presence of two hydroxyl group in the *ortho*-position (i.e. catechol structure) is crucial for the stability of phenoxy radical and an easy oxidation of electroactive species (e.g. hydroxycinnamic acids). All these facts lead to a significant increase of oxidation potential of ferulic acid in comparison to that of caffeic acid.

It can be seen from Fig. 2 that FQAs have the more positive oxidation potentials than FA (peak 1). The explanation of such behaviour is the same as for CQAs, and that is the strong electron-withdrawing effect of ester group (-COOR group) presented in the structure of FQAs, which move the electron density from aromatic ring to the ester group in the side chain (see Scheme 1). Therefore, the oxidation potential of FQAs is moved to the more positive value in comparison to that of FA. It is interesting to note that the position of esterification of FA with QA has influence on the values of oxidation potentials of FQAs, in comparison with CQAs where this effect was not observed (Fig. 1).

### *3.1.2. Effect of successive scans*

The effect of successive polarization scans in the same solution, without cleaning the GCE surface between the scanning, on the shape of the DPVs of all investigated CGAs was investigated and shown in Figure 3.

Figure 3A shows this effect for 5-CQA, representing the behaviour of all other CQAs and diCQAs. The behaviour of CFA as structural moiety of CQAs and diCQAs was shown for comparison. It is evident for both species that during the second and third scans the anodic oxidation current decreases. Electrochemical oxidation of CQAs and diCQAs (and CFA too) on the GCE surface probably formed an adsorption film of oxidation product(s). Oxidation of CQAs and diCQAs molecules in the second and third polarization scan is more difficult, because occurs through the layer of adsorbed product(s). Therefore, the peak currents decreases and peak potentials move to a little more positive potentials during the successive scans. Such adsorption behaviour has been observed for all investigated CQAs and diCQAs.

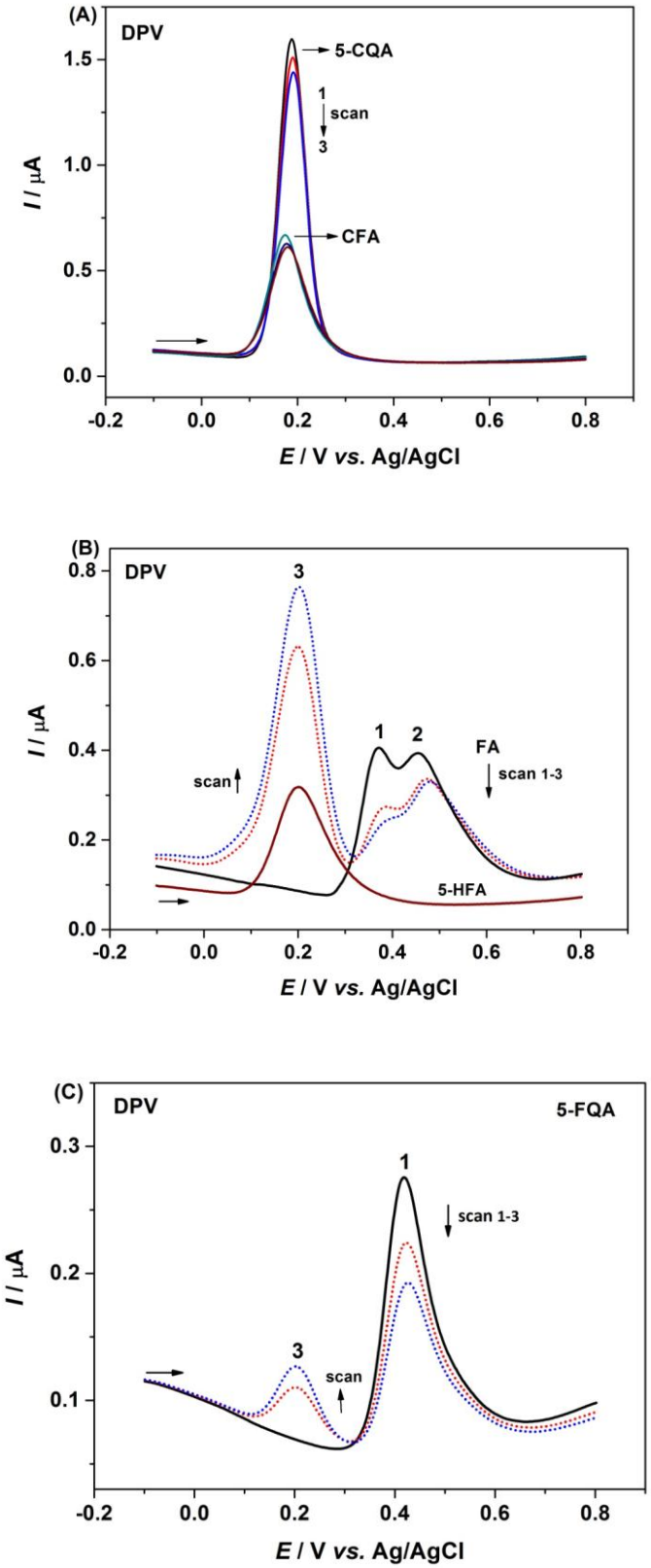



**Figure 3.** The effect of successive scans on DPVs in $3 \cdot 10^{-5}$ mol $L^{-1}$ solution of: 5-CQA and CFA (Figure 3A), FA (Figure 3B), and 5-FQA (Figure 3C) in 0.1 mol $L^{-1}$ PBS pH 7. The experimental conditions for DPV are as in Figure 1.

