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Electrochemical Evaluation of the Synergistic Effect of the Antioxidant Activity of Capsaicin and Other Bioactive Compounds in *Capsicum* sp. Extracts

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The redox features of capsaicin and *Capsicum* sp. fruit extracts (hot peppers extracts) have been studied at glassy carbon electrode by means of cyclic (CV) and square-wave voltammetry (SWV). We also studied voltametrically the interactions between capsaicin and some vitamins present in pepper extracts. Redox features of vitamin E, ascorbic acid, and quercetin have been taken for consideration in voltammetric experiments of capsaicin with CV and SWV. From the features of the voltammograms we could observe indications of interactions between capsaicin and co-extracted vitamins and quercetin. We proposed a simple voltammetric methodology for estimation of the antioxidative potential of these compounds. By using the features of the voltammetric responses of equimolar mixture containing all four compounds (capsaicin, vitamin E, quercetin and ascorbic acid) as a referent system, we could estimate the antioxidative potential of the hot peppers extracts.

Keywords: peppers, capsaicin, antioxidants, voltammetry, reactive oxygen species.

1. INTRODUCTION

Peppers (*Capsicum annuum* L.) are common vegetables that are widely used in nutrition, as they represent a very good source of antioxidants and nutrients [1, 2]. There are many epidemiological evidences confirming a positive correlation of the higher intake of foods with antioxidant abilities and

lower incidence of various human diseases. Many studies have shown that natural products, such as some spices and medical plants containing various antioxidants, posses a high potential for prevention and control of diseases [3, 4]. Capsaicin (8-methyl-N-vanillyl-6-nonenamide) is the main compound in the group of alkaloids known as capsaicinoids, which include dihydrocapsaicin and nordihydrocapsaicin, and it is responsible for over 90% of *Capsicum* sp. fruit pungency. Its molecule is composed of a vanylil moiety and alkenyl chain connected through an amide group [5]. Besides being rich with many vitamins (A, C, E), peppers also contain phenolic compounds as flavonoids (quercetin, myricetin, luteolin, apigenin and kaempferol) and carotenoids [5, 6]. Capsaicinoids are responsible for many physiological and pharmacological actions in human organism. They show anticancer effects, act against high cholesterol levels and obesity, and they are commonly used to treat arthritis pain [7, 8, 9]. Among these, flavonoids are ubiquitous phytochemicals found in plants with a wide group of exploitable activities, including antimicrobial activity, antibiotic synergism and bacterial virulence removal [10].

The antioxidative potential of a given chemical system is defined as ability of particular compound (or a mixture of compounds) to inhibit oxidative degradation of various molecules. The assessment of antioxidative ability of a compound is based on the study of a direct reaction between studied compounds and free radicals (quenching or scavenging) or some transition metals [12-14]. Spectroscopic methods in which colored reagents are used, such as ferric reducing antioxidant power (FRAP), 1,1-diphenyl-2-picryl-hydrazyl (DPPH) and 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assays, have been mainly explored for analyses of the antioxidative properties of capsaicin. Electrochemical methods provide a rapid, simple and sensitive alternative approach to study not only the antioxidative properties, but also the kinetics of the redox reaction of capsaicin and the mechanism of its oxidation. Considering that antioxidant compounds act as reduction agents, they have a tendency to be oxidized on working electrodes. Hence, the correlation between electrochemical behavior of a compound with the antioxidant activity is plausible, as the "low oxidation potential" corresponds to the "high antioxidant power" [15].

The choice of a given electrochemical technique depends on the assumed concentration of the analytes in the studied samples. For capsaicin, cyclic voltammetry (CV) seems to be suitable technique for both electrochemical detection and studying of its redox features [16]. CV is a potentiodynamic techniques which is quite suitable for various mechanistic studies [17]. The current produced in the course of the voltammetric experiment is commonly proportional to the concentration of the redox compounds studied [18, 19]. Combined with a variety of carbon electrodes, CV has been applied to estimate the antioxidative properties of various biological samples [20, 21] in a fast and simple experimental methodology. According to Calvillo et al. [22], capsaicin undergoes two oxidation processes at carbon electrodes; the first process appears at potential of about +516 mV and the second one at +560 mV (vs. Ag./AgCl), measured by pulsed voltammetric techniques. These potentials qualify capsaicin as a potential antioxidant agent, which might be linked to its chemoprotecting properties [22].

The aim of the present work is to study the electrochemical features of capsaicin by means of cyclic and square-wave voltammetry in extracts obtained from several pepper species planted in the region of Republic of Macedonia. As known from the literature [23], beside capsaicin, the extracts of

these plants represent a complex mixture of many other bioactive compounds (Vitamin E, vitamin C, quercetin). The basic electrochemical features of some of the co-extracted compounds of capsaicin are also shortly elaborated. The current study also aims to address the possible interactions and the synergistic antioxidant effects of the co-extracted compounds with capsaicin. To the best of our knowledge, synergetic antioxidative ability of capsaicin with other phytochemicals present in the extracts of hot peppers has not been reported so far.

2. EXPERIMENTAL

2.1 Reagents

Stock solutions of capsaicin, vitamin E, quercetin and ascorbic acid were freshly prepared by using standard substances obtained from Sigma - Aldrich and 96% ethanol (reagent grade). Appropriate dilutions have been made by using buffer solutions (pH from 3.5 to 10.5; c = 0.1 mol/L). Specifically, acetate buffer (pH = 3.6 and 5.5), phosphate buffer (pH form 6.6 to 7.8), and carbonate buffers (pH = 9.4 and 10.6) were used. All aqueous solutions were prepared with de-ionized water obtained by TKA Ultrapure Water System (Germany). All solutions were stored at 4 ˚C. In all solutions used for electrochemical measurements, KCl was added as an additional electrolyte at concentration of 0.010 mol/L.

