*Int. J. Electrochem. Sci.*, 11 **(2016)** 10644 – 10658, doi: 10.20964/2016.12.73

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

# Determination of Antioxidant Activity of Caffeic Acid and *p*-Coumaric Acid by Using Electrochemical and Spectrophotometric Assays

*Anna Masek*[1,*] , *Ewa Chrzescijanska*[2], *Malgorzata Latos*[1]

[1] Technical University of Lodz, Institute of Polymer and Dye Technology, Faculty of Chemistry, 90-924 Lodz, ul. Stefanowskiego 12/16, Poland
[2] Technical University of Lodz, Institute of General and Ecological Chemistry, Faculty of Chemistry, 90-924 Lodz, ul. Zeromskiego 116, Poland
[*]E-mail: anna.masek@p.lodz.pl

*Received:* 18 August 2016 / *Accepted:* 20 September 2016 / *Published:* 10 November 2016

The aim of our study was to investigate the mechanisms of electrooxidation selected hydroxycinnamic acids. The curves made on the basis of cyclic and differential pulse voltammetry and quantum chemical calculations were given the opportunity to determine the scheme of oxidation of the molecule caffeic acid. The described scheme shows, that caffeic acid changes two electrons in the first stage of electrooxidation and for *p*-coumaric acid is exchanged one electron. It was determined the antioxidant potential of the tested compounds using spectrophotometric methods such as: ABTS, DPPH, FRAP and CUPRAC. Both caffeic acid and *p*-coumaric acid were characterized by a high capacity for scavenging free radicals as well as the reduction of iron and copper ions. However, caffeic acid exhibited improved antioxidant properties, which increase linearly as a function of their concentration. Definitely, however, caffeic acid was a lot stronger reducing agent in the oxidation processes.

**Keywords:** electrooxidation; antioxidant; spectrophotometric assay, caffeic acid, *p*-coumaric acid

## 1. INTRODUCTION

Hydroxycinnamic acids are widely distributed in the vegetable in abundance in fruits and vegetables. Caffeic acid occurs most often in the food mainly in chlorogenic acid form. Biosynthesis of hydroxycinnamic acids (caffeic, ferulic, 5-hydroxyferulic and sinapic acids) is generated from the *p*-coumaric acid through the hydroxylations and methylation reactions. In recent decades, the polyphenols are intensively studied, because of their beneficial effects on human health[1-9]. Natural compounds can act as anti-aging substances by several different mechanisms, including free radical

scavenging, metal chelation and protein binding. Hydroxycinnamic acid may also exhibit prooxidative capacity, which is especially interesting aspect of their properties [10-15].

There are ongoing studies on the wide determination of the relationship between antioxidant activity and the structure of the molecule. On the antioxidant activity of polyphenols is affected primarily by the conformation of the molecule. First of all, it takes into account the ethyl substituent to the aromatic ring, localization of the phenolic groups relative to the plane of the ring, orientation of the carbonyl group and the carbon chain C=C bond. The literature shows that the quasi-planar geometry of the molecule contributes to a better stabilizing effect through delocalization of the *p*-electron between aromatic ring and C=C bond. Antioxidant activity of the polyphenols is usually determined count and the position of the hydroxyl groups [16-24]. The aim of this study was to determine the structure of hydroxycinnamic acids on antioxidant activity. In many papers have been reported the antioxidant activity of various plant extracts. In the presented publication was described the chemistry of oxidation reaction and activity in these reactions of caffeic acid and *p*-coumaric acid. Understanding the mechanisms of action of antioxidant is extremely important to explore new active drug ingredients. Measurements of the electrochemical behavior can be excellent guide with a lot of useful information about antioxidant activity of hydroxycinnamic acids [25-31]. During the analysis of results rated the relationship between the measured potential and antioxidant properties. Electrochemical studies in conjunction with the analysis spectrophotometric great complement to allow a detailed description of the oxidation mechanism of phytochemicals. Different spectrophotometric assays as ABTS, DPPH, FRAP and CUPRAC was discussed in the present study [32-35].

## 2. EXPERIMENTAL AND MATERIALS

### *2.1. Chemicals*

The caffeic acid (3,4-dihydroxycinnamic acid, $C_9H_8O_4$) and *p*-coumaric acid (4-hydroxycinnamic acid, $C_9H_8O_3$) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Experiments were performed in non-aqueous media. The substrates solutions were prepared by dissolving in 0.1 mol $L^{-1}$ $(C_4H_9)_4NClO_4$ in acetonitrile ($CH_3CN$, pure p.a.). Concentrations of acids it was $2.0 \times 10^{-3}$ mol $L^{-1}$. All reagents used were of analytical grade supplied from Fluka (France) and Sigma-Aldrich (Germany).

The substrates solutions were prepared by dissolving in 0.1 mol $L^{-1}$ $((C_4H_9)_4NClO_4$ in acetonitrile. 2,2-diphenyl-1-picrylhydrazyl (DPPH), neocuproine ≥98% and TPTZ 2,4,6 –tris(2-pyridyl)-s-triazine for spectrophotometric def of Fe≥99.0% were purchased from Sigma-Aldrich GmbH (Sternheim, Germany). 2,2'-azino-bis-(3-ethylobenzthioazoline-6-sulfonic acid) (ABTS) diammonium salt was purchased from AppliChem (Germany). Trolox®, 97%, 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid was purchased from Acros Organics (USA). Copper chloride (CUPRAC) standard solution 0.01 mol $L^{-1}$, hydrochloric acid standard solution 0.04 mol $L^{-1}$ and iron (III) chloride hexahydrate pure (FRAP) p.a. 99.0%, $CH_3COONa$ – $CH_3COOH$ buffer solution pH 3.6, 0.3 mol $L^{-1}$ were purchased from CHEMPUR.

### *2.2. Measurement methods*

#### *Cyclic voltammetry*

Voltammetric techniques are the most widely used stationary methods used in the analysis of phenolic compounds. Voltammetric measurements were performed with an Autolab PGSTAT 30 (EcoChemie, The Netherlands) potentiostat. All experiments were carried out in a conventional electrochemical cell using traditional three-electrode system. The working electrode used in experiments was a platinum electrode with geometric surface area of 0.5 $cm^2$. The platinum wire was used as the counter electrode and ferricinium/ferrocene electrode ($Fc^+$/Fc) as reference. Ferricinium/ferrocene electrode ($Fc^+$/Fc) as reference electrode is recommended by IUPAC [36]. The effect of the scan rate on the electrooxidation of acids in an non-aqueous medium was assessed. Cyclic voltammetry (CV) were recorded in the potential from 0 to 1.8 or 2.2 V with various scan rates (0.02 to 1 V $s^{-1}$). All of the solutions were degassed with argon prior to the measurements. During the measurements, an argon blanket was maintained over the solution. All experiments were performed at room temperature.

