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Electrochemical and Spectrophotometric Determination of Total Polyphenol Content in Croatian Apple Varieties

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Abstract: Oxidation mechanism of quercetin in three different electrolytes (KCl, NaCl and LiCl) was studied by cyclic and differential pulse voltammetry. The results have shown that quercetin oxidation is reversible and diffusion controlled process in all three investigated electrolytes. Calibration diagram of quercetin was obtained in KCl, so this electrolyte was selected for total polyphenol content determination in apple peel extracts. Total polyphenol content was analysed by differential pulse voltammetry (dpv) and spectrophotometric Folin-Ciocalteu method (FC) in several ancient Croatian apple varieties and a wild apple variety grown in Croatia by using quercetin as a standard. Very high correlation for TP content obtained by two methods was observed since the TP_{dpv}/TP_{FC} ratio was around 1 and a correlation coefficient (R^2) for TP_{dpv} *vs.* TP_{FC} was 0.98. The highest total polyphenol content was found in Slavonska srčika variety.

Keywords: apple extracts, differential pulse voltammetry, spectrophotometry, quercetin, polyphenol determination.

INTRODUCTION

PHENOLIC compounds or polyphenols are a group of compounds that have more than one phenolic hydroxyl group attached to one or more aromatic rings. In plants, they occur in both free and bound form as esters or glycosides. Polyphenols are called natural antioxidants and they have a potential to show anti-inflammatory, antiallergic, anticancer and antihemorrhagic properties displaying some of their prominent roles in vivo as antioxidants. Many epidemiological studies suggest that regular consumption of fruits and vegetables rich in polyphenols may reduce the risk of cancer and cardiovascular diseases. The beneficial effects of polyphenols may be based on several factors, however, quenching free radicals and inhibition of lipid peroxidation (antioxidant properties) are one of the important biological activities of phenolic compounds.[1,2] Recent studies have shown that the ancient apple varieties are a rich source of phenolic compounds.[3,4] Polyphenols most often found in apples are flavan-3 ols in monomeric ((+)-catechin, (–)-epicatechin) and in oligomeric form (proanthocyanidins), flavonols, phenolic acids, dihydrochalcones, and anthocyanidins.[4,5] Apple peel extracts mostly contain quercetin derivatives which belong to the flavonol subgroup of polyphenols.[6] Individual polyphenols together with quercetin derivatives from the peel were identified and quantified in our earlier studies.[4,6,7] It was found that the peel contained galactoside, glucoside, xyloside and rhamnoside derivatives of quercetin, together with some unidentified quercetin derivatives. Moreover, quercetin derivatives occupied 24 % to 74 % of total polyphenols.[4] Polyphenol content in apples was studied by reversed phase- high performance liquid chromatography (RP-HPLC) with photodiode array detection (PDA),[4] HPLC with UV-Vis diode array (DAD) detector coupled to ion trap (IT) mass spectrometer,[7] ultra performance liquid chromatography (UPLC) coupled to quadrupole time of flight (QTOF) mass spectrometer[7] and Folin-Ciocalteu spectrophotometric method.[8,9] In the last decade, cyclic voltammetry,[10-14] differential pulse voltammetry,[15-17] linear

sweep voltammetry,[18] square wave voltammetry[19,20] and adsorptive stripping voltammetry[21,22] were also used for determination of phenolic compounds in their standard solutions and food samples (*e.g.* wine, fruit juices, tea, coffee). Since polyphenols as antioxidants are able to donate electrons, they are easily oxidized at inert electrodes which enable their detection with electrochemical methods.

The purpose of this study, was to investigate oxidation mechanism of quercetin in three different electrolytes (KCl, NaCl and LiCl) by cyclic and differential pulse voltammetry in order to find optimal electrolyte for the total polyphenol content determination. The results were applied to total polyphenol content determination in apple peel extracts of ancient Croatian apple varieties (Lještarka, Ljetna rebrača, Slavonska srčika, Zimnjara, Adamova zvijezda, wild variety (crabapple)), since those extracts mostly contain quercetin derivatives. Results obtained by differential pulse voltammetry were compared to results obtained with spectrophotometric Folin-Ciocalteu method.