Figure 3B shows the effect of successive scans on the electrochemical behaviour of FA. The first DP voltammogram has shown two anodic oxidation peaks (peak 1 and 2), formed by two consecutive charge transfer reactions. In successive voltammograms a new anodic peak 3 appeared at significantly lower anodic potential (0.200 V). It is interesting to note, that at the same time the current of peak 1 disappeared in the successive scans and the current of peak 3 increased. Obviously, the formation of a new electroactive compound (peak 3) is in close connection with the oxidation product of FA formed at potential of peak 1. The current of peak 2 decreased during successive scans due to adsorption of FA oxidation product formed at potential of this peak. The new electrochemically formed compound which is oxidised at peak 3 can be probably assigned to the 5-hydroxyferulic acid (5-HFA) molecule. Such assignation can be proposed on the base of shape of DP voltammogram of commercialy available 5-HFA (see Fig. 3B). 5-HFA show the anodic oxidation peak at the same potential value (0.200 V) as the new compound formed by electrochemical oxidation of FA (peak 3).

Similar behaviour, i.e. formation of a new more electroactive species during successive polarization of FA was also reported by Manaia et al. [37], but was also observed for some other molecules with similar chemical structure (-$OCH_3$ and –OH group on the benzene ring), e.g. curcumin [37, 41], capsaicin [37, 42,43] and eugenol [44].

Figure 3C show the effect of successive polarization scans on the electrochemical behaviour of FQAs, represented by DPVs of 5-FQA. It can be seen that FQAs show similar behaviour as FA. One well expressed anodic peak (peak 1) was observed during the first polarization scan. During the successive anodic polarization a new peak 3 appeared, at the same potential value (0.200 V) as in the case of FA (Fig. 3B). Obviuosly, the new compound formed during successive polarization of 5-FQAs has similar moiety in the structure as the species formed during successive polarization of FA, i.e. 5-HFA moiety. It has been observed, as in the case of FA, that the current of peak 1 decreases in successive scans and at the same time the current of peak 3 increases. Obviously, the formation of a new electroactive compound (peak 3) is in close connection with the oxidation product of 5-FQAs formed at peak 1. The possible mechanism of anodic oxidation of 5-FQA (and all other FQAs and FA) will be presented later in the text.

### *3.1.3. Effect of solution pH*

The effect of solution pH on the electrochemical properties of the investigated CGAs was represented by the behaviour of 5-CQA (a standard used for quantification of total CGAs content in coffee). DPVs of 5-CQA in 0.1 mol $L^{-1}$ PBS of pH range 8-3 were performed and shown in Figure 4. It can be seen that the oxidation peak current of 5-CQA increased from pH 3 to 7, reaching a maximum at this pH value. At the pH higher than 7, the peak current decreased. The same type of behaviour of 5-CQA was observed by the SWV measurements [15] and can be explained as follows.

Three possible centres for dissociation are contained in the structure of 5-CQA: one carboxyl group on the cyclohexane ring and two -OH groups on the catechol moiety (see Scheme 1.). Therefore, three macroscopic acid dissociation constants of 5-CQA were reported in the literature. Thus, Maegawa et al. [45] reported p$K_a$ values for 5-CQA: p$K_1$=3.50, p$K_2$=8.42; p$K_3$= 11.0. Using these p$K_a$

values the distribution diagram of 5-CQA species in $5 \cdot 10^{-5}$ mol $L^{-1}$ solution of 5-CQA (ionic strength, $I$=0.1) was drawn by appropriate software [46]. It is evident from the distribution diagram (not shown here), that in the solutions of pH 3 and 4 neutral molecules of 5-CQA and 5-$CQA^-$ anion are contained. In the pH range from 5 to 7, anion 5-$CQA^-$ species prevailed in the bulk solution (reaching at pH 7 their maximum concentration of 95 %). In the solution of pH 8, 5-$CQA^-$ and 5-$CQA^{2-}$ anions are contained in approximately equimolar ratio. 5-$CQA^-$ anion is formed by dissociation of carboxyl group (deprotonation of one proton, $H^+$) in the cyclohexane ring of the structure of 5-CQA (see Scheme 1). Since phenolate anions are more easily oxidised than neutral phenol molecules, it seems logically that oxidation current of 5-$CQA^-$ anion reached its maximum at pH 7 (see inset of Fig. 4). Maximum oxidation current for 5-$CQA^-$ anion at pH 7 was also observed in the SWV measurements [15].

It is evident from the DPV measurements (Fig. 4) that the oxidation of 5-CQA is a pH-depended process. The oxidation peak potential was linearly shifted to less positive values with the increase of pH values from pH 3 to pH 8 (inset in Fig. 4), showing that deprotonation plays a significantly role in the oxidation process. The slope of d($E$)/d($pH$) line is close to -59 mV/pH unit, showing that the oxidation of 5-CQA at the GCE involves the same number of electrons ($n$) and protons ($m$) [47]. Considering the observed width at half height of anodic peak of $W_{1/2}$~60 mV which correspond to exchange of two electrons, $n$=2, (Fig. 1.), it can be concluded that oxidation process of 5-CQA (in fact 5-$CQA^-$ anion) at the GCE involves transfer of two electrons and two protons (i.e. $2e^-$-$2H^+$ oxidation mechanism).