#### *Kinetic parameters of dyes*

The recorded voltammograms, under linear diffusion of the first step of electrooxidation, were used to determine the peak potential ($E_{pa}$), half-peak potential ($E_{pa/2}$) and half-wave potential ($E_{1/2}$). Additionally, the voltammograms were used to calculate an anodic transition coefficient ($\beta n_\beta$) and heterogeneous rate constant ($k_{bh}$) of the electrode process for the half-wave potential. The parameters were calculated from the following equations [36-38]:

$$\beta n_\beta = \frac{1.857RT}{F(E_{pa} - E_{pa/2})} \quad (1)$$

$$E_{pa} = -1.14\frac{RT}{\beta n_\beta F} - \frac{RT}{\beta n_\beta F}\ln\frac{k^0_{bh}}{D^{1/2}_{red}} + \frac{RT}{2\beta n_\beta F}\ln\beta n_\beta v \quad (2)$$

$$k_{bh} = k^0_{bh}\exp\left(\frac{-\beta n_\beta FE}{RT}\right) \quad (3)$$

where $D_{red}$– diffusion coefficient of reduced form, $cm^2\ s^{-1}$, $v$ – scan rate, V $s^{-1}$; $F$ – Faraday constant (96,487 C $mol^{-1}$); $R$ – universal gas constant (8.314 J $K^{-1}$ $mol^{-1}$); $T$ – Kelvin temperature.

#### *Quantum chemical calculations*

The quantum chemical calculations were performed by the application of the semi-empirical method using the AM1 with HyperChem program packages. The molecular structures of flavones in gas phase were fully optimized by using semi-empirical optimization by the applications algorithm Polak-Ribiere (conjugate gradient) for RMS gradient equal 0.0001 (responsibility for the accuracy of the calculations). The $E_{HOMO}$ highest occupied molecular orbital energies were calculated for the optimization structures of studied acids.

*DPPH radical-scavenging activity*

The ability to scavenge free radicals was examined using the method DPPH. The ethanol solution of the DPPH (2.0 ml) with a concentration of 40 mg/ml (0.1 mM) was added to 0.5 ml of an alcohol solution (80% ethanol) that contained 0.02 mg/ml antioxidant. Then, 10 minutes after mixing, the absorbance of the solutions was determined by UV-Vis spectra at 516 nm. UV-VIS spectra were recorded with a Thermoscientific Evolution 220 spectrophotometer (2015, USA). As a blank, 70% ethanol was used [39-40]. The capability to scavenge the DPPH radical (AA%) was determined using following equation:

Inhibition (% ) = $[(A_o-A_1)/A_o]\times100$

where $A_o$ was the absorbance of the control and $A_1$ was the absorbance in the presence of the sample of antioxidant.

*ABTS radical-scavenging activity*

The antioxidant activity of hydroxycinnamic acids was investigated using the ABTS•+ method. ABTS was dissolved in water to a 6 mM concentration and then mixed with 2.45 mM potassium persulfate. The mixture was incubated in the dark for 16 h. The ABTS /radical solution was diluted with ethanol to an absorbance of 0.70 at 734 nm. UV-VIS spectra were recorded with a Thermoscientific Evolution 220 spectrophotometer, produced in 2015 in the USA. After the diluted ABTS•+ solution (6.0 ml) was mixed with a 50 μL aliquot of each investigated solution (6 mg/ml) or Trolox in ethanol, the absorbance was measured at 734 nm after 2 min at room temperature. The inhibition level (%) of absorbance was obtained using the standard curve, which was prepared with Trolox (% inhibition level- μM Trolox). Results are presented as Trolox equivalent antioxidant capacity (TEAC), mmol Trolox/100 g antioxidant.

*FRAP assay*

Another reducing power assay is ferric reducing antioxidant power, which is based upon reduction of $Fe^{3+}$-TPTZ complex under acidic conditions. In this method, increase in absorbance of blue-colored ferrous form ($Fe^{2+}$-TPTZ complex) is measured at 595 nm. FRAP reagent was freshly prepared by mixing 25 mL of acetate buffer (0.3 M, pH 3.6), 2.25 ml of TPTZ solution (10 mM TPTZ in 40 mM HCl) and 2.25 mL of $FeCl_3$ (20 mM) in water solution. Sinapic acid and ferulic acid dissolved in ethanol. The reaction mixture was incubated at 37$^o$C. The solution was stirred and incubated for 4 min. Finally, the absorbance of mixture was measured at 595 nm.

*Cupric ions ($Cu^2$+) reducing-CUPRAC assay*

$CuCl_2$ was mixed with 0.25 ml (0.01 M), 0.25 ml of an ethanol solution neocuproine (7.5x10-3 M) and 0.25 ml of buffer solution $CH_3COONH_4$ (1 M) in the test tube, followed by addition of various concentrations of samples of antioxidants. Then, the total volume was made up to 2 ml with distilled water and mixed. The tubes were sealed and left at room temperature. Absorbance was measured at

450 nm against a reagent blank (water), after waiting for 30 minutes. Increased absorbance of the reaction mixture indicates increased capacity reduction. Reducing power copper ions ($Cu^{2+}$).

*Statistical analysis*

Results are presented as the means and the standard deviation of three independent extractions (n=3). Statistical analysis was applied for the comparison of the means using the Fischer LSD test (significance level was set at $p< 0.05$).

## 3. RESULTS AND DISCUSSION

### *3.1. Electrochemical behaviour of caffeic acid and p-coumaric acid at Pt electrode*

To investigate electrochemical reactivity of the compounds, first we study these by cyclic voltammetry technique. Electroanalytical techniques are preferred for the analysis of phenolic compounds. The application of Pt electrode is the initial stage in our investigations for comparison with other electrode materials which will be used in electrooxidation. In the case of caffeic acid and *p*-coumaric acid, graphite electrode [41], modified carbon electrodes [42], carbon paste electrode [43] are often suggested in electrochemical analysis. However, platinum seems to be one of the most suitable electrode materials due to its good electrochemical inertness and ease of fabrication into many forms. Many studies have reported that biological activities of polyphenolic compounds are in very close connection with their electrochemical properties. Antioxidant properties of phenolic compounds are related to their ability to donate electrons. Thus, e.g. compounds with lower oxidation potentials showed higher antioxidant activity. Cyclic voltammetry (CV) has become an important and widely used technique electrochemical many fields of chemistry and biochemistry. In particular it is widely used to study the electrode reactions of the compounds, as well as to obtain information on the nature of the intermediates and stability of the reaction products.

