

Characterization of bioactive compounds contained in vegetables of the Solanaceae family by capillary electrophoresis

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Abstract. Because of their antioxidative capability polyphenols and vitamins are the most important naturally occurring compounds. Several widely consumed vegetables are rich in various phenolic compounds and vitamins. In this study, such vegetables as tomato (*Solanum lycopersicum*), eggplant (*Solanum melongena*), chilli pepper (*Capsicum annuum*), and potato (*Solanum tuberosum*) of the Solanaceae family were investigated. The phenolic compounds and vitamins were separated and their composition was determined by capillary electrophoresis (CE). The total phenolic content was measured according to the Price and Butler method. In addition, the antioxidative capability of phenolic compounds was monitored and evaluated by CE using a coloured free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH).

Key words: antioxidativity, capillary electrophoresis, polyphenols, vitamins.

INTRODUCTION

The increasing interest in naturally occurring antioxidants (polyphenols, vitamins) is attributed to their capability of scavenging free radicals that are formed in various biochemical processes. The reactive oxygen species like superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH^{\bullet}) cause an extensive oxidative damage to biomolecules such as nucleic acids, proteins, and lipids. These highly unstable radicals have been found to be related to oxidative stress-related diseases like cardiovascular diseases, cancer, inflammatory disorders, neurological degeneration (Parkinson's and Alzheimer's diseases), premature ageing, etc. [1–6].

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Polyphenols (cinnamic acid derivatives, flavonols, anthocyanins) and vitamins are present in vegetables, fruits, berries, and herbs, which are the main source of natural antioxidants in our daily diet. The basic structure of polyphenols is composed of one or more phenolic rings that are substituted with several hydroxyl groups and these are highly correlated with their strong antioxidant activity [3, 4, 7–9]. Vitamins are structurally a heterogeneous group of compounds, which are essential in the diet for the maintenance of healthy growth and development. In general, vitamins are divided into two main categories, fat and water-soluble ones [10–12].

Among vegetables, tomato (*Solanum lycopersicum*), eggplant (*Solanum melongena*), chilli pepper (*Capsicum annuum*), and potato (*Solanum tuberosum*), which belong to the Solanaceae family, are important for their richness in healthy components due to which they are also widely consumed. Tomato is rich in phenolic compounds (flavonoids, flavones, cinnamic acid derivatives), phytoalexins, protease inhibitors, glycoalkaloids, and carotenoids, but especially in lycopene and β -carotene. In addition, vitamins C, E, and A have been determined in tomato [5, 7, 9, 13–16]. The main polyphenols found in eggplant are phenolic acids (chlorogenic acid, caffeic acid, *p*-coumaric acid), but this vegetable is poor in provitamin A and vitamin E. However, the presence of vitamins C and B in eggplant has been established [17–20]. It is also rich in anthocyanins like nasunin and delphinidin conjugates [21]. Chilli pepper has been reported to contain flavones (luteolin, quercetin), flavonols (myricetin, quercetin), and capsaicinoids [22–24]. Of phenolic compounds, chlorogenic and caffeic acid, catechin, and also glycoalkaloids have been reported to be the main compounds present in potato [25, 26]. Vitamin C has been also determined in potato [26].

As plant matrices have a complex composition, development of methods of their separation is of crucial importance. Therefore several methods like thin-layer chromatography (TLC), gas chromatography (GC), high-pressure liquid chromatography (HPLC), and mass-spectrometry (MS) have been used to separate polyphenols and vitamins. Nowadays hyphenated techniques like HPLC–MS have been developed, which enable the characterization/determination of the structure of a compound [1, 2, 5, 6, 15, 27–31]. Also microbiological assays have been developed for the determination of vitamins [10]. Due to its relatively short analysis time, ease of operation, minimum sample and reagent consumption, non-use or use of a very low amount of organic solvents capillary electrophoresis (CE) has been found to be a powerful tool allowing the separation of bioactive compounds in biological matrixes. Moreover, CE permits simultaneous analysis of different kinds of analytes in a single run [3, 27, 28, 32–34].

The antioxidative capability of the compounds under study has been evaluated by using different assays like a ferric reducing/antioxidant power assay (FRAP), an oxygen radical adsorption capacity method (ORAC), colourization assays using stable coloured free radicals, such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), a 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS)

radical cation or a reactive oxygen species, due to their intensive absorbance in the visible region [2, 35–38].