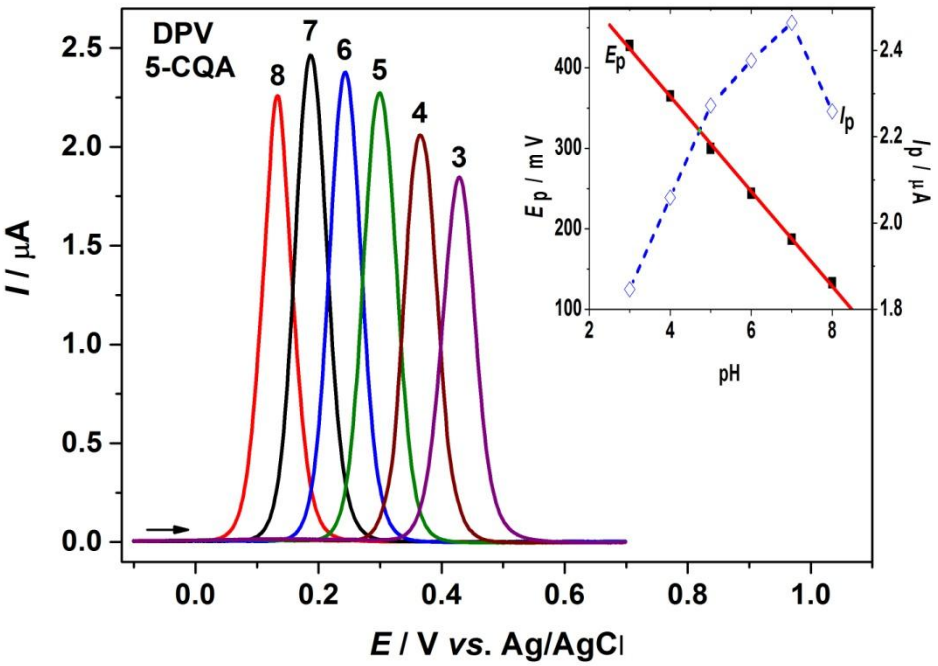



**Figure 4.** DPVs of $5 \cdot 10^{-5}$ mol $L^{-1}$ solution of 5-CQA in 0.1 mol $L^{-1}$PBS of different pH. Inset: Plots of $E_p$ and $I_p$ *vs*. pH. The experimental conditions for DPV are as in Figure 1.

*3.1.4. Effect of concentration*

The effect of concentration of 5-CQA (standard for determination of total CGAs content in coffees) on the profile of DPVs was performed in 0.1 mol $L^{-1}$ PBS of pH 7. Figure 5 shows DPVs obtained by successive additions of 5-CQA into the PBS over the 5 to 50 μmol $L^{-1}$ concentration range. The peak current of anodic oxidation peak, at a potential 0.187 V, increased linearly with the 5-CQA concentration. (Fig. 5, inset). At higher concentration ($c$> 50 μmol $L^{-1}$) this relationship is not more linear (the peak current decreases), due to the strong adsorption of oxidation product(s). The calibration plot in the linear concentration range (5-50 μmol $L^{-1}$) can be expressed by the equation: $I_p$ (μA) = 0.02216+ 0.04908 $x$, where $I_p$ is peak current and $x$ is the concentration of 5-CQA expressed in μmol $L^{-1}$ (correlation coefficient, $r$=0.9998; $p$<0.0001). The sensitivity of DPV method was determined based on the values of the limit of detection and the limit of quantification. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated from the parameters obtained from the calibration curve using the formulas: $LOD$= 3 $s_a/b$ and $LOQ$= 10 $s_a/b$, where $s_a$ is the standard deviation of the $y$-intercept of the regression line and $b$ is the slope of the calibration curve [48]. The calculated LOD and LOQ values of 5-CQA were $1.2 \cdot 10^{-6}$ mol $L^{-1}$ and $4.0 \cdot 10^{-6}$ mol $L^{-1}$, respectively.

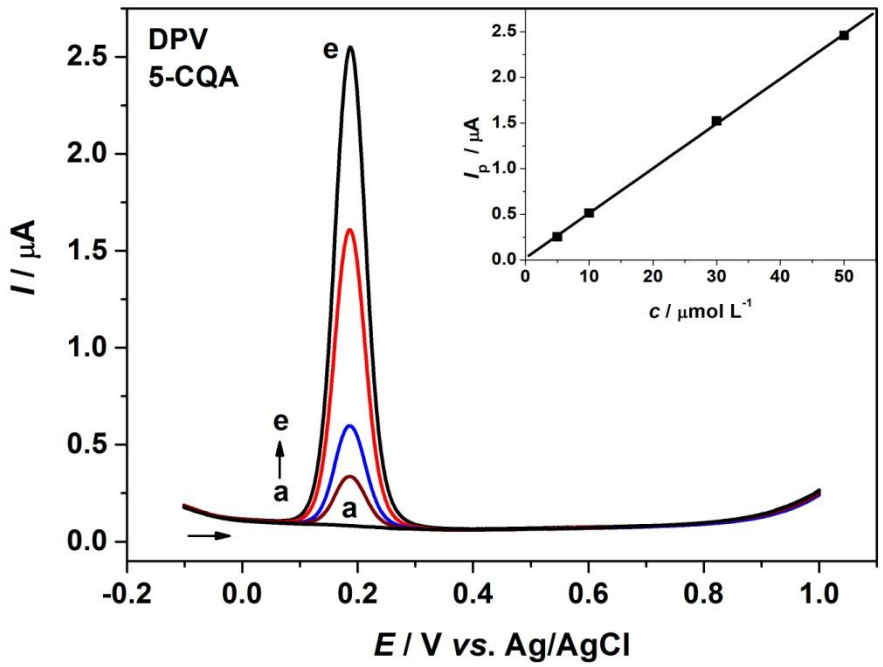


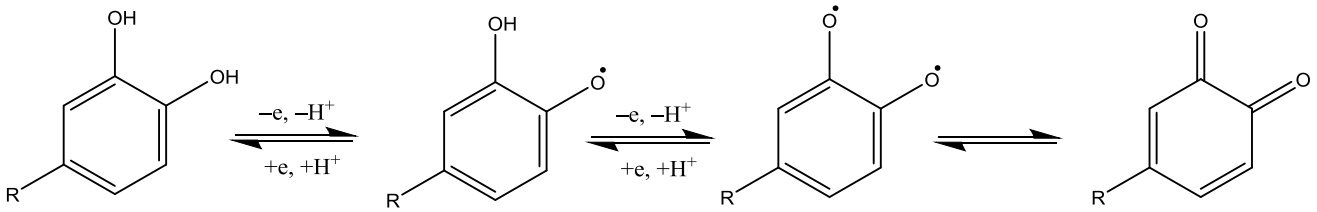

**Figure 5.** DPVs of 5-CQA solutions of different concentration: 0 (a), $5 \cdot 10^{-6}$ (b), $1 \cdot 10^{-5}$ (c), $3 \cdot 10^{-5}$ (d), and $5 \cdot 10^{-5}$ mol $L^{-1}$ (e), in PBS of pH 7. Inset: calibration plot of 5-CQA. The experimental conditions for DPV are as in Figure 1