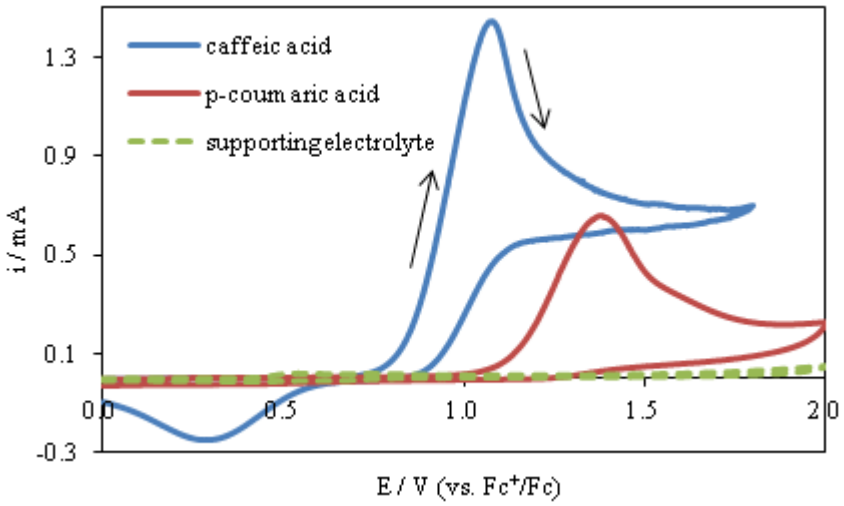



**Figure 1.** Cyclic voltammograms oxidation of caffeic and p-coumaric acids in non-aqueous environment; c = 2 x $10^{-3}$ mol $L^{-1}$ in 0.1 mol $L^{-1}$ $(C_4H_9)_4NClO_4$ in acetonitrile, v = 0.1 V $s^{-1}$.

Therefore we performed to study the electrochemical behavior of caffeic acid and *p*-coumaric acid using cyclic voltammetry (CV). Sample CV shown in Fig. 1. In the current depending on the potential of the electrode that the caffeic and p-coumaric acids may be irreversibly oxidized in at least one stage before the potential of electrode decomposition of the electrolyte. Caffeic acid is oxidized more easily than p-coumaric acid at a potential of about 0.3 V lower. Peak potential ($E_p$) electrooxidation caffeic acids is 1.07 V, while the $E_p$ electrooxidation p-coumaric acids is 1.38 V. The effect of the polarization rate of the reaction of the oxidation of fatty acids was tested. In the cyclic voltammograms made for various speeds determined polarity peak potential ($E_p$), half-peak potential ($E_{p/2}$), half-wave potential ($E_{1/2}$) and peak current ($i_p$).

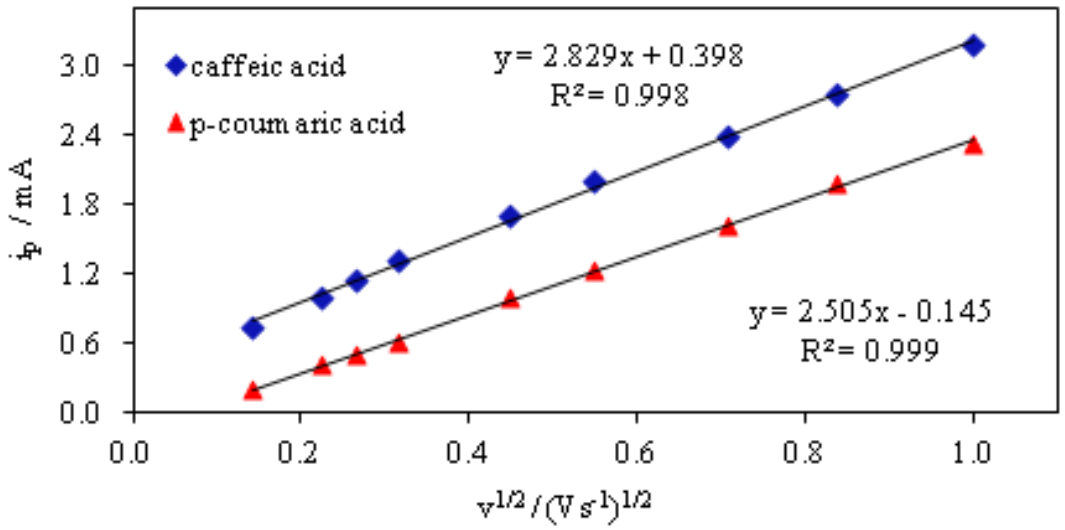



**Figure 2.** The dependence of the peak current ($i_p$) on the square root of the polarization speed ($v^{1/2}$) electrooxidation of the studied acids; c = 2 x $10^{-3}$ mol $L^{-1}$ in 0.1 mol $L^{-1}$ $(C_4H_9)_4NClO_4$ in acetonitrile.

Useful information about the electrooxidation mechanism of compounds can usually be obtained from the relationship between the peak current and the scan rate. Thus, the investigations included determination of the impact of the scan rate on the potential and current of the acids electrooxidation. The dependence of the peak current ($i_p$) on the square root of scan rate ($v^{1/2}$) of the studied acids in electrooxidation presented in Fig. 2 is linear and described by the following equation:

for caffeic acid $i_p = 2.829 \times v^{1/2} + 0.398$ $R^2 = 0.998$

for *p*-coumaric acid $i_p = 2.505 \times v^{1/2} - 0.145$ $R^2 = 0.999$

The dependencies described by equations do not intercept the origin, which indicates that the electrode reactions are diffusion or adsorption-controlled. The dependences of ln $i_p$ vs. ln v is presented in Fig. 3 indicates whether an electrode reaction is controlled by diffusion. The regression equations of the ln $i_p$ = f(ln v) plots for the anodic of caffeic and *p*-coumaric acids are: ln $i_p$ = 0.375 × ln v + 1.144; $R^2$ = 0.998 and ln $i_p$ = 0.604 × ln v + 0.908; $R^2$ = 0.997, respectively (Fig. 3). The slope values of these plots near to 0.5 confirmed also the observation that the electrochemical oxidation of caffeic and *p*-coumaric acids on Pt electrode is diffusion controlled.