EXPERIMENTAL

Chemicals

All chemicals were of reagent grade and used as purchased from commercial sources. Quercetin dihydrate ($C_{15}H_{10}O_7 \cdot 2H_2O$) and Sodium Chloride (NaCl) were obtained from Sigma-Aldrich (St. Louis, MO, SAD). Lithium Chloride (LiCl) was purchased from BDH Prolabo (Leuven, Belgium), Potassium Chloride (KCl), Hydrochloric acid (HCl, w = 36.2 %), Sodium Carbonate (Na_2CO_3) and Folin–Ciocalteu reagent from Kemika (Zagreb, Croatia) and Methanol (CH_3OH) from Carlo Erba (Val de Ruil, France).

Standard Solutions

Quercetin was used as a standard in electrochemical total polyphenol content determination. For the electrochemical determination, a stock solution of quercetin (γ =0.3 g L^{-1}) was prepared in methanol and kept in the refrigerator (solution was stable for at least 1 month). Prior to use, the stock solution was diluted to the desired concentration with supporting electrolytes (I_c = 0.34 M), which were diluted in high purity water from a TKA, GenPure Ultra Pure Water System (TKA, Niederelbert, Germany), resistivity greater than or equal to 18 MΩ cm.

For spectrophotometric determination, quercetin (γ =1 g L^{-1}) was prepared in methanol. The stock solution was diluted with methanol (0 to 0.5 g L^{-1}) and used for the calibration curve preparation.

Sample and Extract Preparation

Ancient, local apple varieties (Malus domestica) (Lještarka, Ljetna rebrača, Slavonska srčika, Zimnjara, Adamova zvijezda, wild variety (crabapple)) (around one kilogram) were picked in October / November 2015 in a family orchard (M. Veić, Mihaljevci, near Požega, Croatia). Apples were peeled with a consumer-grade peeler, and the peel was pooled and homogenized using a blender. The homogenized sample was used for the polyphenol extract preparation. Polyphenols were extracted with 0.1 % HCl in methanol.[6] Shortly, homogenized samples were weighed (0.2 g), mixed with 1.5 mL of extraction solvent and vortexed (Grant Bio, Cambridgeshire, England). Samples were then placed in an ultrasonic bath (Bandelin Sonorex, RK 100, Berlin, Germany) for 15 minutes. After that, samples were centrifuged (Minispin, Eppendorf AG, Germany). Extracts were removed and the residue was extracted one more time with the same procedure in 0.5 mL of extraction solvent. Two extracts were combined. From one homogenized apple peel sample, two parallel extracts were prepared and each was analyzed two times with spectrophotometric method and with cyclic and differential pulse voltammetry.

Spectrophotometric Method for the Total Polyphenol Determination

Total polyphenols were determined by the use of Folin-Ciocalteu micro method[23] according to the following procedure. Distilled water (1580 μL) was mixed with extract (20 μL), Folin-Ciocalteu reagent (100 μL) and a sodium carbonate solution (200 g L^{-1}) (300 μL). Reaction solution was incubated in a water bath at 40 °C (30 min). After that, the absorbance was read at 765 nm on a UV-Vis spectrophotometer (JP Selecta S.A., UV 2005, Barcelona, Spain). In order to create a calibration curve, quercetin solutions (0 to 0.5 g L^{-1}) were measured with the same procedure. The results were therefore expressed in mg of quercetin equivalents (QE) per kg of fresh peel weight (FW).