2.2 Plant materials

Fruits obtained from eight different genotypes of peppers from the genus *Capsicum annuum* L. Solanaceae, (seven hot peppers and one mild) were cultivated and collected from the field in the phase of botanical maturity. Plant seeds were stored in the gen bank of Goce Delcev University, Faculty of Agriculture, Macedonia. Plant names have been checked on the web: www.theplantlist.org on February 10, 2015. Eight genotypes of peppers with local names: *dzinki, feferona, bombona, aiseff f1, vezena luta, piran, hybrid type 13515* and *kurtovska kapija* (as mild control) were dried at room temperature for about two weeks to a constant mass. Afterwards, they were grinded and the powdered material was used for Soxlet extraction.

2.3 Apparatus

Extraction of capsaicin and other compounds from different genotypes of peppers mentioned above has been performed with help of Soxlet apparatus, on a water bath. Quantitative determination of capsaicin was done by using HPLC/DAD (High Pressure Liquid Chromatography/diode array detector) Agilent 1200, (Agilent Technologies Palo Alto, CA, USA), with a binary pump and a Zorbax SB-C18 column (4.6 × 250 mm × 5μm), connected to an Agilent ChemStation software. For the Voltammetric experiments we used a Palm Sense Potentiostat connected to a PS Trace system version 3.0. All voltammetric measurements were carried out using a three electrode electrochemical cell.

Glassy carbon electrode (GCE) (diameter = 1.5 mm) was used as a working electrode, Ag/AgCl (KCl 3 mol/L) was the reference electrode, while a Pt wire served as a counter electrode.

2.4 Extraction and determination of capsaicin

Soxlet extractions have been performed by using a Soxlet apparatus on a water bath, with ethanol 96% (*v/v*) as an extraction solvent, at temperature of 68-70 ˚C, for a period of 5 hours. The determination of capsaicin was made by HPLC. Chromatographic conditions were based on the validated method reported previously [24]. The analyses of capsaicinoids were made at 25 ˚C. Elution step was performed with an isocratic mixture of water: acetonitrile (50 : 50), with a flow rate of 1.5 mL/min. Detection was set at 220 nm. Injection volume was 20 μL. All peaks related to capsaicinoids were eluted in about 15 min. Quantitative analysis was performed following the external standard method. Calibration curves were constructed by injecting a triplicate of nine increasing concentrations of a standard.

2.5 Voltammetric methods

Electrochemical characterization of capsaicin was performed by means of cyclic voltammetry (CV) and square-wave voltammetry (SWV) at a glassy carbon working electrode. Experiments in cyclic voltammetry were conducted over a potential range from -0.200 to 1.000 V, with a scan rate of v = 10 mV/s (if not otherwise stated). Experimental conditions for SWV were: potential step dE = 0.001 V, pulse height (SW amplitude) Esw = 0.050 V and frequency of f = 10 Hz. Prior to each electrochemical experiment the working electrode was polished by using $AlCl_3$ on a polishing cloth, followed by rinsing of the electrode with water and acetone and drying in air.

3. RESULTS AND DISCUSSION

3.1 Chromatographic results for quantification of capsaicin

Quantitative determination of capsaicin was made by using two standards-capsaicin and dihydrocapsaicin, for which corresponding calibration curves have been constructed. Typical chromatograms obtained with standard solutions, together with the chromatogram of *feferona* hot peppers extract, are presented in Fig.1. Capsaicin at concentration of 10.640 µg/mL features a peak with a retention time (t_r) of 7.65 min and a peak area of 88 mAU, while dihydrocapsaicin (7.812 µg/mL) exhibits a peak with t_r = 10.82 min, and a peak area of 60 mAU. After measuring the area under peaks for each extract separately, the content of capsaicin and dihydrocapsaicin has been determined, and the corresponding data are presented in Table1. The peak ascribed to the *feferona* extract is associated with the peak area of 361 and 184 mAU, for capsaicin and dihydrocapsaicin, respectively.

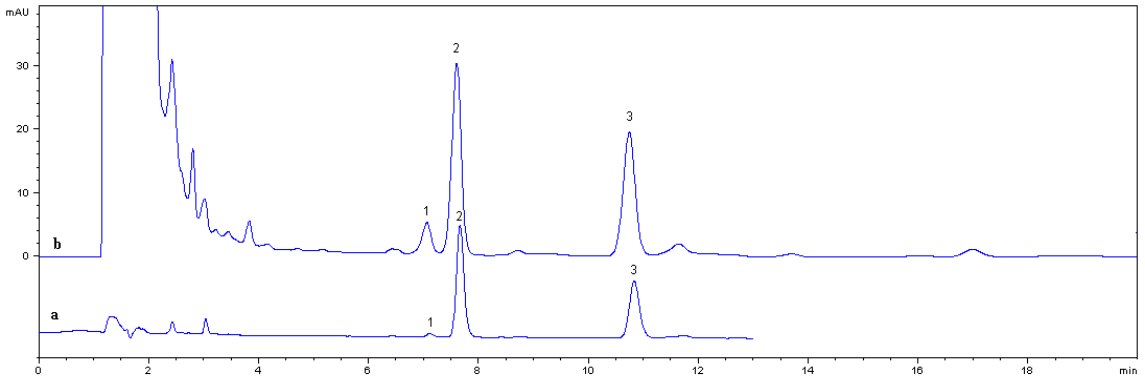

Figure 1. (**a**) Chromatogram obtained from standard solutions of capsaicin and dihydrocapsaicin (10 µg/mL both), and (**b)** Ethanolic extract of feferona. Assignation of the peaks is as follows: (1) nordihydrocapsaicin, t_r = 7.1 min; (2) capsaicin t_r = 7.65 min and (3) dihydrocapsaicin, t_r = 10.82 min.

Table 1. The content of capsaicin, dihydrocapsaicin and nordihydrocapsaicin in the ethanolic extracts, calculated from AUC (area under curve) from HPLC analyses, and the net peak current of the extracts obtained with SWV under experimental conditions given in section 2.5 (potential step dE = 1 mV, pulse height (SW amplitude) E_{sw} = 0.05 V and frequency of f = 10 Hz).