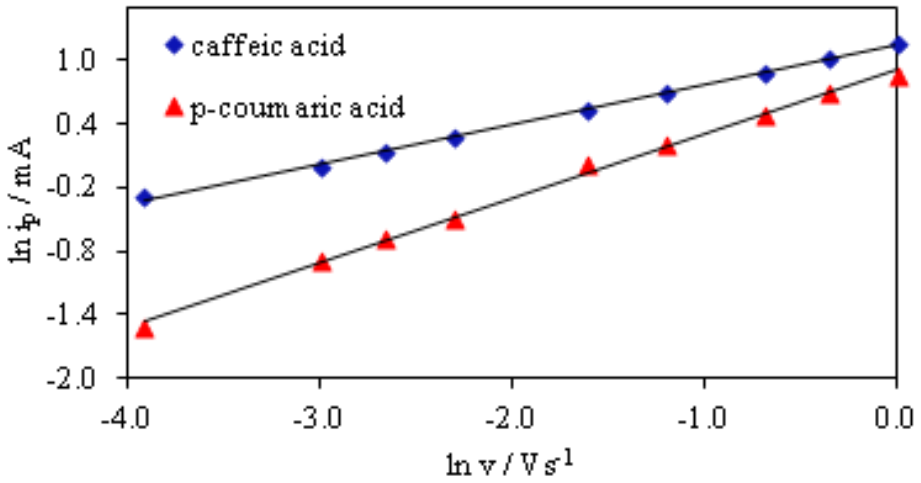



**Figure 3.** Dependence ln $i_p$ on ln *v* for electrooxidation of the studied acids; c = 2 x $10^{-3}$ mol $L^{-1}$ in 0.1 mol $L^{-1}$ $(C_4H_9)_4NClO_4$ in acetonitrile.

The effect of the scan rate on the potential of of caffeic and *p*-coumaric acids electrooxidation was also investigated.

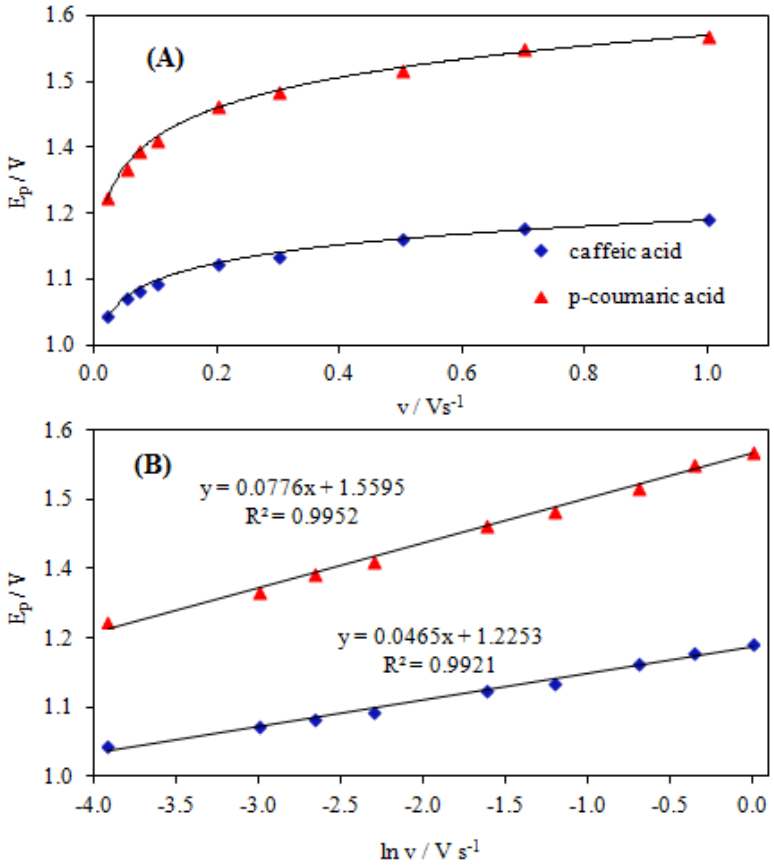



**Figure 4.** (A) Dependence of the anodic ($E_{pa}$) peak potential on the scan rate (*v*) for the oxidation of caffeic acid and *p*-coumaric acid, (B) Dependence of $E_{pa}$ on ln *v*; c = 2.0 × $10^{-3}$ mol $L^{-1}$ 0.1 mol $L^{-1}$ $(C_4H_9)_4NClO_4$ in acetonitrile at a Pt electrode.

Fig. 4 A presents the dependence of the peak potential ($E_p$) vs. the scan rate (v) for of caffeic and *p*-coumaric acids electrooxidation. The anodic peak potentials shift towards more positive potentials as scan rate increases, which points to a degree of irreversibility in the electrochemical processes.

Moreover, the value of the electron transfer coefficient ($\beta n_\beta$) for the reaction of acids electrooxidation was calculated to the equation [36]:

$$E_p = (\frac{RT}{\beta n_\beta F})\ln v + const \quad (4)$$

where: $E_p$ - peak potential (V), $R$ - universal gas constant (8.314J $K^{-1}$ $mol^{-1}$), $F$ - Faraday constant (96,487 C $mol^{-1}$), $T$ - Kelvin temperature (298 K), $\beta n_\beta$ –anodic transfer coefficient, $v$ - scan rate (V $s^{-1}$).

The dependence of $E_p$ vs. ln $v$ is presented in Fig. 4 B. In the range of the scan rate, $E_p$ and ln v showed a linear relationship. The regression equations were:

for caffeic acid $E_{pa} = \{0.047 \ln v \text{ (V s}^{-1})\}$ V + 1.2225 V, $R^2 = 0.992$

for *p*-coumaric acid $E_{pa} = \{0.078 \ln v \text{ (V s}^{-1})\}$ V + 1.559 V, $R^2 = 0.995$.

The value of $\beta n_\beta$ is 0.56 for caffeic acid and 0.32 for *p*-coumaric acid.

According to Bard and Faulkner [36], $\beta$ is found as,

$$\beta = \frac{47.7}{E_p - E_{p/2}} \quad (5)$$

where $E_{p/2}$ is the potential where the current is at half the peak value. From this result, we obtained the values of $\beta$ of 0.28 for caffeic acid and 0.26 for *p*-coumaric acid. Further, the number of electrons (n) transferred in the electrooxidation of compounds was 2 caffeic acid and 1.1 for *p*-coumaric acid.