The objective of the present study was to determine the composition of polyphenols (phenolic acids, flavonols, flavones) and vitamins of the skin extracts of the plant family of Solanaceae by capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC). The conditions of the separation of water-soluble vitamins such as thiamine (B₁), nicotinamide and nicotinic acid (B₃), D-pantothenic acid (B₅), and pyridoxine (B₆), which are also known as vitamins B complex, as well as vitamin C (L-ascorbic acid) were optimized. Besides, the antioxidative capability of the compounds in question was determined spectrophotometrically, using a free radical like 2,2-diphenyl-1-picrylhydrazyl (DPPH). Additionally, the method for the monitoring of the scavenging capability of a DPPH radical was developed and evaluated by CZE.

MATERIALS AND METHODS

Chemicals

All reagents were of analytical grade and were used as received. Rutin (3,3',4',5,7-pentahydroxyflavone-3-rutinoside), quercetin (3,3',4',5,7-tetrahydroxyflavonol), naringenin (4',5,7-trihydroxyflavanone), genistein (4',5,7-trihydroxyisoflavone), cinnamic acid ((*E*)-3-phenyl-2-propenoic acid), luteolin (3',4',5,7-tetrahydroxyflavone), myricetin (3,3',4',5',7-hexahydroxyflavone), chlorogenic acid (1,3,4,5-tetrahydroxycyclo-hexanecarboxylic acid 3-(3,4-dihydroxycinnamate)), *p*-coumaric acid (4-hydroxycinnamic acid), ferulic acid (3-methoxy-4-hydroxycinnamic acid), caffeic acid (3,4-dihydroxycinnamic acid), L-ascorbic acid, thiamine, pyridoxine, sodiumdodecylsulphate (SDS), ferric chloride, potassium ferricyanide, aluminium chloride, sodium nitrite, DPPH, tannic acid, sodium tetraborate, and sodium hydroxide were obtained from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Catechin (5,7,3',4'-tetrahydroxyflavane), nicotinamide, nicotinic acid, and D-pantothenic acid hemicalcium salt were from Fluka Chemie GmbH (Switzerland). Boric acid was from Riedel-de Hën (Germany). The structures of phenolic compounds are given in Fig. 1. Methanol was purchased from Rathburn Chemicals Ltd (Walkerburn, Scotland). Deionized water (MilliQ, Millipore S. A. Molsheim, France) was used for the preparation of all solutions.

Instrumental

All experiments were performed using an Agilent CE System (Agilent Technologies, Waldbronn, Germany) with a diode array detection. A CE Chemstation (Agilent Technologies) was used for instrument control, data acquisition, and data handling. The separation of polyphenols was performed in a fused silica capillary (Polymicro Technology, Phoenix, AZ, USA) with a total length of