Cyclic and Differential Pulse Voltammetry

Electrochemical experiments were performed on a PalmSens potentiostat/galvanostat (PalmSens BV, Utrecht, The Netherlands). The instrument was driven by PSTrace 4.2 software. A conventional three-electrode cell was used. Glassy carbon (geometrical area 0.018 cm^2) was used as a working electrode, Ag/AgCl as a reference electrode, and a platinum wire as a counter electrode. The glassy carbon working electrode was polished with coarse diamond polish (1 μm, ALS, Japan) and after that with polishing α-Al_2O_3 (0.05 μm, ALS, Japan) before each measurement. Cyclic voltammetry scan rate varied from 50 mV/s to 300 mV s^{-1}. The used differential pulse voltammetry conditions were: scan increment 5 mV, pulse amplitude 25 mV, pulse width 70 ms, and scan rate 5 mV s^{-1}.

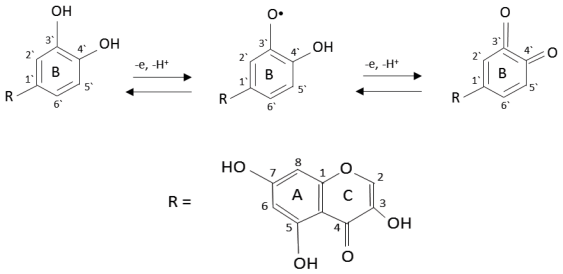
Statistical Analysis

Two parallel extracts were prepared from the peel of each apple variety. Each extract was measured two times with spectrophotometric method and with differential pulse voltammetry (n = 4). Mean values and coefficient of variation were reported. Regression analysis was done with Microsoft Excel.

RESULTS

Cyclic Voltammetry

In order to find the best electrolyte for the electrochemical total polyphenol content determination, cyclic voltammograms of quercetin (Figure 1) in three different electrolytes (KCl, NaCl and LiCl) were recorded. All voltammograms revealed two oxidation peaks and one oxidation shoulder of quercetin (Figure 2), which correspond to oxidation of 5-OH groups of quercetin. The first oxidation peak (A1), at 0.328 V corresponds to oxidation of catechol group (3`,4`-dihydroxy) at the B-ring of quercetin, second oxidation peak (A2), at 0.695 V corresponds to oxidation of 3-hydroxy group at the C- ring and the oxidation shoulder (A3), at 1.010 V corresponds to the oxidation of 5- and 7-hydroxy groups at ring A of quercetin.[24] A reduction shoulder (C1) at 0.272 V, corresponds to the reduction of the oxidation product formed in the first anodic peak. Obtained ΔEp value was 56 mV indicating reversible oxidation reaction in all three electrolytes. The quercetin oxidation process is diffusion controlled since linear dependence (R^2 = 0.98), in all investigated electrolytes, between anodic peak current and the square root of scan rate was found (Figure 3). A possible oxidation mechanism of quercetin, which corresponds to the oxidation of 3',4'dihydroxy substituent on the B-ring and includes transfer of two electrons and protons is:[25]



Differential Pulse Voltammetry

Differential pulse voltammograms also revealed two oxidation peaks (A1, A2) and one broad oxidation shoulder (A3) in three investigated electrolytes which correspond to oxidation of 5 hydroxy groups in quercetin. The first oxidation peak (A1) was at 0.256 V, the second oxidation peak (A2) was at 0.622 V and the oxidation shoulder (A3) was at 0.938 V. Two oxidation peaks and oxidation shoulder decreased in a second scan (Figure 4), since active surface of the working electrode was partially blocked by quercetin`s oxidation product.[24] Partial blockage of the

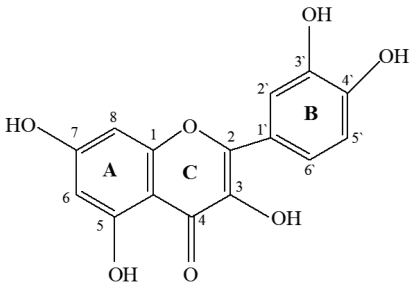


Figure 1. Chemical structure of quercetin (3,3`,4`,5,7-pentahydroksyflavone).