When the electrooxidation of caffeic acid in the first step are exchanged electrons, whereas in the case of p-coumaric acid is mentioned one electron.The voltammograms recorded under linear diffusion of the first step of the electrooxidation were used to determine the $E_{pa}$, $E_{pa/2}$, and $E_{1/2}$ for the electrooxidaton of caffeic acid and *p*-coumaric acid. Additionally, calculated the $\beta n_\beta$ and the heterogeneous rate constant ($k_{bh}$) of the electrode process for the $E_{1/2}$ [37]. Heterogeneous rate constant determines the electron transfer rate from the studied molecules to the electrode. It depends on the structure of the oxidized compound and on the types of solvents, used for the electrode reaction.

The parameters were calculated using the equations (1), (2) and (3). The $E_{1/2}$ of caffeic acid is 1.05±0.02 V and of p-coumaric acid is 1.28 ± 0.02 V. The calculated $\beta n_\beta$ is 0.56 for caffeic acid and 0.32 for p-coumaric acid, and the $k_{bh}$ of the electrooxidation of investigated acids at the $E_{1/2}$ is (3.92±0.05) × $10^{-4}$ cm $s^{-1}$ and (3.45±0.05) × $10^{-4}$ cm $s^{-1}$, respectively.

The obtained electrode reaction kinetic parameters ($E_{1/2}$ for electrooxidation of acids) can be correlated with the $E_{HOMO}$ obtained by quantum-chemical calculations. Distribution of electron density in researched molecule is not uniform, because of the heterogeneous structure of the compound. Electron density indicates the most reactive positions of the studied compound. The highest electron density of studied molecules is on the carbon atoms in the benzene ring, which are linked to the hydroxyl groups. This fact suggests that the hydroxyl groups at these positions are easily oxidized (Fig. 5).

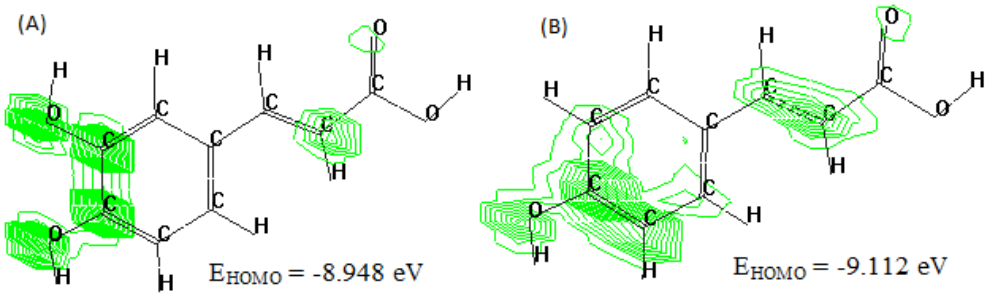
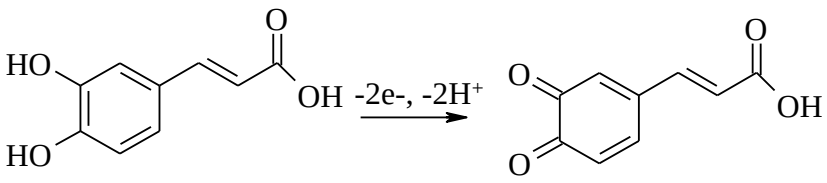



**Figure 5.** The HOMO frontier molecular orbitals of the (A) - caffeic acid and (B) - *p*-coumaric acid.

The $E_{HOMO}$ values is correlated with the values of $E_{1/2}$ that are determined from the cyclic voltamperograms for the test acids. Caffeic acid, having a two hydroxyl groups in ring, is oxidized easier and at the faster rate, and the calculated $E_{HOMO}$ has the largest value, which is -8.948 eV. In contrast, *p*-coumaric, which has one hydroxyl group, is the harder to oxidize, and the calculated $E_{HOMO}$ is the smallest, which is -9.112 eV.

Based on electroanalytical measurements and literature [42, 44-54] data, an oxidation mechanism of caffeic acid may be proposed (Scheme 1).




**Scheme 1.** Proposed oxidation mechanism of caffeic acid.

In the first step electrooxidation of caffeic acid they are exchanged two electrons and two protons, resulting in a o-benzoquinone acid.

*3.2. Evaluation of the antioxidant capacity of the cinnamic derivatives by using ABTS, DPPH, FRAP and CUPRAC spectrophotometric assays.*

We have analyzed the ability of the cinnamic derivatives to scavenge free radicals by using ABTS and DPPH assays. Antioxidant activity of the polyphenols is highly dependent on the structure of the position and number of hydroxyl groups. These two methods ABTS and DPPH are complementary and provide a lot of information on the capability of reacting with free radicals.

Free radicals are responsible for generating the negative processes of oxidation and therefore an extremely important element in the analysis of polyphenols compounds becomes describe the mechanism of the antioxidant actions. ABTS and DPPH radicals unfortunately have very limited relevance to compare the effect of the polyphenol in biological systems.

We set-activity curves for the scavenging in the function of the concentration of acids. Antioxidant activity of the compound depends on their concentration and reaction time. ABTS is measured as a reduction of cation radicals as the percentage inhibition of absorbance at 734 nm. Due to

the fact that solutions of the compounds listed are therefore clear without any interference and may have used to determine more precisely the free radical scavenging activity.

**Table 1.** Antioxidant activity (ABTS, DPPH) of the caffeic acid and *p*-coumaric acid.

| Concentration (μg ml$^{-1}$) | Inhibition (%) | | | |
|---|---|---|---|---|
| | Caffeic acid | | *p*-Coumaric acid | |
| | ABTS | DPPH | ABTS | DPPH |
| 5 | 9.5±0.08 | 5.7±0.26 | 15.5±0.54 | 0.5±0.30 |
| 10 | 16.0±0.40 | 14.5±1.07 | 18.7±0.07 | 2.1±0.54 |
| 15 | 19.0±0.73 | 16.0±0.26 | 31.2±0.46 | 2.8±0.86 |
| 20 | 22.2±0.96 | 21.8±0.82 | 34.3±0.82 | 3.3±0.28 |
| 25 | 29.8±0.72 | 25.2±1.03 | 46.6±0.94 | 5.2±0.30 |
| 30 | 32.1±0.83 | 28.5±0.51 | 51.7±0.41 | 5.3±0.50 |