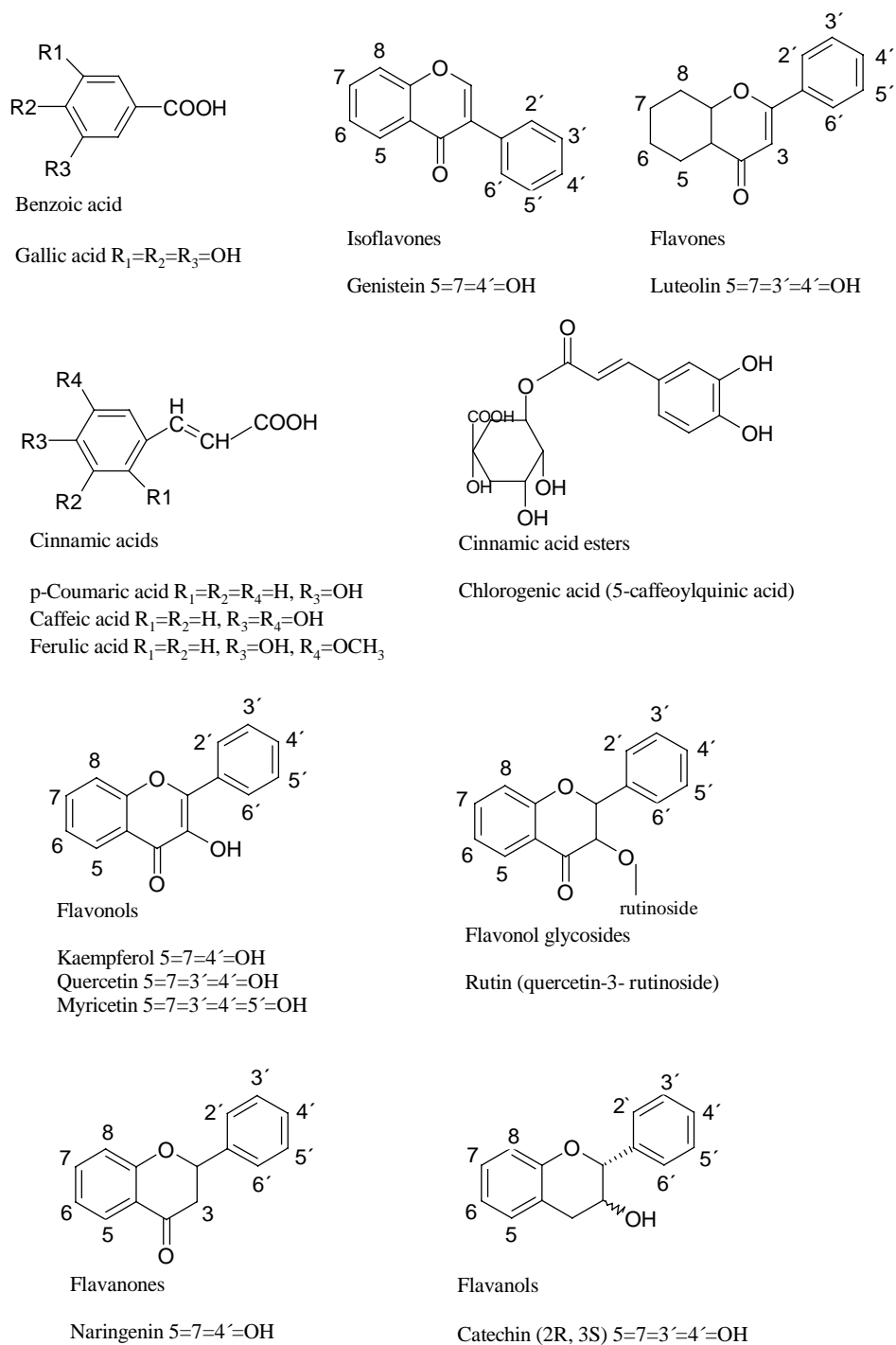


Fig. 1. The structures of polyphenolic compounds found in plants of the Solanaceae family.

75 cm (the effective length 50 cm) and i.d. of 75 μm . A fused silica capillary with a total length of 60 cm (the effective length 52 cm) and i.d. of 50 μm was used to separate vitamins. Prior to use, the capillary was rinsed with a 0.1 M NaOH solution for 5 min and with the separation buffer for 5 min. As a separation buffer 25 mM sodium tetraborate (pH 9.3) was used in the case of polyphenols and 40 mM boric acid with 50 mM SDS (pH 8.5) in the case of vitamins. The voltage applied for the separation of polyphenols and vitamins was +25 and +16 kV, respectively.

Sample preparation

Tomato, eggplant, chilli pepper, and potato were purchased at a local market in the autumn of 2006 and 2007. The vegetable skins were dried at room temperature and for analysis the weighed portions of the dried sample were homogenized into powder. Ultrasonic extraction was performed using a 80:20 mixture of methanol and water. For the extraction 0.5 g of the ground skin was weighed and 5 mL of the extraction mixture was added. The sample was left at room temperature for 60 min and in an ultrasonic bath at room temperature for 20 min. The extract was filtered through a 0.45 μm filter and stored at +4°C in dark. L-ascorbic acid was determined in the extracts of the fresh skin of vegetables under investigation.

Determination of total phenolic content

The total phenolic content of the extracts was determined spectrophotometrically (Jasco V-530, USA) according to the Price and Butler method [39]. Tannic acid was used as a standard (linear range 0.01–2.5 mM). The sample (250 μL) was added to 25 mL of deionized water and mixed. After that 3 mL of FeCl_3 was added and, additionally, after 3 min, 3 mL of $\text{K}_3[\text{Fe}(\text{CN})_6]$ was added. The solution was mixed and incubated at room temperature for 18 min. The absorbance was measured at 720 nm spectrophotometrically.

Determination of flavonoid content

The determination of the flavonoid content of plants was performed using the colorimetric assay [40]. At first 50 μL of the skin extract was diluted with 0.5 mL of deionized distilled water and 0.03 mL of 5% NaNO_2 was added. Then 0.06 mL of 10% AlCl_3 and 0.2 mL of 1 M NaOH were added after 5 min and a further 6 min, respectively. Finally, 0.21 mL of deionized distilled water was added. The absorbance was recorded at 510 nm spectrophotometrically. Rutin was used as a standard.