*3.1.5. Oxidation mechanisms of investigated CGAs*

To explain the oxidation mechanism of investigated CQAs and diCQAs, we must look for the electroactive centre(s) in these molecules. All investigated CQAs and diCQAs have in their structure

one or two catechol moiety with two −OH groups in the *ortho*-position (Scheme 1.). It is well known that catechol and polyphenols with catechol moiety in their structure (e.g. caffeic acid and flavonoids catechin, quercetin and rutin) undergo reversible oxidation to *ortho*-quinone structure by a transfer of two electrons and two protons during anodic polarization [26-30]. But it should be noted here that according to recently published paper of Lin et al. [31], the mechanism of electrochemical oxidation of catechol (and consequently all molecules with catechol moiety in structure) is not a simple one. This mechanism proceeded through different pathways in dependence on pH of solution and includes many intermediate (transition) species as radicals, radical cations, anion and cation species, etc. In total this mechanism finished with *o*-quinone structure, as were usually reported. Speaking generally, it seems that electrochemical oxidation of investigated CQAs and diCQAs proceeded *via* the same (or very similar) mechanism as that of above mentioned catechol, flavonoids and caffeic acid, first of all due to the presence of catechol group in their structures.

Therefore, it could be concluded that electrochemical oxidation of CQAs (5-CQA, 4-CQA and 3-CQA) is a process which proceeds *via* a reversible $2e^{-}$-$2H^{+}$ mechanism, involving catechol moiety, i.e. oxidation of two -OH groups in the *ortho*-position in the structure of CQAs to *ortho*-quinone structure. This is a pH-dependent process and the maximum of anodic peak current is observed at pH 7, at the pH value where deprotonated anion species prevailed in the bulk solution. The oxidation product(s) strongly adsorbed on the GCE surface, thus causing decreases of the oxidation peak current during successive anodic scans (Fig. 3A). Considering the results of the SWV measurement [15], it can be concluded that during the reverse scan, i.e. cathodic polarization, *ortho*-quinone structure was reduced by a two-electron-two-proton mechanism back to catechol structure. Thus, the mechanism of total oxidation/reduction processes of CQAs can be defined as reversible $2e^{-}$-$2H^{+}$ mechanism (see Scheme 2).



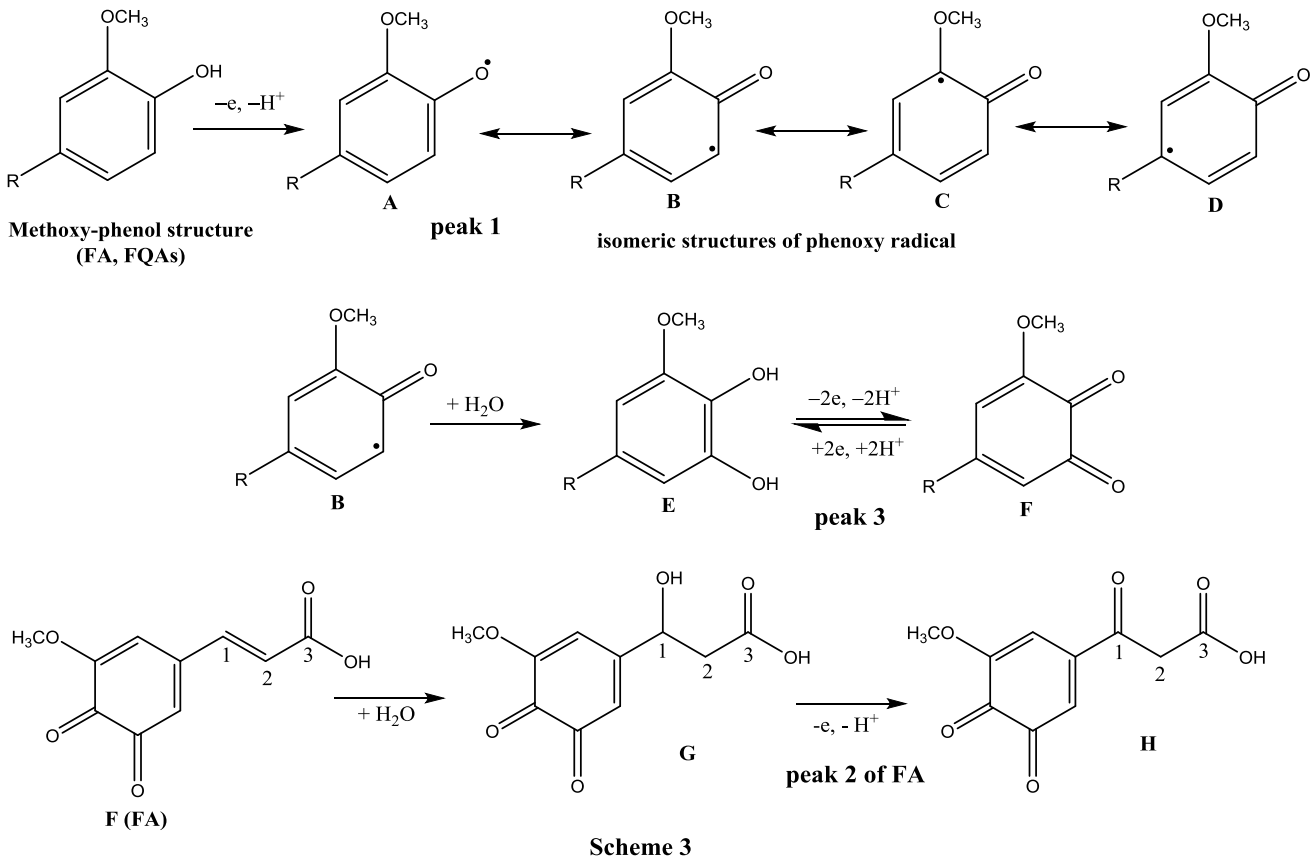

**Scheme 2**

**Scheme 2**. Proposed mechanism for the electrochemical oxidation/reduction of CQAs, diCQAs and CFA

Dicaffeoylquinic acids (3,4-diCQA, 3,5-di-CQA and 4,-di-CQA) have two catechol moieties in their structure (Scheme 1), but show only one anodic peak similar to the mono-esters, CQAs (Fig. 1). This suggests that the oxidation potential of both catechol moieties have the same or very close value, and the peaks become indistinguishable. Considering the DPV results obtained in this paper and the SWV results published earlier [15], the probable oxidation reaction occurs *via* two, reversible, $2e^{-}$-$2H^{+}$