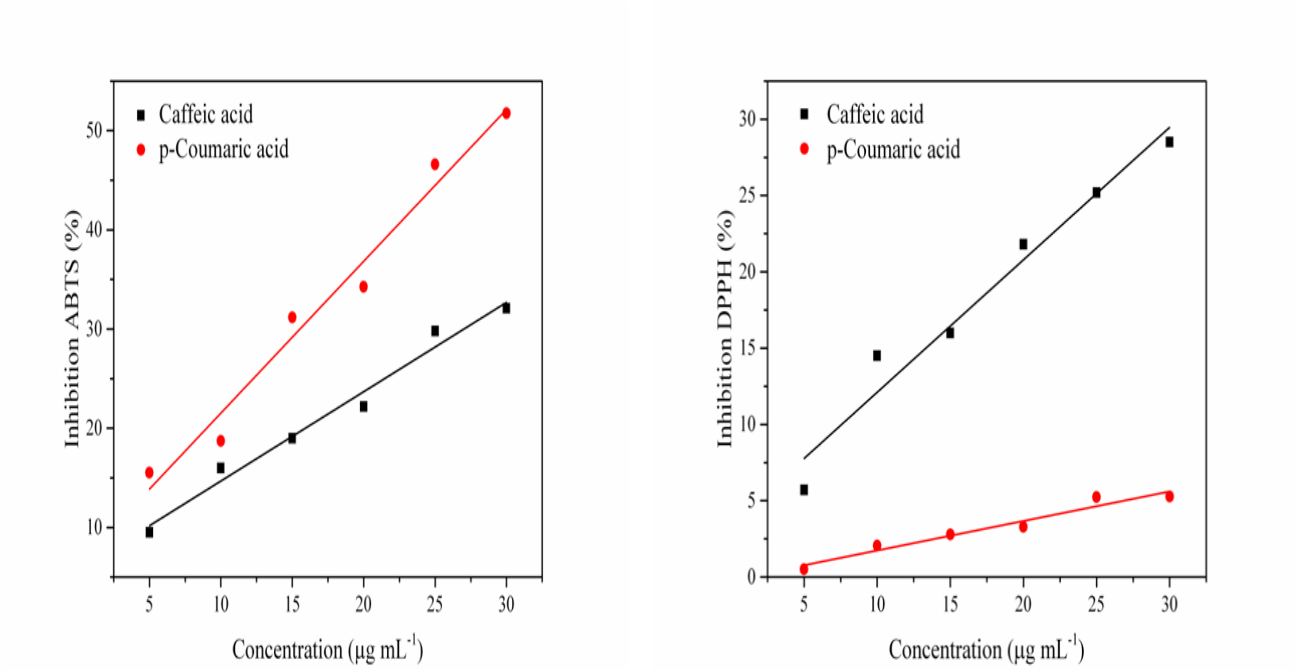



**Figure 6.** Radical-scavenging activities of caffeic acids and p-coumaric acid on (A) 2,2-diphenyl-1-picrylhydrazyl• (DPPH•), as measured by changes in absorbance at 517 nm and (B) on 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•+), as measured by changes in absorbance at 734 nm.

Caffeic acid shows high activity to deactivate radical DPPH, while less for radical ABTS. The opposite tendency was observed for p-coumaric acid. The biggest differences were noted for the method of DPPH, for which the activity of caffeic acid increased from 5.7 ± 0.26% for a concentration of 5 μg/ml to 28.5 ± 0.51% at a concentration of 30 μg ml$^{-1}$. In contrast, the activity for ABTS reduction for both tested acids differs pronounced at higher concentrations in terms of 15-30 μg ml$^{-1}$ of antioxidants (Table 1, Fig. 6). Ilhami Guelcin has been presented in his paper, that the caffeic acid exhibits as well as our research shows exceptionally high antioxidant activity measured by various in

vitro antioxidant assays. Also it indicates that these properties both in terms of scavenging free radicals and reducing or chelating metal ions with variable valency are comparable to that on commercial antioxidants such as BHT, BHA [55]. Elena Kurin reported interactions effects of mixture compounds (synergy; antagonism; additive), which can have a negative influence of their quenching properties. Therefore, action of the individual compounds do not always reflect the action of the mixture, for example in the plant extracts [56]. High potential of studied acids for scavenging ABTS radicals and preventing hydroperoxidation of a lipidic substrate was described by Jaqueline Badanai [57].

**Table 2.** Antioxidant activity (FRAP) of the caffeic acid and *p*-coumaric acid.

| Concentration ($\mu g\ ml^{-1}$) | FRAP ($\Delta A*100\%$) | |
|---|---|---|
| | Caffeic acid | *p*-Coumaric acid |
| 5 | 4.3 | 2.4 |
| 10 | 13.7 | 3.0 |
| 15 | 17.3 | 3.3 |
| 20 | 21.5 | 4.0 |
| 25 | 26.8 | 4.4 |
| 30 | 30.8 | 4.6 |

FRAP is a method enabling to determine the ability to reduce the iron ions of biological fluids and aqueous solutions of compounds. This method allows to determine the reduction potential of selected polyphenols, it consists on reduction of ferric-tripyridyltriazine ($Fe^{3+}$-TPTZ) complex to the ferrous ($Fe^{2+}$) with an maximum absorption at 593 nm.

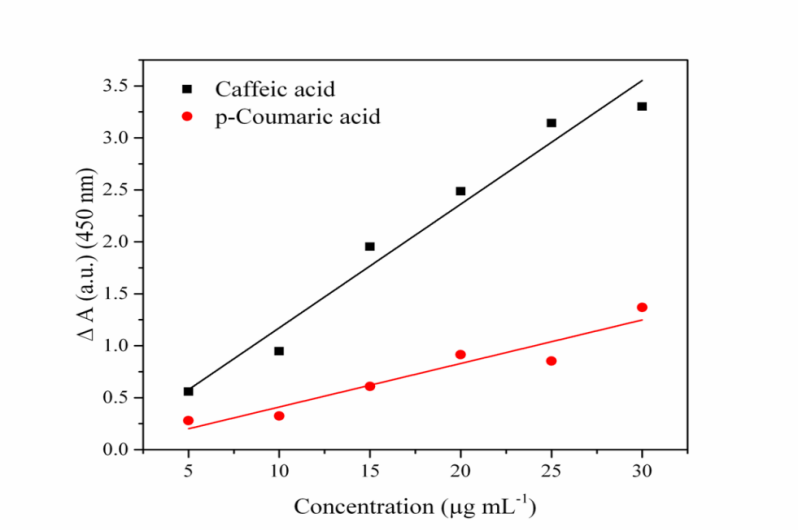



**Figure 7.** Antioxidant activity of the caffeic acid and p-coumaric acid samples measured by FRAP method.

The table number has been presented values of iron reduction ability of p-coumaric acid and caffeic acid. Definitely more production capacity caffeic acid. It was noticed the trend of increased reduction

activity in the function of the concentration both tested acids. For caffeic acid at 30 μg ml-1 equal to the power reduction was up to 30.8 % and for p-coumaric acid at the same concentration ΔA (*100%) was equal only 4.6 % (Table 2, Fig. 7).