DPPH radical scavenging capability

The free radical scavenging capability of the compounds under investigation was evaluated using a stable free radical DPPH for the decolorization assay

[41–43]. The assay is based on the reduction of DPPH by phenolic compounds and the adsorbance of DPPH radical at 515 nm. To the cuvette 3.9 mL of a DPPH methanolic solution (6.02×10^{-5} M) was transferred and 0.1 mL of the extract was added. The absorbance at 515 nm was recorded at certain time intervals until a steady state of the reaction was reached. The blank reference cuvette contained a 80:20 mixture of methanol and water. The percentage of the DPPH radical remaining at the steady state was determined by the following equation:

$$\% \text{DPPH} = \left(1 - \frac{A_f}{A_0} \right) \times 100,$$

where A_0 and A_f correspond to the absorbance at 515 nm of the radical at the beginning of the reaction and at the steady state, respectively. The time needed to reach the steady state at an EC_{50} concentration of the compound (EC_{50} is the amount of an antioxidant needed to decrease the initial DPPH radical concentration by 50%) was calculated graphically [41–43]. All the determinations were done in triplicate.

RESULTS AND DISCUSSION

Total phenolic and flavonoid contents and the antioxidant capability

The total phenolic and flavonoid contents and antioxidant capability of the skin extracts of vegetables of the Solanaceae family are given in Fig. 2. The results indicate eggplant to have the highest total phenolic and flavonoid contents – 1.5 g/L (900 mg/100 g) and 1.1 g/L (660 mg/100 g), respectively, followed by chilli pepper – 0.8 g/L (480 mg/100 g) and 0.4 g/L (240 mg/g), respectively. The

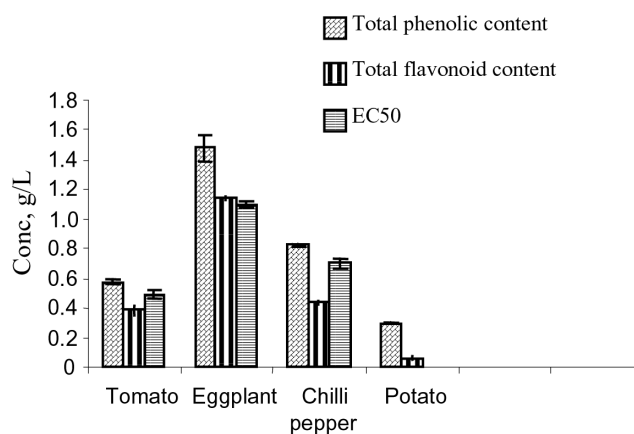


Fig. 2. Comparison of total phenolic and flavonoid content and antioxidant capability of plants of the Solanaceae family.

total phenolic content of the tomato skin extract was 0.6 g/L (360 mg/100 g) and that of flavonoids, 0.4 g/L (240 mg/100 g). Potato had the lowest total phenolic content, 0.3 g/L (180 mg/100 g), and its flavonoid content was 0.06 g/L (36 mg/100 g).

EC₅₀ is one of the most frequently measured parameter characterizing the antioxidant capability of plants [41–43]. Its value is inversely related to the antioxidative capability of a compound. Thus, the lower the EC₅₀, the higher the antioxidant power. Figure 2 shows tomato to have the lowest EC₅₀, followed by chilli pepper and eggplant. It is interesting that the decolorization reaction of the potato extract with DPPH was negligible, therefore no data are shown.

Separation of bioactive compounds by CE

Separation of polyphenols

Various phenolic compounds were separated and identified by CZE. Based on the results of our earlier studies, an efficient separation buffer, borate, was used in the case of polyphenols [44, 45]. The electropherogram of the standard mixture of polyphenols was obtained and is shown in Fig. 3. A satisfactory separation was achieved in 12 min.

Polyphenols contained in vegetables of the Solanaceae family were separated and identified. The results are demonstrated in Fig. 4. The phenolic compounds were identified by the spiking of the standard solution to the extract, which resulted in an increase of the analyte peak. As an example, the electropherograms of spiking for the identification of chlorogenic acid in potato extract are