mechanism. In other words, oxidation occurs simultaneously (or successively) on both catechol groups in the structure of diCQAs (see Scheme 1). Similar oxidation mechanism was proposed for rosmarinic acid [49], which molecule also has two catechol moieties in the structure. Due to the fact that total oxidation reaction involves four electrons, the net peak currents of diCQAs are significantly higher than that of mono-esters CQAs (see Fig. 1). A possible oxidation mechanism for diCQAs was shown in Scheme 2.

The oxidation mechanism of feruloylquinic acids (FQAs) is different than that of CQAs, probably due to the presence of only one –OH group at benzene ring in connection with $–OCH_3$ group (Scheme 1).To understand the oxidation mechanism of investigated FQAs, their electrochemical properties were compared with that of ferulic acid (FA), a structural moiety of the investigated FQAs. As was said before, the mechanism of electrochemical oxidation of FA is only rarely investigated in the literature and is still not quite clearly explained [34-37]. From DPV voltammograms of FA (Figs. 2 and 3B) it can be concluded that FA is oxidised during the first anodic scan in an irreversible one-electron and one-proton process (peak 1) to a phenoxy radical, which can exist in different isomeric forms (A, B, C and D, see Scheme 3) [36].




**Scheme 3**. Proposed mechanism for the electrochemical oxidation/reduction of FQAs and FA

The irreversibility of peak 1 was confirmed in the SWV measurements of FA (not shown here), where cathodic peak was not observed. All phenoxy radical isomers (Scheme 3) formed at potential of peak 1 are not thermodynamically stable and can be stabilized by a further oxidation, a chemical reaction of dimerisation [34, 35], and/or by chemical reaction with water molecules (probably by

hydrolysis) [26, 37]. The chemical process of hydrolysis leads to the hydroxylation of benzene ring [37]. The highest electron density in the benzene ring of phenoxy radical is located on both *ortho*- and *para*-positions, with respect to the -OH group presented in the phenol structure before oxidation [26]. Because *para*-position in the FA phenoxy radical is occupied by the side chain (R), hydroxylation can be favoured at *ortho*-position (on radical B in Scheme 3). After hydroxylation a new electroactive species with methoxy-catechol structure (5-hydroxyferulic acid, 5-HFA) was formed (species E in Scheme 3). Such assignation was confirmed by the DP voltammogram of commercially purchased 5-HFA compound. The new species formed during electrochemical oxidation of FA has the same value of oxidation potential (0.200 V) as the commercially purchased 5-HFA (see Fig. 3B). Both these species were reversible oxidised during the second and further scans (Fig.3B, peak 3) by a two electrons and two protons mechanism (Scheme 3, species F). Such explanation of the oxidation mechanism of FA was confirmed by our SWV measurements (not shown here), where redox pair of peak 3 was clearly observed. Similar explanation of oxidation mechanism of FA was also reported in the paper of Manaia et al. [37]. The oxidation potential of new formed species (5-HFA) at peak 3 is lower than that of FA anodic peak 1 (see Fig 3B.). This is expected because introduction of one additional –OH group in the structure of FA stabilized corresponding phenoxy radical and decreased the oxidation potential. It can be concluded that electrochemical oxidation of FA to 5-HFA proceeded *via* the electrochemical-chemical (EC) mechanism (see Scheme 3.).

The assignation of anodic peak 2 observed in the DPVs of FA still remain unclear. One of possible explanation is that this peak is related with the double bound of the hydrocarbon chain of the FA molecule (see Scheme 1), as was reported by Manaia et al. [37]. After hydroxylation at position 1 in the hydrocarbon chain of FA molecule the–OH group is formed (species G in Scheme3), and during further anodic polarization this group is oxidised to the oxo group (species H in Scheme 3). It has been found by SWV (not shown here) that this oxidation is one electron-one proton irreversible process (presented in Scheme 3) with no cathodic peak in the reverse scan, while Manaia et al. [37] reported a reversible oxidation/reduction process for this peak 2.

It seems reasonable concluded that oxidation of FQAs proceeded by the same or very similar mechanism as that of FA. The reason for such a conclusion is that electroactive centre for oxidation of FQAs was ferulic acid moiety in the structure of FQAs (see Scheme 1). The DPVs presented in Figs. 2, 3B and 3C, show that FQAs have the similar electrochemical behaviour as FA. The difference is only in the number of peaks. FQAs show only one anodic oxidation peak (peak 1) during the first scan, while FA additionally show the second anodic peak (peak 2). The formation of the second oxidation peak (peak 2) is not possible in the FQAs structure, probably due to a strong electron-withdrawing effect of ester (-COOR) group presented in their structure (see Scheme 1). All other behaviours of FQAs are the same or very similar to that of FA, including the formation of 5-HFA moiety (peak 3) during their electrochemical oxidation in the successive scans (compare Figs. 3B and 3C). It is interesting to note that the value of peak 3 potential was the same (0.200 V) for FQAs and FA, although FQAs have more positive potential of peak 1 in comparison to FA, due to the effect of esterification with quinic acid (see earlier explanation for CQAs). Generally, it can be concluded that electrochemical oxidation of FQAs to their methoxy-*o*-quionone structure proceeded by the EC mechanism, as was proposed in the Scheme 3.