**Table 3.** Antioxidant activity (CUPRAC) of the caffeic acid and *p*-coumaric acid.

| Concentration (μg $ml^{-1}$) | CUPRAC (ΔA) | |
|---|---|---|
| | Caffeic acid | p-Coumaric acid |
| 5 | 0.56 | 0.28 |
| 15 | 0.95 | 0.33 |
| 10 | 1.95 | 0.61 |
| 20 | 2.49 | 0.85 |
| 25 | 3.14 | 0.91 |
| 30 | 3.30 | 1.37 |

Mechanisms of the studied compounds reaction with radicals are quite different. DPPH assay involves electron transfer, while on CUPRAC is based on the redox reactions. Both methods are not compatible and do not give linear results. CUPRAC method can be used to analyze the different antioxidants such as: hydrophilic and lipophilic. The CUPRAC is stable reagent and selective, because it has a lower redox potential than that of the Fe(III)/Fe(II).

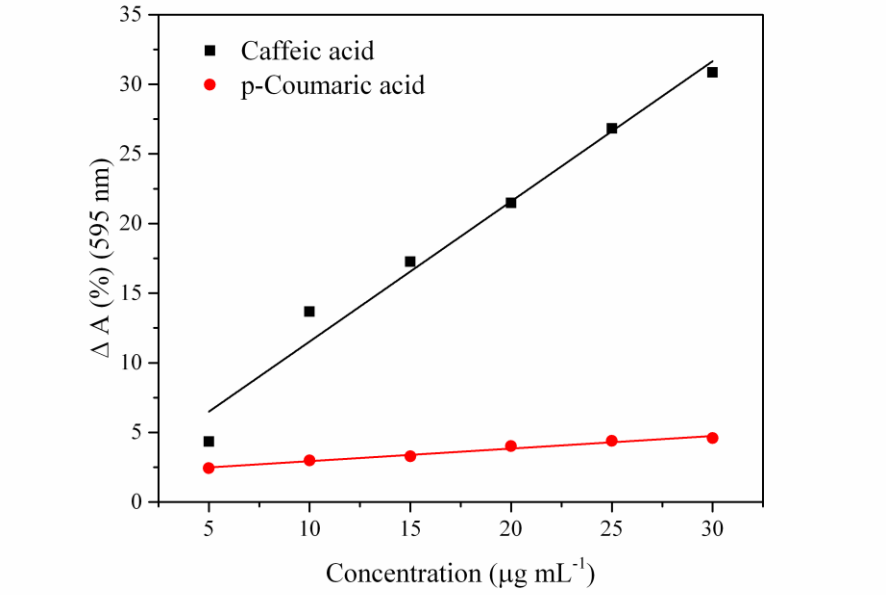



**Figure 8.** Antioxidant activity of the caffeic acid and *p*-coumaric acid samples measured by CUPRAC method.

According to the demostrated data in the table, it shows that the caffeic acid has almost twice as great potential for the reduction of Cu (II) ions, than p-coumaric acid. In the case of caffeic acid was

recorded linear and very large increase of ΔA to their concentration functions. P-coumaric acid is also characterized by a linear increase of reduction activity, for concentration 30 μg ml-1, ΔA is equal to 1.37 (Table 3, Fig. 8).

## 4. CONCLUSIONS

The electrochemical behaviour of caffeic acid and p-coumaric was investigated CV method at a Pt electrode. Acids were irreversibly oxidized in at least one stage electrode. Caffeic acid oxidizes more easily and with greater speed than the p-coumaric. In the case of oxidation of caffeic acid in the first stage they were exchanged electrons, while in the case electrooxidation of *p*-coumaric acid was replaced one electron. Underwent oxidation of hydroxyl groups in the molecules of acids. The number of hydroxyl groups contained in the molecular affects the antioxidant properties of the test compound. Caffeic acid has two hydroxyl groups and therefore has a better antioxidant properties than the *p*-coumaric acid, which has one hydroxyl group. This studies makes possibility to describe the dependence of the hydroxycinnamic acids structure on their antioxidant properties. In addition, through a combination of electrochemical techniques of spectrophotometric assays give a opportunity to predict the mechanism of oxidation of the tested compounds. According to the method ABTS or DPPH associated with the electron transfer was much more active caffeic acid, which has two OH groups in its structure. Similarly, in the case of methods FRAP and CUPRAC caffeic acid has been shown a great reduction potential for Fe and Cu ions.

ACKNOWLEDGEMENTS
This study was supported by the National Centre for Research and Development (NCBR), project 0139/L-7/2015.

## References

1. K. Kiessoun, A. Souza, N.T. Meda, A.Y. Coulibaly, M. Kiendrebeogo, A. Lamien-Meda, M. Lamidi, J. Millogo-Rasolodimby and O.G. Nacoulma, *Eur. J. Scientific Res*., 44 (2010) 570.
2. S. Klick and K. Herrmann, *Phytochem*., 27 (1988) 2177.
3. E. Koutsilieri, C. Scheller, E. Grünblatt, K. Nara, J. Li and P. Riederer, *J. Neurol*., 249 (2002) ii01.
4. K. Kowalska, *Postępy Hig. Med. Dośw*., 65 (2011) 515.
5. T.B. Lam, K. Kadoya and K. Iiyama, *Phytochemistr*y, 57 (2001) 987.
6. M. López, F. Martínez, C. Del Valle, C. Orte and M. Miró, *J. Chromatogr. A*., 13;922 (1-2) (2001) 359.
7. R. Tsao, *Nutrients*, 2 (2010) 1231.
8. B. Halliwell, J.M.C. Gutteridge and O. Aruoma, *Anal. Biochem.*, 165 (1987) 215.
9. V. Chobot, J. *Agric. Food Chem*., 58 (2010) 2088.
10. L. Belguendouz, L. Fremont and A. Linard, *Biochem. Pharmacol*., 53 (1997) 1347.
11. N.R. Perron and J.L. Brumaghim, *Cell Biochem. Biophys*., 53 (2009) 75.
12. R. Pulido, L. Bravo and F. Saura-Calixto, *J. Agric. Food Chem*., 48 (2000) 3396.
13. R.C. Hider, Z.D. Liu and H.H. Khodr, *Methods Enzymol*., 335 (2001) 190.
14. Y. Lu and L. Y. Foo, *Food Chem*., 68 (2000) 81.