	Local name of *Capsicum* sp.	capsaicin µg/mL	dihydrocapsaicin µg/mL	nordihydrocapsaicin µg/mL	Net SW peak current[1] of extracts /µA	TAC values form FRAP[2] assay/ Fe^{2+} µmol/L
1.	*vezena luta*	3.791	4.070	0.992	0.473	508.052
2.	*feferona*	22.683	19.541	3.603	1.802	1159.893
3.	*bombona*	19.503	17.844	3.533	0.654	728.527
4.	*aiseff f1*	2.342	0.730	0.461	0.252	340.115
5.	*dzinki*	16.391	23.672	12.072	1.581	938.113
6.	*hybrid 13515*	2.970	1.664	0.264	0.250	325.446
7.	*piran*	7.320	6.004	0.931	0.173	323.849
8.	*kurtovska kapija (mild control)*	ND[3]	ND	ND	0.962	540.950

[1] In the case of more peaks, the net current obtained in SWV for the highest peak of the studied extract.
[2] Total antioxidative capacity obtained as result from *in vitro* FRAP assay.
[3] ND, not detectable.

3.2 Basic voltametric features of capsaicin

The initial voltammetric experiments have been performed in order to get insight into the basic redox features of capsaicin in different media. Figure 2A shows typical cyclic voltamogram of capsaicin recorded at GCE in KCl aqueous solution. Cyclic voltamograms of capsaicin in 96% and 50% (*v/v*) ethanol-water mixture are shown in Figures 2B and 2C, respectively. These experiments have been performed in order to get an insight into the solvent effect on the voltammetric features of

capsaicin and to assess the chemical stability of the generated species. In all cases, the redox transformation of capsaicin features a quasireversible electrode process with a wide potential separation between the anodic and cathodic peaks. The oxidation process of capsaicin was observed at about $E_{p,a}$ = 0.600 V in KCl aqueous solution, while it is shifted to 0.700 V in ethanol-water mixture. Best definition of all voltammetric peaks was obtained in an aqueous solution compared to ethanol solutions, valid for both anodic and cathodic CV peaks. The anodic peak potential of capsaicin in KCl aqueous solution is $E_{p,a}$ = 0.641 V, attributed with a peak current $I_{p,a}$ = 0.388 µA. Under such conditions, capsaicin features a small cathodic peak with $I_{p,c}$ = 0.067 µA . The anodic peak of capsaicin in 50% ethanol solution has the peak potential of $E_{p,a}$ = 0.661 V and the peak current $I_{p,a}$ = 0.250 µA, while the corresponding values in 96% ethanol solution are $E_{p,a}$ = 0.736 V and $I_{p,a}$ = 0.271 µA.

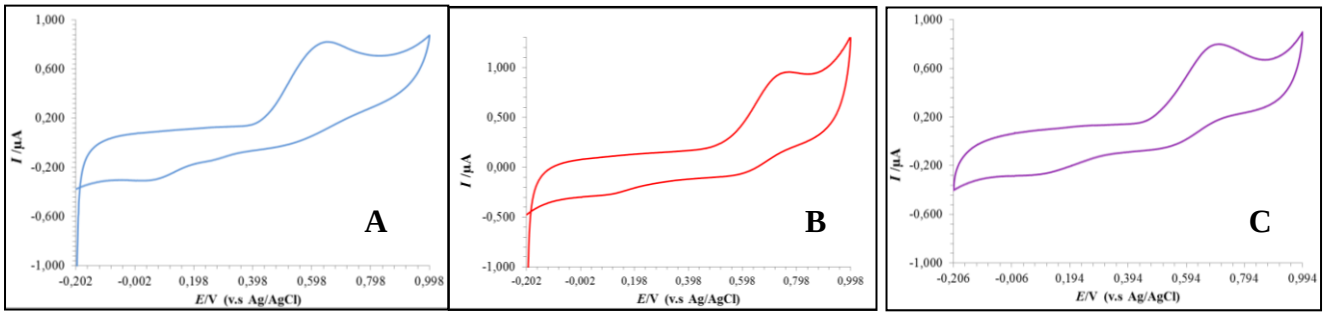

Figure 2. Cyclic voltamograms of 100 µmol/L capsaicin at GCE (scan rate v = 10 mV/s), recorded in **(A)** KCl aqueous solution; **(B)** in ethanol 96% (*v/v*) and **(C)** 50% ethanol-water. All solutions contain 0.010 mol/L KCl as a supporting electrolyte.

In order to reveal the effect of pH to the voltammetric features of capsaicin, the voltammetric behavior of capsaicin in aqueous buffers with different pH values has been examined. Shown in Fig. 3A is the evolution of cyclic voltammograms of capsaicin as a function of pH, together with the dependence of the anodic peak potential as a function of pH (Fig. 3B).

In an acidic acetate buffer at pH = 3.6, capsaicin features an oxidation peak at potential of about 0.613 V, whereas in an alkaline solution (pH = 10.6), the oxidation peak is shifted to the potential of 0.259 V. The oxidation peak in a neutral medium (pH = 7.1) appears at 0.417 V. The shifting of the oxidation potential to lower potential values by increasing pH shows that protons are involved in the electrode reaction, i.e., the deprotonation of capsaicin precedes the oxidation reaction.

To get further insight in the electrochemistry of capsaicin, a scan rate analysis, together with concentration dependence, were carried out in a phosphate buffer at pH = 7.1. The relevant data obtained from these analyses are summarized in Fig. 4. Cyclic voltammograms recorded for 50 µmol/L capsaicin over the scan rate interval from 10 to 100 mV/s show a linear dependency of the anodic peak current on the square root of the scan rate applied. This implies that the electrode process is mainly controlled by diffusion. The results obtained for capsaicin in the range of different concentrations (10, 25, 50, 75, 100, 150 µmol/L) show a linear dependence between the anodic peak current and the

capsaicin concentration, with a correlation coefficient R = 0.986 and a positive slope of 0.005 (µA mol^{-1} L).