### *3.2. Characterization of CGAs in coffee using DPV*

Taking into account the results of electrochemical properties of CGAs contained in coffee obtained by DPV, this technique was applied for identification and quantification of CGAs in different coffee samples. DPVs of coffee samples were recorded in order to investigate the analytical potential of the DPV as a method for determination of total CGAs content in coffee.

#### *3.2.1. Electrochemistry of coffee samples*

DPVs of two different coffee extracts, diluted 50-fold in 0.1 mol $L^{-1}$ PBS pH 7 (Figs. 6 and 7), represent electrochemical behaviour of all investigated coffee samples. DP voltammogram of *C. Arabica* green bean coffee extract (Fig. 6, curve a) also represents the behaviour of *C. Robusta* green bean coffee extract, *C. Arabica* and *C. Robusta* roasted bean extracts and two ground coffee extracts. The DPVs of four investigated instant coffees were represented by voltammogram of Nescafé Classic coffee extract (Fig. 7, curve a).

All DPVs of coffees were characterized by the presence of two anodic oxidation peaks: very high well expressed peak at lower oxidation potential (peak 1, Figs. 6 and 7) and the small second oxidation peak at higher oxidation potential (peak 2). This peak 2 is relatively well expressed in instant coffees (Fig. 7), but only slightly expressed (more as shoulder) in all other coffee samples (Fig. 6).

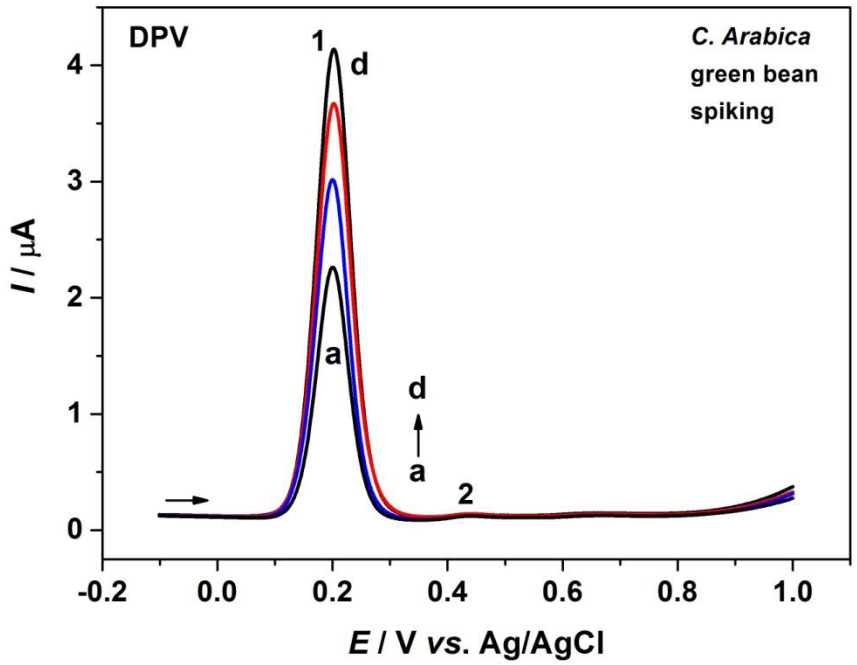



**Figure 6.** DPVs of *C. Arabica* green bean extract (diluted 50-fold), in 0.1 mol $L^{-1}$ PBS pH 7 (a). Spiked samples: (b) *C. Arabica* + $3 \cdot 10^{-5}$ mol $L^{-1}$ of 5-CQA, (c) *C. Arabica* + $1 \cdot 10^{-5}$ mol $L^{-1}$ of 3,4-diCQA, and (d) *C. Arabica* + $1 \cdot 10^{-5}$ mol $L^{-1}$ of 4,5-diCQA. The experimental conditions for DPV are as in Figure 1.

Figure 6 shows the electrochemical behaviour of *C. Robusta* green bean extract. The peak potential ($E_p$) of the first oxidation peak 1 was observed at 0.200 V. Ziyatdinova et al. [14] reported the same value of 0.200 V for the first oxidation peak of their coffee samples also investigated by the DPV. The oxidation peak potentials of peak 1 of all investigated coffees in this paper are in the potential range between the value of oxidation peaks of CQAs (0.187 V) and diCQAs (0.200 V), respectively (see Fig. 1). The equimolar mixtures of CQAs and diCQAs show oxidation potentials between the potentials of these two groups of compounds. Thus, equimolar mixture of $3\ 10^{-5}$ mol $L^{-1}$ of 5-CQA and 3,4-diCQA show oxidation potential at 0.197 V (DP voltammogram not shown here).

It is well known that coffee contained a mixture of different CQAs and diCQAs as the main CGAs, as was reported in many published HPLC analysis of coffee [5-8]. We also analysed our coffee samples by HPLC and found that in these coffees, mixture of CQAs and diCQAs prevailed in the total CGQAs content. Thus, e.g. in green coffee beans extract mixture of CQAs and diCQAs correspond approximately to 95 % of all CQAs contained. Therefore, it can be concluded with almost certainty that the first oxidation peak correspond to the electrochemical oxidation of mixture of different CQAs and diCQAs contained in all investigated coffee samples.

To confirm such assignation of the first anodic oxidation peak 1, some coffee samples were spiked with CQAs and diCQAs. Figure 6 shows the observed behaviour. *Coffea Arabica*, green bean extract (diluted 50-fold, curve a, Fig. 6) was spiked with: $3\cdot10^{-5}$ mol $L^{-1}$ solution of 5-CQA (curve b, Fig. 6); with $1\cdot10^{-5}$ mol $L^{-1}$ solution of 3,4-diCQA (curve c, Fig. 1); and with $1\cdot10^{-5}$ mol $L^{-1}$ solution of 4,5-diCQA (curve d, Fig. 6), in the separate DPV experiments. It can be seen that the spiking of 5-CQA in *C. Arabica* green extract significantly increases the current of peak 1 of *C. Arabica* green bean extract, whereas the peak potential value (0.200 V) is not changed. Spiking of 3,4-diCQA and 4,5-diCQA increases the peak current of *C. Arabica* green bean extract more than that of 5-CQA (in accordance with experiments shown in Fig. 1) and the peak potential is moved to 0.203 V. This is probably due to adsorption of diCQAs oxidation product(s) on the GCE. The experiments with spiking undoubtedly confirm that the first anodic oxidation peak observed in coffee samples by DPV can be ascribed to the electrochemical oxidation of mixture of CQAs and diCQAs contained in each coffee sample. Such assignation of this anodic oxidation peak was also reported in our SWV measurements of the same coffee samples [15].