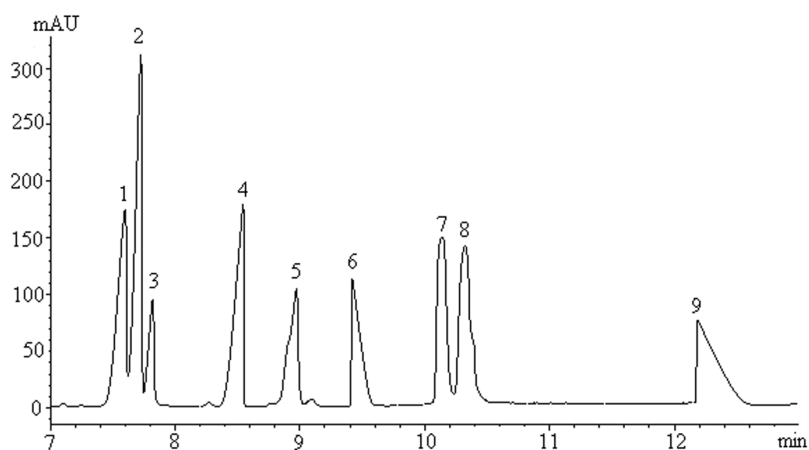


Fig. 3. Electropherogram of the standard mixture of polyphenols (250 mM of each compound): 1 – genistein, 2 – rutin, 3 – naringenin, 4 – cinnamic acid, 5 – chlorogenic acid, 6 – *p*-coumaric acid, 7 – myricetin, 8 – quercetin, 9 – caffeic acid. The separation conditions: separation buffer 25 mM sodium tetraborate (pH 9.3), the effective length of the capillary 50 cm, applied voltage +25 kV, UV detection at 210 nm; injections were performed hydrodynamically 15 s.

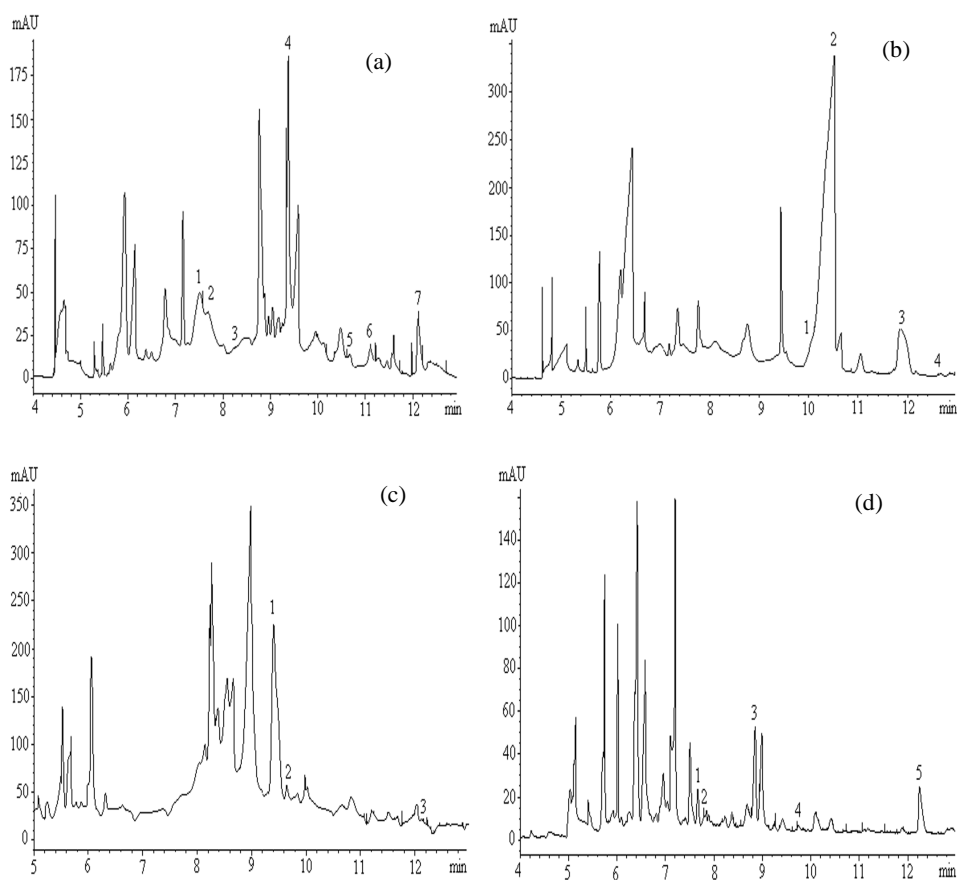


Fig. 4. Electropherograms of skin extracts of plants of the Solanaceae family: (a) skin extract of tomato: 1 – genistein, 2 – rutin, 3 – naringenin, 4 – chlorogenic acid, 5 – myricetin, 6 – quercetin, 7 – caffeic acid; (b) skin extract of eggplant: 1 – cinnamic acid, 2 – chlorogenic acid, 3 – caffeic acid, 4 – ferulic acid; (c) skin extract of chilli pepper: 1 – luteolin, 2 – quercetin, 3 – caffeic acid; (d) skin extract of potato: 1 – catechin, 2 – rutin, 3 – chlorogenic acid, 4 – quercetin, 5 – caffeic acid. The separation conditions were the same as in Fig. 3.

presented in Fig. 5. A certain amount of a standard solution of polyphenols (chlorogenic acid) was added to the extract and a decreased peak was observed. In addition to the spiking procedure, the spectra of the phenolic compounds separated from the skin extracts of vegetables were compared to the spectra of reference compounds using a diode-array detector. Comparison of the electropherograms in Fig. 4 reveals that polyphenols were mainly identified in the tomato skin extract and only a few were identified in the chilli pepper skin extract under the separation conditions applied.