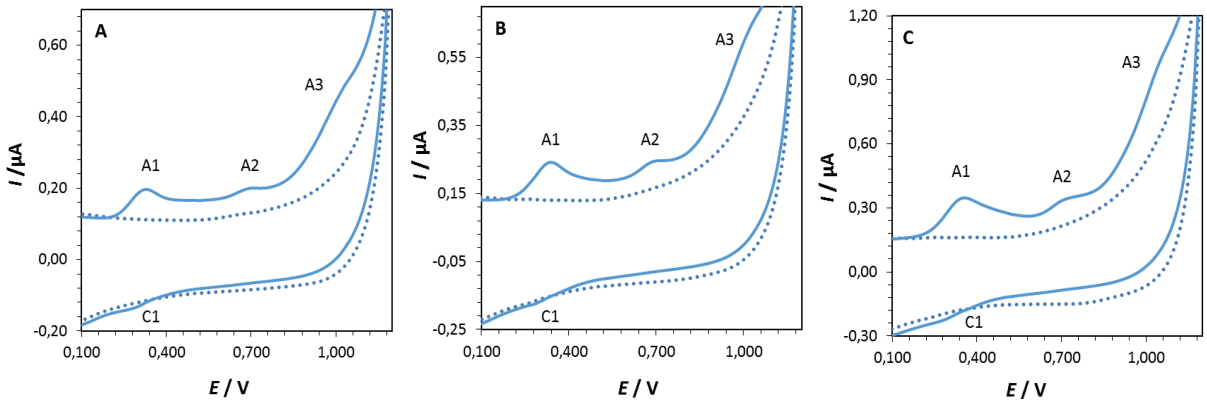


Figure 2. Cyclic voltammograms of quercetin (γ = 3 mg L^{-1}) recorded at glassy carbon electrode, scan rate: 100 mV s^{-1}. I_c = 0.34 M KCl (A), NaCl (B) and LiCl (C).(····) blank, (—) solution with quercetin.

working electrode surface was the most pronounced in LiCl (Figure 4C) since the most drastic decrease of oxidation peaks was observed.

Calibration diagram of quercetin is shown in Figure 5. It was constructed by plotting the oxidation peak current of the first oxidation peak (A1) *vs*. quercetin concentration (R^2= 0.9924, y =1.9833x + 0.1079). Linearity was obtained in quercetin concentration range from 0.3 mg L^{-1} to 4.8 mg L^{-1}. In other two electrolytes (NaCl and LiCl) calibration diagrams were not constructed since linearity of the first oxidation peak (A1) *vs*. quercetin concentration was not obtained.

Analysis of Apple Peel Extracts

The electrochemical results have shown that the calibration diagram of quercetin was obtained in 0.34 M KCl (linearity of the first oxidation peak (A1) *vs*. quercetin concentration was not obtained in NaCl and LiCl), so KCl was selected as a

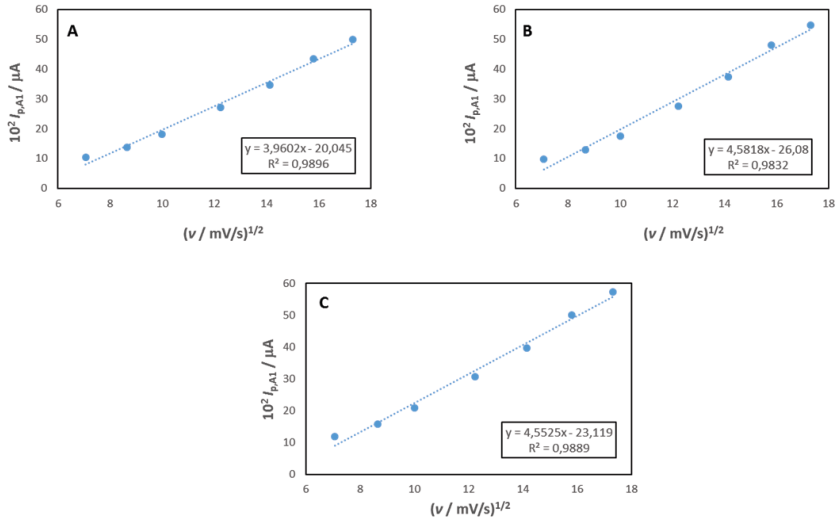


Figure 3. Anodic peak current of the first oxidation peak ($I_{p,A1}$) of quercetin (γ= 3 mg L^{-1}) as a function of the square root of scan rate, $v^{1/2}$. I_c = 0.34 M KCl (A), NaCl (B) and LiCl (C).