The assignation of the second anodic oxidation peak of coffees (peak 2, Fig. 7.) was based on the electrochemical behaviour of FQAs (see Fig 2.), spiking of some coffee samples with FQAs standards (see Fig 7) and HPLC analysis of our coffee samples.

HPLC analysis of the coffee samples that has been investigated in this paper (HPLC results were not presented here) shows relatively minor content of FQAs (e.g. in *C. Arabica* green beans ca. 6%, and in other investigated coffees lesser). Similar results for FQAs content were also reported by other authors in HPLC analysis of different coffee brands [5-8]. In these papers free ferulic acid (FA) was not found, but their esters i.e. FQAs were observed. Therefore, such HPLC results have been the first fact suggesting that the second oxidation peak observed in DPV of coffee could probably be attributed to the FQAs content.

Such assignation was additionally supported by the second fact, i.e. by the values of oxidation potentials of investigated FQAs which had been in the potential range of 0.419-0.430 V, depending on

the type of FQAs (see Fig. 2). The oxidation potentials of FQAs were very close to the potential of the second oxidation peak observed in the investigated coffee samples (potential range of 0.430-0.444 V, depending on the brand of coffees).

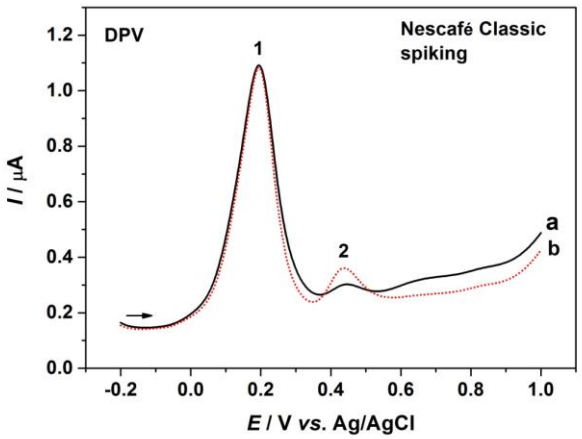



**Figure 7**. DPVs of Nescafé Classic extract (diluted 50-fold), in 0.1 mol $L^{-1}$ PBS pH 7 (a). Spiked sample (b): Nescafé Classic extract + $2 \cdot 10^{-5}$ mol $L^{-1}$ of 5-FQA. The experimental conditions for DPV are as in Figure 1.

Finally, to be completely certain that this second oxidation peak corresponds to the oxidation of FQAs, some coffee samples were spiked with FQAs solutions. Fig. 7 represents one of these experiments. It could be seen that spiking of $2 \cdot 10^{-5}$ mol $L^{-1}$ solution of 5-FQA in Nescafé Classic coffee extract significantly increases the current of the second oxidation peak (peak 2, curve b, Fig. 7), moving the oxidation potential of "pure" coffee only slightly in the cathodic direction (from 0.444 V in "pure" coffee to 0.441 V in the "spiked" coffee).

Some other parameters essential for the analytical methodology, e.g. the effect of pH of buffer solutions, were tested in Nescafé Classic extract (DPVs not shown here). It was observed that the peak potentials ($E_p$) of both oxidation peaks (peak 1 and peak 2) depended on the pH value of buffer solutions. $E_p$ of the first oxidation peak (peak 1) of Nescafé Classic coffee shifts linearly to less positive values from pH 5 to pH 8 with the slope of d($E$)/d(pH) ca. -60 mV/pH unit, the value very close to that observed for 5-CQA (see inset in Fig. 4.). This fact indicated that the oxidation/reduction processes proceeded on the GCE in coffee samples during the oxidation in the potential region of peak 1 are the same or very similar as that observed in the model solution of 5-CQA and others CQAs and diCQAs (see explanation in Section 3.1.5). That means that electrochemical oxidation of CQAs and diCQAs contained in the coffee samples had involved two electron-two proton mechanisms, i.e. oxidation of catechol moiety (moieties) in the structure of these CGAs. The maximum of peak current ($I_p$) of the first oxidation peak in Nescafé Classic coffee extract was observed in PBS of pH 7, similar as in the solution of 5-CQA (see Section 3.1.3). Therefore PBS solution of pH 7 was chosen for quantification of the total CGAs content in all coffee samples.

The pH dependence of the oxidation peak potential $E_p$ of peak 2 in Nescafé Classic extract, had also shown linear relationship in the pH range from 5 to 8. The slope of d($E$)/d(pH) is close to -58 mV/pH unit, which had shown that the oxidation processes proceeded in the potential region of peak 2 in Nescafé Classic coffee extract, are similar to that observed in the solutions of FQAs (see explanation in Section 3.1.5.).

Speaking generally, the electrochemical properties of investigated coffee samples (e.g., oxidation potentials, pH dependence, oxidation/reduction mechanism, etc.) were the same or very similar to that observed in CQAs, diCQAs, and FQAs solutions. Therefore, it could be concluded that the first anodic oxidation peak (peak 1) recorded in all investigated coffee samples could be ascribed to the electrochemical oxidation of mixture of the different CQAs and diCQAs. The second oxidation peak (peak 2) of coffees could be assigned to the electrochemical oxidation of FQAs. All these CGAs were presented in coffees, as were reported by many HPLC analyses of different coffees [5-8]. In addition, HPLC analysis of coffee samples investigated in this paper was performed (results were not presented here), and were found that three CQAs (5-CQA, 4-CQA, 3-CQA) and three diCQAs (3,4-diCQA, 3,5-diCQA, 4,5-diCQA) represented ca. 90-95 % of all CGAs found in coffees; and 5-CQA was prevailed CGA in all investigated coffee samples. Other CGAs found in coffee by HPLC analysis were FQAs, presented in content of ca. 4-5% in instant coffees to 1-2 % in other coffee samples. Similar proportion of CQAs, diCQAs and FQAs content was found also in DPV measurements. Therefore, the sum of CQAs and diCQAs content (from anodic peak 1) and FQAs (from peak 2) represent total CGAs content in coffee samples, and 5-CQA was used as standard to express this content.