The electropherograms in Fig. 4 demonstrate that the extracts of vegetables are very complex in composition and the determination of individual compounds

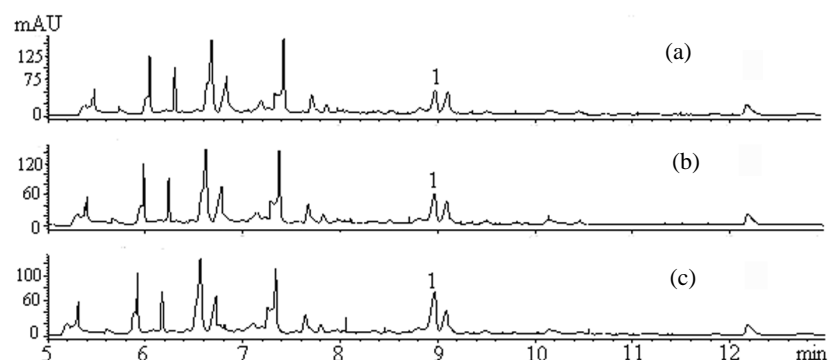


Fig. 5. Identification of a phenolic compound by spiking: (a) – potato skin extract; (b) – 5 μ L of 2.5 mM chlorogenic acid (peak 1) was added to the extract; (c) – 5 μ L of 250 mM chlorogenic acid added to the extract. The separation conditions were the same as in Fig. 3.

will require the use of several analytical methods. A number of flavonoids may also be present as glycosides and their reference compounds are not commercially available. Thus in the present study most glycosides were not identified, but it could be possible applying MS.

Separation of vitamins

Different techniques of CE like CZE, MEKC, and micellar emulsion electrokinetic chromatography (MEEKC) have been successfully applied to separate vitamins [46–50]. In this study, MEKC was used and as a separation buffer 40 mM boric acid with 50 mM SDS was found to be satisfactory in terms of compromise between the separation efficiency and the analysis time. Peak identification was based on the comparison of the migration times of standard compounds and the extracts of vegetables. It was confirmed by spiking (fortification technique) and the spectra of standard compounds and those in the extracts of vegetables. The quantification was based on an external standard method using calibration curves (Table 1). To assess the linearity of the relationship between the concentration and peak area of analytes, four standard solutions (in the range 250 to 1000 μ mol/L) dissolved in milli-Q water were analysed. The limit of detection was evaluated as three times the signal-to-noise ratio.

The composition of vitamins of group B and vitamin C (L-ascorbic acid) was investigated. The fresh skin extracts of vegetables of the Solanaceae family were used for the determination of ascorbic acid as it is easily decomposed. Figure 6 shows the vitamins determined in the skin extracts of tomato, eggplant, and chilli pepper. In the potato extract these vitamins were not detected. L-ascorbic acid was present in the fresh skin extracts of chilli pepper and tomato, but of B-group vitamins, pyridoxine was determined in the dried eggplant skin extract. Table 2 shows quantitative results of vitamin determination in the extracts.

Table 1. The analytical parameters of vitamins

Analyte	Linear range, $\mu\text{mol/L}$	Equation of calibration curve	R^2	LOD*, $\mu\text{mol/L}$	LOQ**, $\mu\text{mol/L}$
Nicotinamide	250–1000	$y = 0.0618x + 5.51$	0.9946	8	23
Pyridoxine	250–1000	$y = 0.1327x + 4.850$	0.9941	4	13
D-Pantothenic acid	250–1000	$y = 0.0405x + 2.95$	0.996	12	36
L-ascorbic acid	250–1000	$y = 0.1037x + 4.4$	0.9948	7	21
Nicotinic acid	250–1000	$y = 0.0905x + 6.96$	0.9963	6	18
Thiamine	250–1000	$y = 0.1527x + 10.45$	0.994	3	10

* Limit of detection (LOD).