15. P.H. Davis, Flora of Turkey and the East Aegean Islands. Edinburgh: Edinburgh University Press (1982) 349–382.
16. A. Masek, E. Chrzescijanska, M. Latos and M. Zaborski, *Food Chem.*, 215 (2017) 501.
17. U. Ozgen, A. Mavi, Z. Terzi, A. Yildirim, M. Coskun and P.J. Houghton, *Pharm. Biol*., 44 (2006) 107.
18. S. Bounatirou, S. Smiti, M.G. Miguel, L. Faleiro, M.N. Rejeb, M. Neffati, M.M. Costa, A.C. Figueiredo, J.G. Barroso and L.G. Pedro, *Food Chem.,* 105 (2007) 146.
19. E.G. Bakalbassis, A. Chatzopoulou, V.S. Melissas, M. Tsimidou, M. Tsolaki and A. Vafiadis, *Lipids*, 36 (2001) 181.
20. F. Natella, M. Nardini, M. Di Felice and C. Scaccini, *J. Agric. Food Chem*., 47 (1999) 1453.
21. S.S. Pekkarinen, H. Stockmann, K. Schwarz, I.M. Heinonen and A.I. Hopia, *J. Agric. Food Chem*., 47 (1999) 303.
22. J. Piljac-Zegarac, L. Valek, T. Stipcevic and S. Martinez, *Food Chem.,* 121 (2010) 820.
23. Y. Nakamura, S. Watanabe, N. Miyake, H. Kohno and T. Osawa, *J. Agr. Food Chem*., 51 (2003) 3309.
24. Y. Wang and C.T. Ho, *J. Agr. Food Chem*., 57 (2009) 8109.
25. A.K. Atoui, A. Mansouri, G. Boskou and P. Kefalas, *Food Chem*., 89 (2005) 27.
26. M.V. Eberhardt, C.Y. Lee and R.H. Liu, *Nature*, 405 (2000) 903.
27. H. Li, X.Y. Wang, Y. Li, P.H. Li and H. Wang, *Food Chem.,* 112 (2009) 454.
28. A.M. Pisoschi, M.C. Cheregi and A.F. Danet, *Molecules*, 14 (2009) 480.
29. M.B. Arnao, A. Cano and M. Acosta, *Free Radical Res*., 32 (1999) 89.
30. V.L. Singleton and J.A. Rossi, *Am. J. of Enol. Viticult*.,16 (1965) 144.
31. G.G. Duthie, S.J. Duthie and J.A.M. Kyle, *Nutr. Res. Rev*., 13 (2000) 79.
32. V.G. Georgiev, J. Weber, E.M. Kneschke, P.N. Denev, T. Bley and A.I. Pavlov, *Plant Foods Hum Nutr*., 65(2) (2010) 105.
33. H.M. Han and B. Koh, *J. Sci. Food Agric*., 91(13) (2011) 2495.
34. K.E. Heim, A.R. Tagliaferro and D.J. Bobilya, *J. Nutr. Biochem*., 13(10) (2002) 572.
35. A. Masek, E. Chrzescijanska and M. Zaborski, *Int. J. Electrochem. Sci*., 9 (2014) 7904.
36. A.J. Bard and L.R. Faulkner, Electrochemical Methods, Fundamentals and Applications, 2nd ed., John Wiley & Sons, New York, 2001, pp. 236, 503, 709.
37. Z. Galus, Fundamentals of Electrochemical Analysis, Ellis Horwood/Polish Scientific Publishers PWN, New York/Warsaw, 1994, pp. 84, 297.
38. E. Chrzescijanska, E. Kusmierek and G. Nawrat, *Polish J. Chem.*, 83 (2009) 1115.
39. A. Masek, E. Chrzescijanska and M. Zaborski, *Electrochim. Acta*, 107 (2013) 441.
40. D. Mihaylova and S. Schalow, *Braz. Arch. Biol. Technol.,* 56 (2013) 431.
41. I.G. David, A.-M.C. Bizgan, D.E. Popa, M. Buleandra, Z. Moldovan, I.A. Badea, T.A. Tekiner, H. Basaga and A.A. Ciucu, *Food Chem*., 173 (2015) 1059.
42. A.B. Moghaddam, M.R. Ganjali, R. Dinarvand, P. Norouzi, A.A. Saboury, A.A. Moosavi-Movahedi, *Biophysical Chemistry* 128 (2007) 30.
43. M. Meshki, M. Behpour and S. Masoum, *Anal. Biochem*., 473 (2015) 80.
44. M. Šeruga, and I. Tomac, *Int. J. Electrochem. Sci*., 9 (2014) 6134.
45. A.B. Moghaddam, M.R. Ganjali, R. Dinarvand, P. Norouzi, A.A. Saboury, A.A. Moosavi-Movahedi, *Biophysical Chemistry* 128 (2007) 30.
46. A. Alizadeh, M.M. Khodaei, M. Fakhari, G. Abdi and S. Ghouzi, *Electrochim. Acta*, 178 (2015) 533.
47. H. Hotta, M. Ueda, S. Nagano, Y. Tsvjino and Koyama, *J. Anal. Biochem*., 303 (2002) 66.
48. C. Bianchini, A. Curulli, M. Pasquali and D. Zane, *Food Chem.,* 156 (2014) 81.
49. S. K. Trabelsi, N. B. Tahar and R. Abdelhedi, *Electrochim. Acta*, 49 (2004) 1647.
50. L.R. Fukumoto and G. Mazza, *J. Agric. Food Chem.,* 48 (2000) 3597.
51. L.R. Fukumoto and G. Mazza, *J. Agric. Food Chem,* 48 (2000) 3597.

52. A.C. Oliveira and L.H. Mascaro, *Int. J. Electrochem. Sci.*, 6 (2011) 804.
53. H.R. Zare, R. Samimi and M. Mazloum-Ardakani, *Int. J. Electrochem. Sci.*, 4 (2009) 730.
54. H. Hotta, M. Ueda, S. Nagano, Y. Tsujino, J. Koyama and T. Osakai, *Anal. Biochem*., 303 (2002) 66.
55. I. Guelcin, *Toxicology*, 217 (2006) 213.
56. E. Kurin, P. Mučaji and M. Nagy, *Molecules*, 17 (2012) 14336.
57. J. Badanai, C. Silva, D. Martins, D. Antunes, M. G. Miguel, *J. App. Pharm. Sci*., 5 (2015) 034.