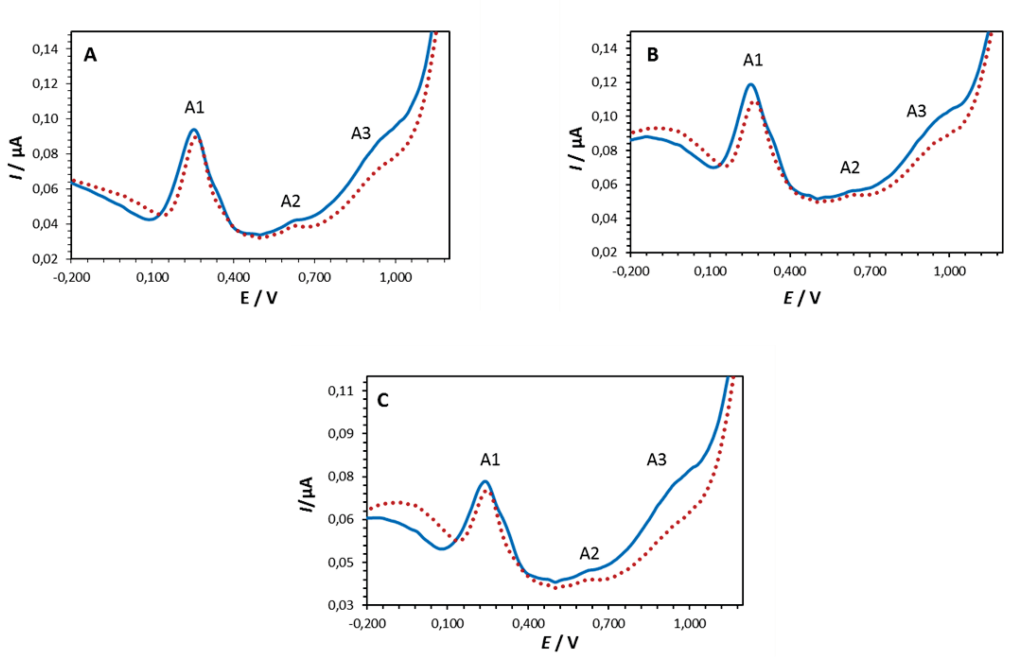


Figure 4. Differential pulse voltammograms of quercetin (γ = 3 mg L^{-1}) recorded at glassy carbon electrode, scan rate: 5 mV s^{-1}. I_c = 0.34 M KCl (A), NaCl (B) and LiCl (C). 1. scan (—), 2. scan (····).

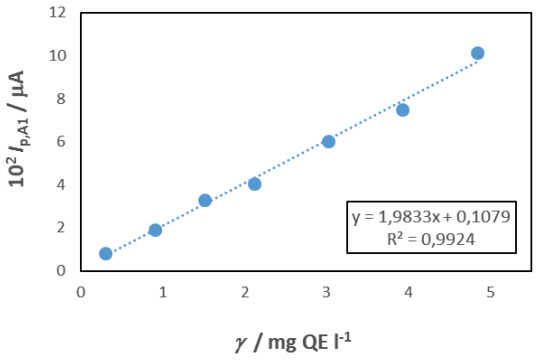


Figure 5. Calibration diagram of quercetin (I_c = 0.34 M KCl) obtained by differential pulse voltammetry, scan rate: 5 mV s^{-1}.