** Limit of quantification (LOQ).

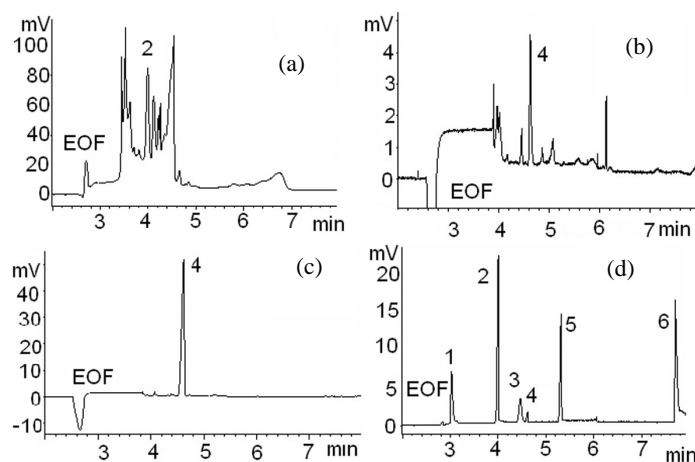


Fig. 6. Electropherograms of the vitamins determined in the plant extracts: (a) – dry eggplant skin extract, peak No. 2 – pyridoxine; (b) – fresh tomato skin extract, peak No. 4 – L-ascorbic acid; (c) – fresh chilli pepper skin extract, peak No. 4 – L-ascorbic acid; (d) – mixture of standard solutions of the vitamins (each 250 μM): 1 – nicotinamide, 2 – pyridoxine, 3 – D-pantothenic acid, 4 – L-ascorbic acid, 5 – nicotinic acid, 6 – thiamine. The separation conditions: separation buffer 40 mM boric acid with 50 mM SDS (pH = 8.5), the effective length of the capillary 52 cm, applied voltage +16 kV; injections were performed hydrodynamically 5 s.

Table 2. Quantification of vitamins found in the skin extracts of vegetables of the Solanaceae family

Fresh tomato	Fresh chilli pepper	Dry eggplant
L-ascorbic acid, 265 nm 4 mg/L \pm 0.2	L-ascorbic acid, 265 nm 196 mg/L \pm 10	Pyridoxine, 210 nm 609 mg/L \pm 10

Monitoring of the free radical scavenging capability by CE

The free radical scavenging capability of phenolic compounds was monitored using CE. Previously, the antioxidative capability had been evaluated mainly spectrophotometrically, but CE allows a simultaneous monitoring of the oxidation of several phenolic compounds over time using a stable free radical like DPPH. Such approach is suitable for qualitative evaluation of the antioxidative capability of phenolic compounds. Due to its richness in polyphenols, the tomato skin extract was used to evaluate the antioxidative capability of compounds.

In Fig. 7 the results of the monitoring of antioxidativity are presented. The electropherogram (Fig. 7b) was taken after 5 min mixing of 10 μ L of the tomato skin extract with 90 μ L of a 0.5 mM DPPH solution. After 5 min the reaction was completed and therefore no considerable changes were observed. In Fig. 7 the main decreasing peaks are shown. The spectra of the decreased peaks were analysed and compared with the original spectra of the extract. The peaks of rutin, naringenin, chlorogenic acid, and caffeic acid mainly decreased. Besides, Fig. 7 shows four unknown peaks that mostly decreased. Comparing the spectra the unknown peaks X, Y, Z, and Q may be attributed to phenolic acids.

The unoxidized part (%) was determined by the original peak areas (Fig. 7a). Table 3 demonstrates that chlorogenic acid (peak 3) and caffeic acid (peak 4) and