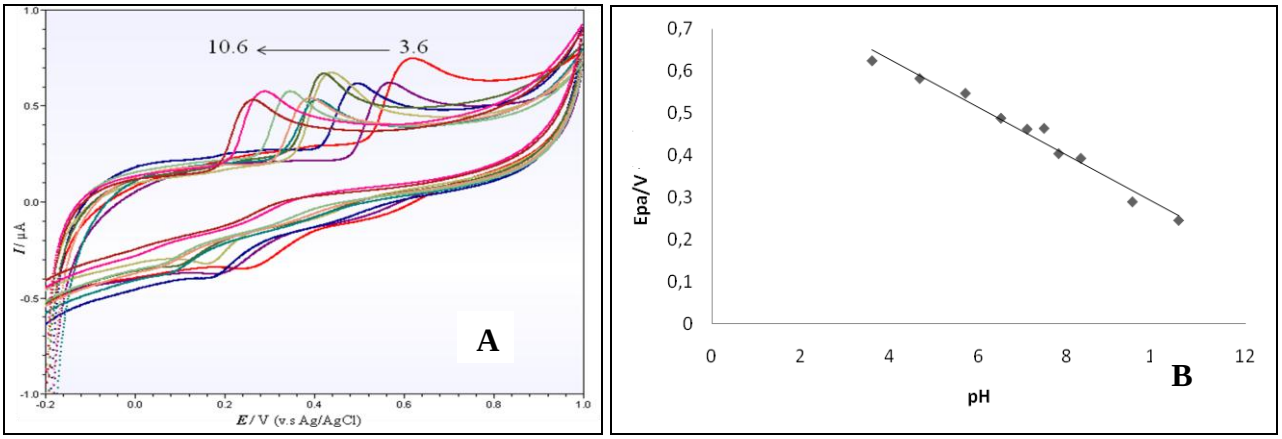

Figure 3. (A) Cyclic voltammograms of capsaicin (50 µmol/L) recorder at different pH (3.6; 5.5; 6.6; 7.1; 7.4; 7.8; 9.4 and 10.6). Scan rate was v = 10 mV/s **(B)** Oxidation peak potential as function of pH of the medium.

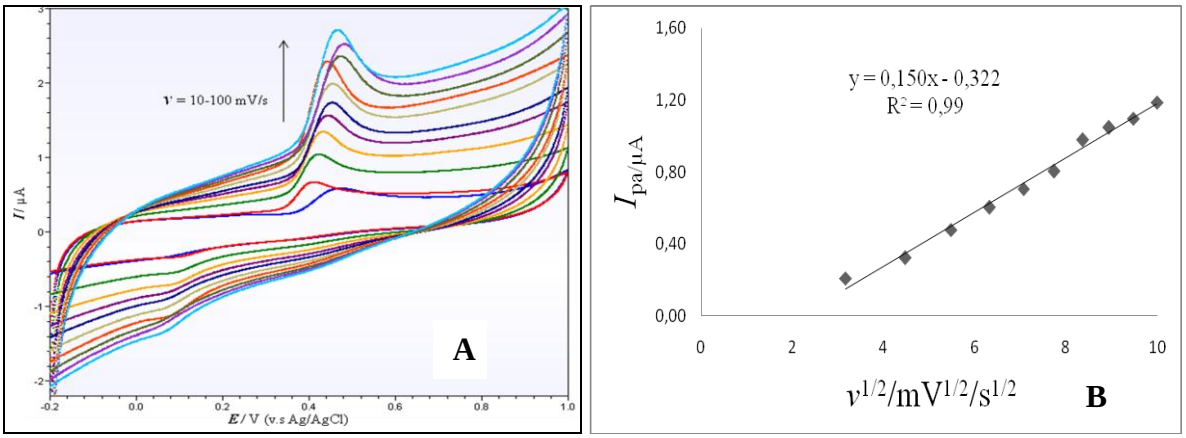

Figure 4. (**A)** Cyclic voltammograms of 50 µmol/L capsaicin recorded in a phosphate buffer at pH = 7.1, containing 0.01 mol/L KCl as a supporting electrolyte over the scan rate interval from 10 to 100 mV/s. (**B)** Dependence of the anodic peak current on the square root of the scan rate.

In order to examine the mechanism of oxidation of capsaicin in more details, repetitive cyclic voltammetry of 50 µmol/L capsaicin at pH of 6.5 has been performed. In the first scan, only one anodic (I_{a1}; $E_{p,a1}$ = 0.44 V) and one cathodic (I_c, $E_{p,c}$ = 0.16 V) peaks were observed (Fig. 5). In the second potential scan, a new oxidation peak at lower potentials emerges (I_{a2}, $E_{p,a2}$ = 0.23 V). In the course of the second scan, the height of reduction peak I_c remained almost the same. Obviously, the second oxidation peak I_{a2} corresponds to oxidation of the reduction product formed as a result of the occurrence of the process I_c. Thus, the peak pair I_{a2}/I_c corresponds to a single redox couple formed in a

follow-up chemical reaction coupled with the initial oxidation of capsaicin at I_{a1}. This assumption is supported by the voltammograms shown in the inset of Fig. 5, which corresponds to the potential cycling over the interval -0.2 V to 0.4 V. Obviously, without initial oxidation of capsaicin at about 0.44 V, the peak pair I_{a1}/I_c does not appear, implying that the precondition for the occurrence of this second process is the occurrence of the initial oxidation of capsaicin.