### *3.2.2. Determination of total CGAs content in coffee samples*

**Table 2.** Total chlorogenic acids (CGAs) content in different brand of coffees determined by DPV and HPLC methods

| Brand of coffee | CGAs (total)-DPV[a] | CGAs (total)-HPLC[b] |
|---|---|---|
| *C. Arabica*, Rio Minas, Brazil (green bean) | 7451 | 7370 |
| *C. Arabica*, Rio Minas, Brazil (roasted bean) | 2630 | 2613 |
| *C. Robusta*, Cherry, India (green bean) | 9115 | 9112 |
| *C. Robusta*, Cherry, India (roasted bean) | 2852 | 2826 |
| Flatscher Olimpia (ground coffee, 100 % Arabica) | 4101 | 3932 |
| Franck Quatemala (ground coffee, 100 % Arabica) | 3574 | 3519 |
| Nescafé Classic (instant coffee) | 3283 | 3203 |
| Nescafé Espresso (instant coffee) | 3229 | 3185 |
| Jacobs Monarch (instant coffee) | 3203 | 3149 |
| Jacobs Intense (instant coffee) | 3465 | 3462 |

Results represent mean value of three independent measurements ($n$ =3)

[a]- values determined by DPV, total CGAs content was expressed as 5-CQA equivalent (mg 5-CQAE/100 g of coffee)

[b]- values determined by HPLC, total CGAs content were sum of individual CGAs content and expressed as mg CGAs/100 g of coffee

The total CGAs content in coffee samples was calculated from the summarized peak current ($I_p$) values of peak 1 and peak 2, using calibration curve for 5-CQA as the standard (see inset of Fig. 5), and expressed as mg 5-CQA equivalents per 100 g of coffee (mg 5-CQAE/100 g of coffee, Table 2.). The results were mean values of three independent measurements. For comparison, the results of HPLC analysis (performed in the separately experiments) were presented. In HPLC analysis the total CGAs content is the sum of content of all individual detected CGAs, and was therefore expressed as mg CGAs/100 g of coffee.

From the results presented in Table 2 it is evident that DPV method gave practically the same or only slightly higher values of total CGAs content than HPLC analysis. The total CGAs content decreases in the following order: green beans, ground coffees, instant coffees, roasted beans. Such results are in agreement with HPLC analysis reported, which have shown significant loss of CGAs content and change in CGAs profile during roasting of green beans and further processing of ground coffees to produce other type of coffees, e.g. instant coffees [5,8].

The comparison of the total CGAs content in coffees obtained in this paper with results from our SWV measurements [15] and results of other authors which have used electrochemical methods for determination of total CGAs content [13,14,16-22], has shown a reasonable agreement. A more detailed comparison with results of other authors was not possible due to the different coffee samples, different method of preparation of coffee extracts, and different method and experimental conditions of analysis.

## 4. CONCLUSIONS

The electrochemical properties of nine CGAs isomers: three CQAs (5-CQA, 4-CQA, 3-CQA), three diCQAs (3,4-diCQA, 3,5-diCQA and 4,5-CQA) and three FQAs (5-FQA, 4-FQA, 3-FQA) were investigated by DPV and compared with the electrochemical properties of CFA and FA (structural moieties of investigated CGAs). The electrochemistry of FQAs was investigated for the first time. The study had shown that electrochemical properties of all investigated CGAs strongly depend on their chemical structure and electronic properties, particularly on electron-donating effect of –OH and –CH=CH– groups, and a strong electron-withdrawing effect of ester (-COOR) group presented in their structure. In addition, the electrochemical properties of FQAs were also influenced by the presence of methoxy group ($–OCH_3$) attached on the aromatic ring. Substitution of the 3-OH group in the catechol structure of CFA and CQAs by a $–OCH_3$ group considerably shifts the redox potential of FA and FQAs toward the more positive values.

DPV measurements have shown that electrochemical oxidation/reduction process of investigated CQAs and diCQAs at the GCE is reversible, pH-dependent, a two electron-two proton process. The oxidation/reduction process occurred on the catechol moiety (*ortho*-dihydroxyl groups) in the structure of these molecules. Electrochemical oxidation of FQAs has proceeded by an EC mechanism. In the first, electrochemical step, a phenoxy radical was formed by irreversible one-electron-one-proton process. In the second, chemical step, phenoxy radical undergoes hydrolysis

generated the new more electroactive species with methoxy-catechol structure. This species was reversibly oxidised to a methoxy-*o*-quionone structure by a two-electron-two-proton process.

The electrochemistry of 5-CQA (major CGAs in coffee) was investigated more in detail. It was observed that anodic peak current of 5-CQA show maximum in PBS solution of pH 7, due to the maximum concentration of 5-CQA$^-$ anion at this pH value. A linear relationship of the anodic peak current with the concentration of 5-CQA in the concentration range of 5-50 μmol $L^{-1}$ was observed, with LOD of 1.2 $10^{-6}$ mol $L^{-1}$. The calibration curve of 5-CQA was used for quantification of total CQAs content in coffee.

The DPV measurements of coffee samples have shown that electrochemical properties of coffee samples were very similar to that of investigated CGAs. Therefore, DPV was used for determination of CGAs in coffees. It was shown that DPV is a very sensitive and selective method for determination of total CGAs content in coffee. A very good agreement between the results obtained by DPV and HPLC methods was observed.

## References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