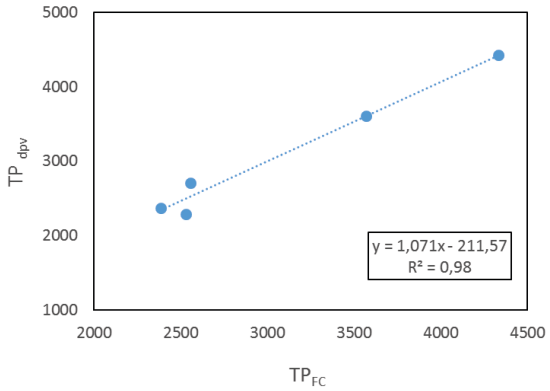


Figure 6. Correlation of the amount of total polyphenols obtained by electrochemical (TP_{dpv}) and spectrophotometric Folin-Ciocalteu (TP_{FC}) method.

suitable electrolyte for electrochemical determination of total polyphenol content (TP_{dpv}) in apple peel extracts of ancient Croatian apple varieties. Peel extracts (0.1 mL) were diluted with 0.34 M KCl to the final working volume 15 mL in order to adapt the expected concentration of peel extracts to match a linear range of the calibration diagram of quercetin (0.3 mg L^{-1} to 4.8 mg L^{-1}). Differential pulse voltammograms were recorded at the same conditions applied for quercetin standard solutions. Total polyphenol content was calculated by measuring the peak current in differential pulse voltammograms of apple extracts and by using the quercetin calibration diagram obtained in 0.34 M KCl (Figure 5A). The results were expressed as quercetin equivalents per kg of fruit weight (mg QE / kg FW) and in milligrammes of quercetin per liter (mg QE / L).

Furthermore, spectrophotometric Folin-Ciocalteu method was used to quantify total polyphenols (TP_{FC}) and the results were also expressed as mg QE / kg FW. The results obtained by differential pulse voltammetry and spectrophotometric method were compared (Table 1). According to differential pulse voltammetry, total polyphenols in apple peel varied from 2294 mg kg^{-1} FW to 4427 mg kg^{-1} FW. Similar results were obtained by Folin-Ciocalteu method where total polyphenols content varied from 2388 mg kg^{-1} FW to 4332 mg kg^{-1} FW. Very high correlation between the results obtained by two methods was observed since the TP_{dpv} / TP_{FC} ratio was around 1 and correlation coefficient, R^2 was 0.98 (Figure 6). The highest total polyphenol content was found in Slavonska srčika variety which agrees with literature data.[7]

Table 1. Total polyphenols determined by differential pulse voltammetry (TP_{dpv}) and spectrophotometric Folin-Ciocalteu method (TP_{FC}) and their ratio TP_{dpv} /TP_{FC}.

Apple	$TP^{(a)}_{dpv}$ mg QE / L	$TP^{(a)}_{dpv}$ mg QE/ kg FW	$TP^{(a)}_{FC}$ mg QE/ kg FW	TP_{dpv}/ TP_{FC}
Lještarka	234.4	2372.9	2387.6	0.99
Ljetna rebrača	365.2	3612.0	3569.3	1.01
Slavonska srčika	585.7	4426.7	4331.6	1.02
Adamova zvijezda	366.5	2706.0	2557.3	1.06
Wild	250.8	2293.7	2531.6	0.91

[a] Mean values of four determinations. Coefficient of variation was 0.24 for TPdpv and 0.27 for TP_{FC} respectively.

CONCLUSION

In this study, electrochemical oxidation of quercetin in three different electrolytes was investigated. The results have shown that quercetin oxidation is reversible and diffusion controlled process in all three investigated electrolytes. Calibration diagram was constructed in KCl (linearity was obtained in quercetin concentration range from 0.3 mg L^{-1} to 4.8 mg L^{-1}). The first oxidation peak of quercetin was chosen for quantification of total polyphenol content in apple peel extracts and the results were compared with spectrophotometric Folin-Ciocalteu method. Very high correlation between the results obtained by two methods was observed (R^2 = 0.98). The highest total polyphenol content was found in Slavonska srčika apple variety.