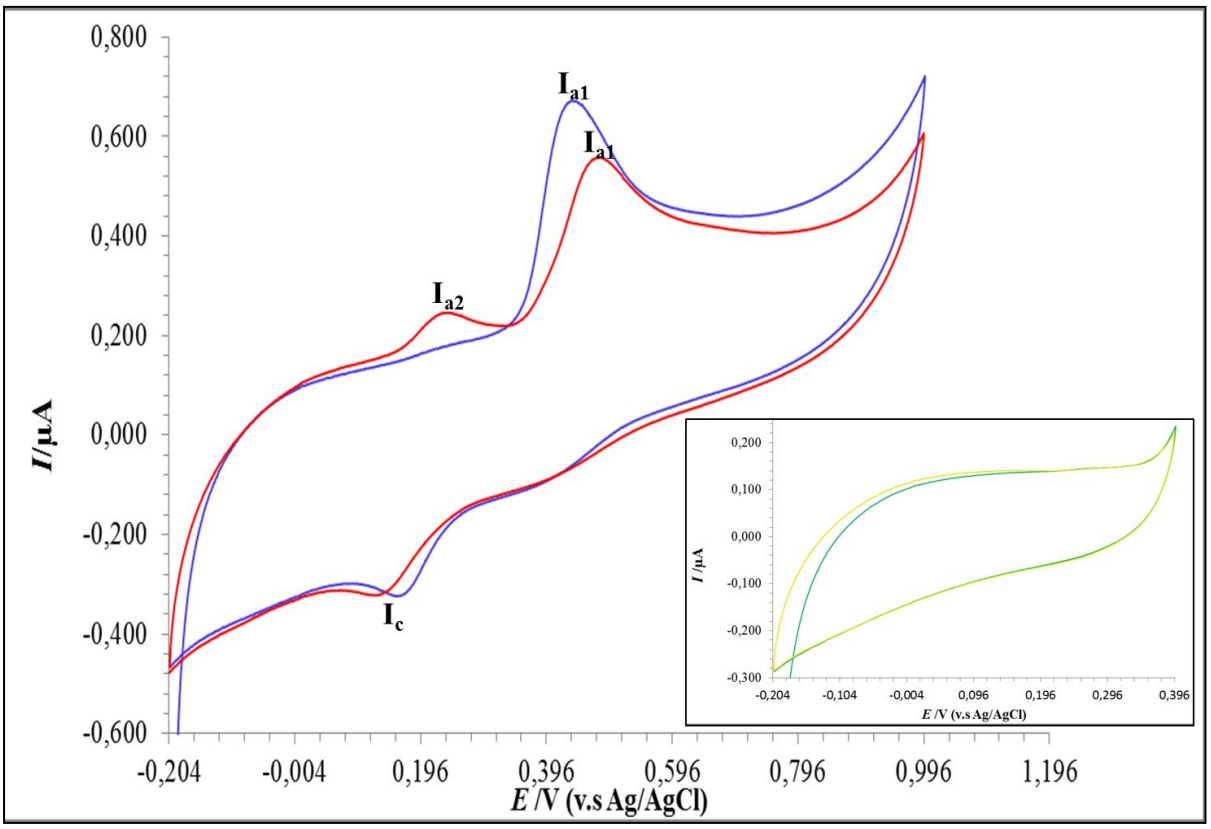

Figure 5. Repetitive cyclic voltammograms of capsaicin (50 µmol/L) in a phosphate buffer at pH = 6.5 (blue line – first scan; red line second – scan) containing 0.010 mol/L KCl as a supporting electrolyte. Scan rate was 10 mV/s. The inset represents cyclic voltammograms of capsaicin recorded overt the potential range from -0.2 to 0.4 V.

In a medium with pH = 7.4, the second oxidation peak is almost invisible. At pH ≥ 7.8, the cyclic voltammograms of capsaicin did not show any oxidation peak in the second scan. This indicates that the follow-up chemical reaction of capsaicin depends on pH of the medium. Yardim et al., [25] proposed a similar oxidation mechanism for capsaicin and dihydrocapsaicin in acidic and alkaline media. In that work [25] it has been suggested that the capsaicin undergoes first two-electron irreversible oxidation leading to formation of a phenoxonium cation radical. The latter is unstable species, being quickly converted to *o*-benzoquinone by a nucleophilic attack of H_2O. Besides *o*-benzoquinone, the hydrolysis reaction results in formation of methanol as a side product. The formed benzoquinone of capsaicin is further reduced in the course of the cathodic potentail scan to *o*-hydroxyphenol (catechol) form of the capsaicin giving a raise to the cathodic peak I_c (Fig. 5). In the second potential scan, the catechol form is oxidized to *o*-benzoquinone form resulting in appearance of

the anodic peak I_{a2}. Hence, the peak pair I_{a2}/I_c corresponds most likely to the *o*-quinone/catechol redox couple of capsaicin, which can be formed only after initial oxidation of capsaicin coupled with hydrolitic reaction. It was suggested that the hydrolitic chemical reaction occurs when capsaicin is dissolved in acidic or in solution with pH around 9, as the pK_a of *o*-methoxyphenol is 9.93 [26]. Unlike the latter report, our results indicate that capsaicin undergoes a second oxidation only in acidic or neutral media. It should be also noted that catechol form of capsaicin could react with methanol in the vicinity of the electrode surface, yielding a methoxyphenol form of the native capsaicin.

3.3 Basic electrochemical characterization of some compounds co-extracted with capsaicin

After the initial study on the electrochemical behavior of capsaicin and its oxidation mechanism, we also studied the voltammetric features of a few other molecules that are commonly co-extracted from hot pepper fruits, such as vitamin E, ascorbic acid and quercetin. A brief electrochemical characterization of these compounds has been performed by means of cyclic voltammetry, while their potential synergetic antioxidative effect has been analyzed with square-wave voltammetry.

Cyclic voltammogram of vitamin E (100 µmol/L, in a phosphate buffer at pH = 7.1, see Fig. 6A) consists of a single irreversible oxidation peak positioned at $E_{p,a}$ = 0.206 V with the peak current $I_{p,a}$ = 1.032 µA. The anodic peak of vitamin E is significantly higher than the anodic peaks of other compounds under the same experimental conditions. These data are in agreement with the data reported by Giacomelli et al. [27], who have reported that vitamin E exhibits three oxidation peaks in alcoholic solutions at glassy carbon electrode, in contrast to a single oxidation process in other solvents, including aqueous medium.