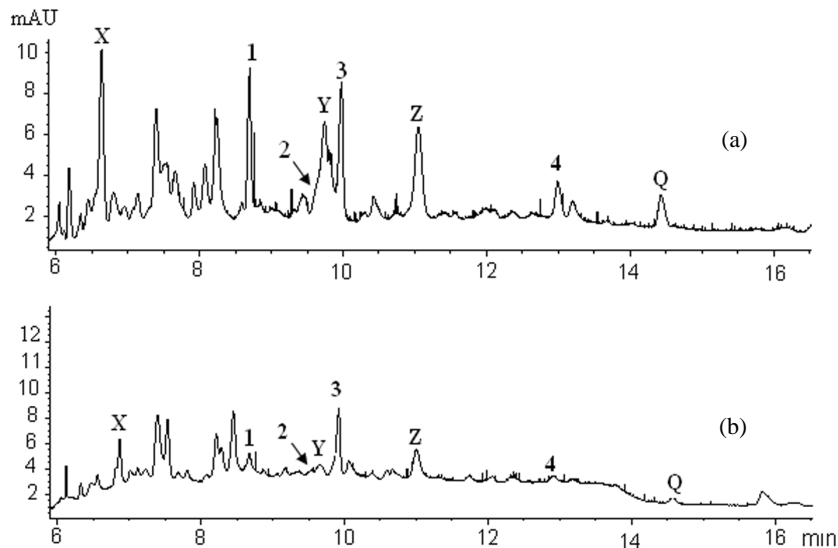


Fig. 7. Electropherograms of the monitoring of the antioxidativity of tomato skin extract using DPPH by CE: (a) 10 μ L of tomato skin extract mixed with 90 μ L of 80:20 methanol/water. Because of dilution the separation should be compared with Fig. 4. Peaks: 1 – rutin, 2 – naringenin, 3 – chlorogenic acid, 4 – caffeic acid; X, Y, Z, Q – unknown compound; (b) the reaction after 5 min. The mixture of the reaction: 10 μ L of tomato skin extract mixed with 90 μ L of 0.5 mM DPPH. Separation conditions were the same as in Fig. 3.

Table 3. Oxidation of the tomato skin extract by DPPH using CE

Peak No.	Migration time, min	Compound	Unoxidized part*, %
X	6.7	Unknown compound	22.0
1	8.7	Rutin	78.6
2	9.5	Naringenin	81.8
3	9.7	Chlorogenic acid	57.6
Y	9.6	Unknown compound	77.6
Z	11.0	Unknown compound	41.7
4	12.9	Caffeic acid	28.2
Q	14.5	Unknown compound	34.3

* Reaction time 5 min.

also unknown compounds (X, Y, Z, Q) disappeared almost immediately after adding a DPPH solution to the tomato skin extract. It can be said that the antioxidative capability of these compounds is higher compared to the other compounds existing in the extract. The antioxidative capability of phenolic compounds is associated with their structure. Polyphenols, whose hydroxyl group is in the *ortho* or *para* position, undergo the oxidation reaction more easily [28].

CONCLUSIONS

The extracts of vegetables of the Solanaceae family were studied using CE. The presence of several phenolic compounds in tomato, eggplant, chilli pepper and potato skin extract was established. Of vitamins, pyridoxine was present only in the dried eggplant skin extract, but L-ascorbic acid was present in fresh tomato and chilli pepper skin extracts. A traditional method, a decolorization assay, using a stable free radical DPPH, was applied to determine the antioxidative capability of compounds. The monitoring of the oxidation reaction of the extract by CE allows the evaluation of the role of every single phenolic compound separately in this process.

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Bioaktiivsete komponentide määramine Solanaceae perekonna taimedes kapillaarelektroforeesi abil

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Fenoolsed ühendid (kaneelhappe derivaadid, flavonoolid, flavoonid, antotsüaanid) ja vitamiinid on laialdaselt levinud mitmetes taimsetes materjalides ning nende tähtsaima omaduse – käituda antioksidandina – tõttu on nende uurimise olulisus pidevalt kasvanud. Antioksidatiivsus seisneb vabade radikaalide sidumises, mis tekivad pidevalt biokeemiliste protsesside tulemusena. Liiga suures hulgas või sobimatus keskkonnas võivad vabad radikaalid muutuda toksiliseks, kahjustades biomolekule, nagu DNA, lipiidid, valgud, süsivesikud. Tagajärjeks on kudedes tekivad degeneratiivsed muutused, mis on mitmete haiguste tekkepõhjuseks: põletikud, immuunsüsteemi nõrgenemine, südame- ja veresoonehaigused, vähktõbi, Parkinsoni ning Alzheimeri tõbi ja ka vananemisprotsess.

Käesolevas töös on uuritud maavitsaliste perekonda Solanaceae kuuluvaid taimi, nagu tomat (*Solanum lycopersicum*), baklažaan (*Solanum melongena*), tšillipipar (*Capsicum annuum*) ja kartul (*Solanum tuberosum*), seoses nende bioaktiivsete ühendite rikkaliku sisaldusega (polüfenoolid, vitamiinid). Fenoolsed ühendid ja vitamiinid on ekstraheeritud vastavast taimest ultraheli ekstraktsioonil metanooli-veeseguga (80:20). Uuritavate ühendite lahutamisel ja identifitseerimisel on kasutatud kapillaarelektroforeesi (KE). Üldfenoolide sisaldus on määratud Price'i ja Butleri meetodi abil. Lisaks on hinnatud antioksidatiivsust 2,2-difenüül-1-pikrüülhüdrasüül- (DPPH-)radikaali abil nii spektrofotomeetriliselt kui ka kapillaarelektroforeesiga, mis võimaldab jälgida iga ühendi antioksidatiivsust eraldi.