REFERENCES

[1] V. L. Singleton, F. H. Kratzer, *J. Agric. Food Chem.* **1969**, *17*, 497.
[2] I. Sensoy, R. T. Rosen, C.T. Ho, M.V. Karwe, *Food Chem.* **2006**, *99*, 388.
[3] P. Iacopini, F. Camangi, A. Stefani, L. Sebastiani, *J. Food Compos. Anal.* **2010**, *23*, 518.
[4] L. Jakobek, R. A. Barron, *J. Food Compos. Anal.* **2016**, *45*, 9.

[5] R. M. Alonso-Salces, C. Herrero, A. Barranco, L.A. Berrueta, B. Gallo, F. Vicente, *Food Chem.* **2005**, *93*, 113.
[6] L. Jakobek, M. Boc, R. A. Barron, *Food Anal. Met.* **2015**, *8*, 2612.
[7] L. Jakobek, R. García-Villalba, F. A. Tomás-Barberán, *J. Food Compos. Anal*. **2013**, *31*, 199.
[8] L. K. Beh, Z. Zakaria, B. K. Beh, W. Y. Ho, S. K. Yeap, N. B. M. Alitheen, *J. Med. Plants Res.* **2012**, *6*, 5857.
[9] C. Henríquez, S. Almonacid, I. Chifelle, T. Valenzuela, M. Araya, L. Cabezas, R. Simpson, H. Speisky, *Chil. J. Agr. Res.* **2010**, *70* (4), 523.
[10] P. A. Kilmartin, H. L. Zou, A. L. Waterhouse, *Am. J. Enol. Viticult*. **2002**, *53*, 294.
[11] O. Makhotkina, P. A. Kilmartin, *Anal. Chim. Acta* **2010**, *668*, 155.
[12] K. E. Yakovleva, S. A. Kurzeev, E. V. Stepanova, T. V. Fedorova, B. A. Kuznetsov, O. V. Koroleva, *Appl Biochem. Microbiol*. **2007**, *43*, 661.
[13] W. R. Sousa, C. da Rocha, C. L. Cardoso, D. H. S. Silva, M.V.B. Zanoni, *J. Food Compos. Anal.* **2004**, *17*, 619.
[14] V. Roginsky, T. Barsukova, C. F. Hsu, P. A. Kilmartin, *J. Agric. Food Chem.* **2003**, *51*, 5798.
[15] M. Šeruga, I. Novak, L. Jakobek, *Food Chem*. **2011**, *124*, 1208.
[16] A. J. Blasco, M. C. Gonzalez, A. Escarpa, *Anal. Chim. Acta* **2004**, *511*, 71.
[17] K. Bisetty, M. I. Sabela, S. Khulu, M. Xhakaza, L. Ramsarup, *Int. J. Electrochem. Sci.* **2011**, *6*, 3631.
[18] M. Cortina-Puig, H. Gallart-Ayala, S. Lacorte, *Curr. Anal. Chem*. **2012**, *8*, 436.
[19] A. R. Malagutti, V. G. Zuin, E. T. G. Cavalheiro, L. H. Mazo, *Electroanalysis* **2006**, *18*, 1028.
[20] E. de Rijke, P. Out, W. M. A. Niessen, F. Ariese, C. Gooijer, U. A. T. Brinkman, *J. Chromatogr. A* **2006**, *1112*, 31.
[21] M. Nardini, A. Ghiselli, *Food Chem*. **2004**, *84*, 137.
[22] K. Tyszczuk, A. Skalska-Kamniska, A. Wozniak, *Food Chem.* **2011**, *125*, 1498.
[23] A. Waterhouse, **2016**. *Folin-Ciocalteau micro-method for total phenol in wine.* (http://waterhouse.ucdavis.edu/faqs/folin-ciocalteau-micro-method-for-total-phenol-in-wine) (accessed April 16 2016).
[24] A. M. Oliveira Brett, M.-E. Ghica, *Electroanalysis* **2003**, *15*, 1745.
[25] M. Medvidović-Kosanović, M. Šeruga, L. Jakobek, I. Novak, *Croat. Chem. Acta* **2010**, *83*, 197.