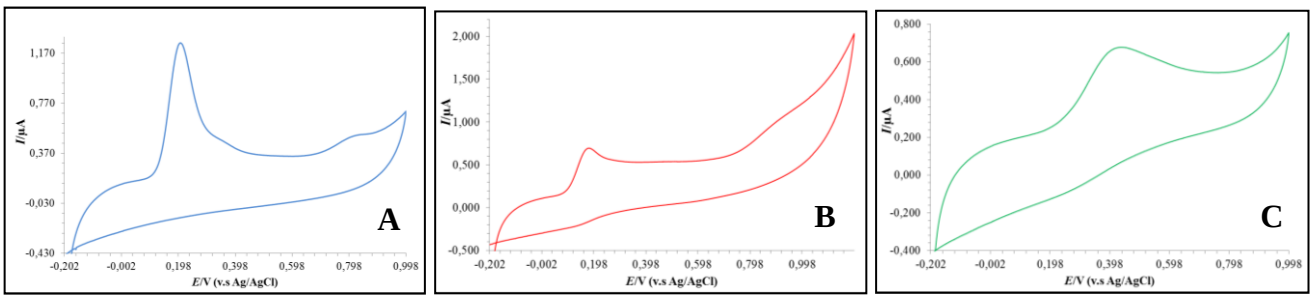

Figure 6. Cyclic voltammograms of **(A)** vitamin E, **(B)** quercetin (both at a concentration of 100 µmol/L), and **(C)** ascorbic acid (200 µmol/L). Voltammograms are recorded under the same experimental conditions as for Fig. 5.

It was further demonstrated that quercetin in a phosphate buffer (pH = 7.1) exhibited one oxidation peak shown on Fig. 6B, similar to the findings of Timbolla et al. [28]. The potential at which the main oxidation of quercetin occurs is $E_{p,a}$ = 0.176 V, while the peak current is $I_{p,a}$ = 0.466 µA. A typical voltammetric response of ascorbic acid (200 µmol/L) is depicted in Fig. 6C. As it is already

known, the oxidation process is attributed with an irreversible peak at $E_{p,a}$ = 0.426 V with the peak current of $I_{p,a}$ = 0.321 μA, being in agreement with the literature data [29-31].

When these compounds are present in a mixture with capsaicin (as in *Capsicum* fruit extracts), a redox communication between them is possible. Therefore, a synergetic antioxidative effect between these compounds is also plausible. In order to get an insight into the assumed synergetic effect, electrochemical properties of mixtures of these compounds have been studied by applying square-wave voltammetry, as one of the most advanced and sensitive voltammetric techniques. All experiments have been conducted at physiological relevant pH close to 7.

Typical net SW voltammograms of vitamin E, quercetin, and ascorbic acid (each at concentration of 10 μmol/L) recorded at a glassy carbon electrode in a buffer solution at pH = 7.0 are depicted in Fig. 7. The net SW peaks of vitamin E and quercetin are closely positioned at potentials of $E_{p,net}$ = 0.146 V and $E_{p,net}$ = 0.108 V, respectively, while the peak of ascorbic acid is located at $E_{p,net}$ = 0.409 V. Vitamin E gives raise to the highest net peak current ($I_{p,net}$ = 1.894 μA) compared to the maximal peak currents of quercetin and ascorbic acid (the 0.580 μA for quercetiny, and 0.193 μA for ascorbic acid).

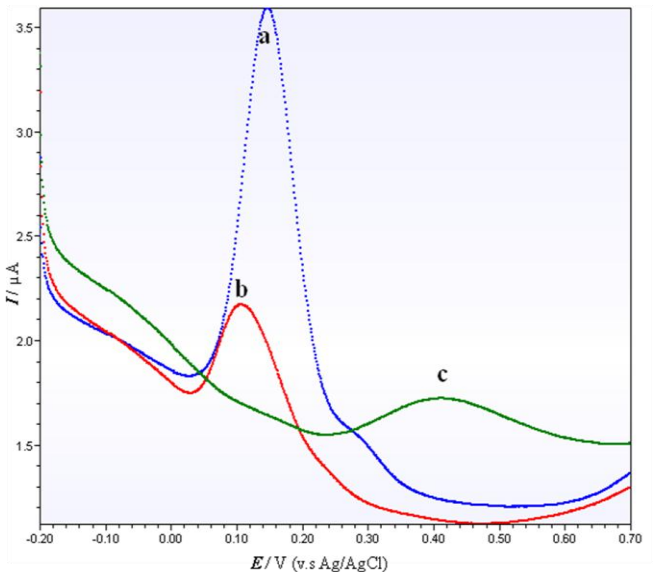

Figure 7. Square-wave voltammogram of: (**a**) vitamin E; (**b**) quercetin, and (**c**) ascorbic acid (10 μmol/L) recorded at a glassy carbon electrode in a buffer solution at pH = 7.1. Instrumental parameters were: step potential dE = 0.001 V, square-wave amplitude E_{sw} = 0.05 V and frequency of 10 Hz.

Interestingly, when capsaicin and vitamin E were present at equimolar concentrations in the electrolyte solution (10 μmol/L), that net peak current of vitamin E decreased slightly from 1.894 to 1.182 μA and a small new peak at $E_{p,net}$ = 0.327 V ($I_{p,net}$ = 0.106 μA), corresponding to capsaicin, emerges (Fig. 8). The decreasing of the vitamin E response is probably a result of capsaicin adsorption and partial blocking of the electrode surface. On the other hand, when capsaicin is present in a significant excess (50 μmol/L), the net peak current of vitamin E increased significantly from 1.894 to

2.270 µA (Fig. 8, curve c). These results imply a synergetic effect of capsaicin and vitamin E, most probably because of the redox interaction between oxidized form of vitamin E and capsaicin.

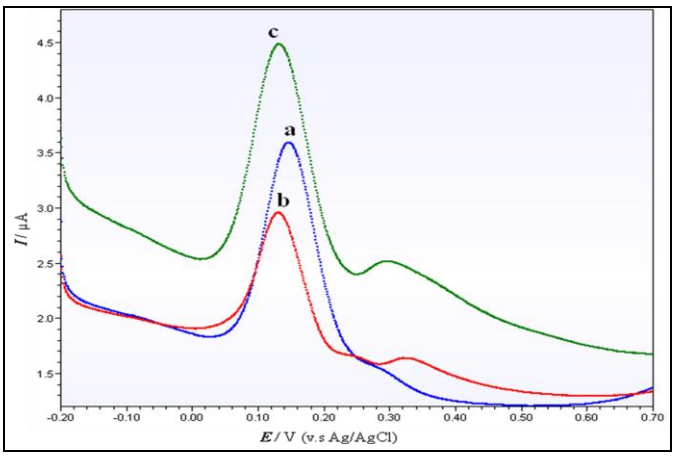

Figure 8. Square-wave voltammograms of (**a**) vitamin E at concentration of 10 µmol/L, (**b**) vitamin E and capsaicin (equimolar, 10 µmol/L), (**c**) 50 µmol/L capsaicin and 10 µmol/L vitamin E. Experimental conditions are the same as for Fig**. 7**.

The effect of capsaicin to the SW voltammetric features of quercetin has been studied in an analogous way as for vitamin E. Shortly, when capsaicin and quercetin are present at equimolar concentration in the electrolyte solution (10 µmol/L) the net SW peak current of quercetin increased from 0.584 to 0.648 µA, in addition to the appearance of a small peak of capsaicin at $E_{p,net}$ = 0.367 V ($I_{p,net}$ = 0.095 µA) (see Fig. 9). In addition, the net peak of quercetin becomes broader due to a shoulder positioned at more positive potentials relative to the main peak (Fig. 9).

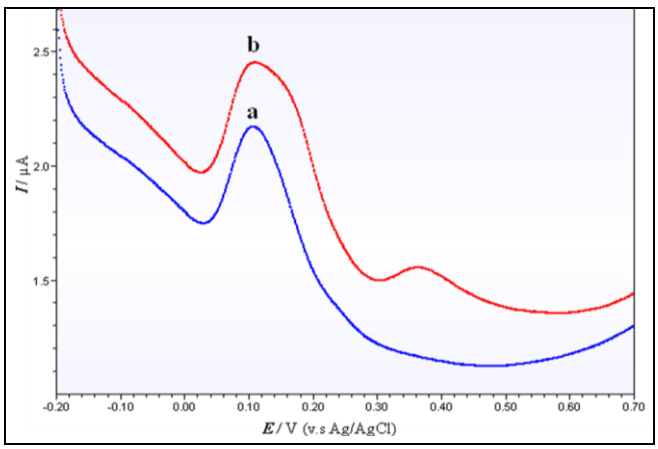

Figure 9. Square-wave voltammograms of (**a**) quercetin (10 µmol/L) and (**b**) a mixture of quercetin and capsaicin at equal concentration of 10 µmol/L. Experimental conditions are the same as for Fig**. 7**.

Typical square-wave voltammograms of ascorbic acid, capsaicin, and their mixture are given in Fig.10. As it can be seen, the response of ascorbic acid increased from 0.193 to 0.345 µA, whereas the peak potential shifted from 0.408 V to 0.367 V in the presence of capsaicin implying a redox communication between them and potential synergetic antioxidative effect.

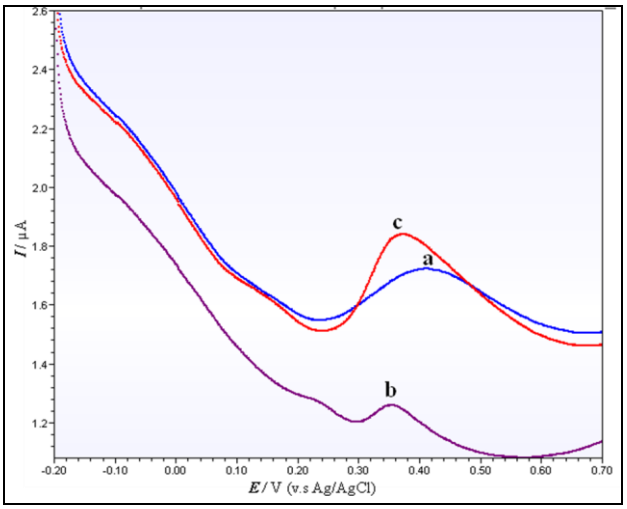

Figure 10. Square-wave voltammograms of (**a**) ascorbic acid (10 µmol/L), (**b**) capsaicin (10 µmol/L), and (**c**) a mixture of ascorbic acid and capsaicin (equimolar, 10 µmol/L). Experimental conditions are the same as for Fig**. 7**

Taking into account that all these compounds are present in the extracts of *Capsicum* fruit, it is particularly intriguing to study the voltammetric response in a mixture containing all four compounds at equimolar level (Fig. 11). The SW voltammetric response of 10 µmol/L equimolar mixture consists of a single SW peak at the potential about 0.128 V, which is between the typical peak of Vitamin E and quercetin. Thus, it could be assumed that the redox communication with the electrode proceeds via the electrode reaction of vitamin E. The net peak current obtained for the mixture of four compounds ($I_{p,net}$ = 3.313 µA) is exceeding the sum of the individual peak currents recorded separately, which implies that these compounds being present simultaneously in the electrolyte solution can exhibit a synergistic antioxidative effect.

In order to use the voltammetric response of the four compounds mixture as a reference for estimating the antioxidant potential of the pepper fruit extracts, a calibration line was constructed over the concentration interval from 1 to 10 µmol/L. The net SW peak current of the mixture linearly increases with the concentration of all species present at equimolar level, the linear regression line being associated with the coefficient of R = 0.984 and the slope of 0.691 (□A mol^{-1} L).

Finally, square-wave voltammograms were recorded for the ethanolic extracts obtained from 8 different species of genus *Capsicum*. The experimental procedure involved evaporation of a 10 mL ethanolic extracts to the volume of 1 mL, which was added to the buffer solution in the electrochemical cell. Voltammograms of these extracts are given in Fig.12. The results showed that

extracts (1, 2, 3, 5, and 6) which contained higher concentrations of capsaicin (Table 1) are associated with a voltammetric peak at about 0.400 V, typical for the oxidation of capsaicin.

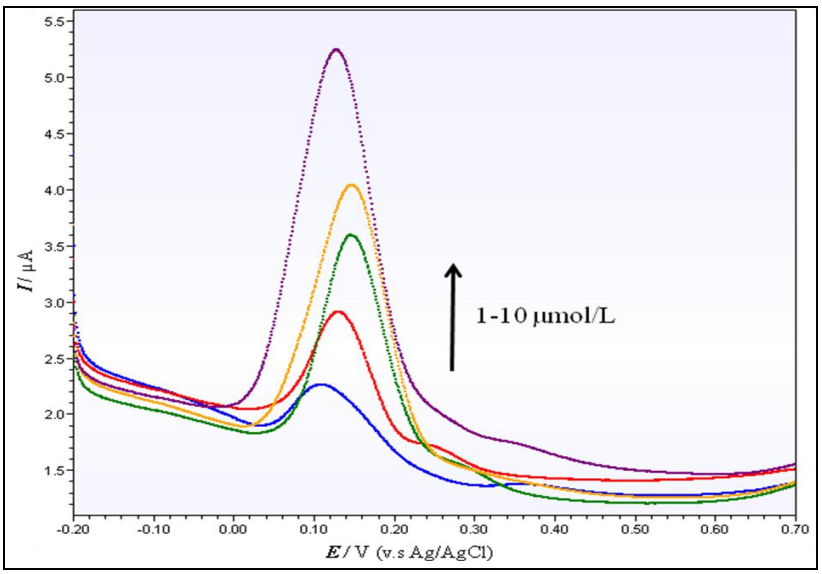

Figure 11. Square-wave voltammograms recorded for equimolar mixtures containing vitamin E, quercetin, ascorbic acid and capsaicin at increasing concentration from 1 to 10 µmol/L, in a phosphate buffer at pH = 7.1 (dE = 0.001V, E_{sw} = 0.050 V and frequency of 10 Hz).

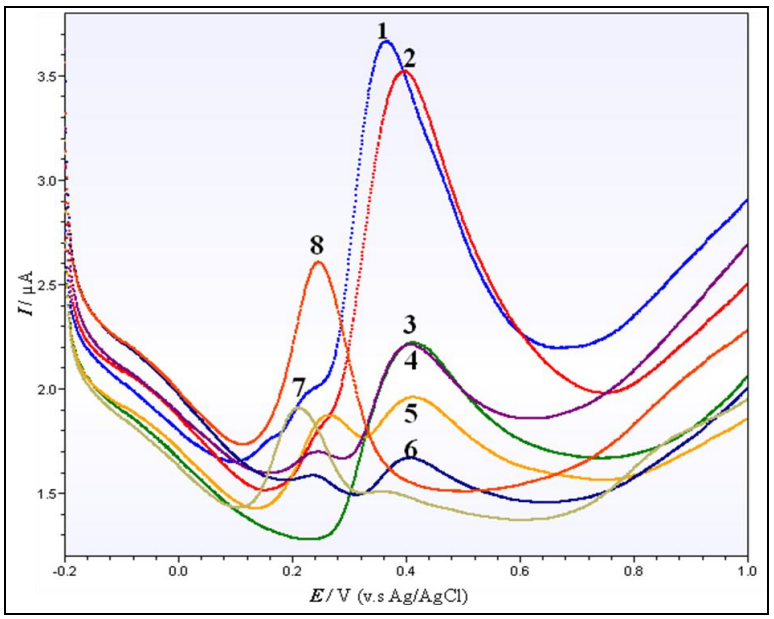

Figure 12. Square-wave voltammograms obtained from ethanolic *Capsicum* extracts: (1) dzinki; (2) feferona; (3) bombona; (4) aiseff f1; (5) vezena luta; (6) piran; (7) hybrid 13515; (8) kurtovska kapija (control). Voltammograms were recorded under the same conditions as shown in Fig. 7.

The extracts 4, 7 and 8, which contained less capsaicin, exhibited their SW peak at potentials close to those characteristic peaks for the oxidation vitamin E and quercetin (i.e., at about 0.200 V)

(Fig. 12). These results suggest that the antioxidative potential of the extracts 1, 2, 3, 5, and 6 is mainly assigned to the capsaicin content (see Tab.1). The highest voltammetric peak was obtained for the extract from peppers with local name *feferona,* being virtually identical with the standard mixture containing all four compounds at concentration of 6 μmol/L. The extracts 4, 7 and 8 contained low concentration of capsaicin; consequently their voltammetric response have been observed at potential about 0.200 V, where the electrode reactions of vitamin E and quercetin dominate.

4. CONCLUSION

In the present work, a comprehensive voltammetric study has been performed in order to predict the antioxidative capacity of compounds present in the *Capsicum* extracts. The cyclic voltammetry experiments have been conducted to investigate some of the electrochemical features of capsaicin. This study gives some hints about the complex synergistic effects between capsaicin and vitamin E, quercetin and ascorbic acid, separately or together present in a complex mixture. Further investigations are needed in order to explain in more details the phenomenon of synergism between the studied compounds. Yet, these results suggest that consuming peppers in the everyday nutrition brings higher antioxidative effect to the body than some other vitamin supplements ingested separately. Knowing the fact that there are many others phytochemicals present in peppers alongside capsaicin [32], we suggest that *Capsicum* extract is highly potent source of antioxidants with a synergistic effect.

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