

## Separation of polyphenols and L-ascorbic acid and investigation of their antioxidant activity by capillary electrophoresis

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**Abstract.** Growing evidence of the role of free radicals and antioxidants in health and ageing has focused great interest on these compounds. Vitamins C and E and various carotenoids are known to act as strong antioxidants. In recent years however interest in various polyphenols as antioxidants has also increased. Capillary electrophoresis, an effective analytical method, was used to separate the standard mixture of polyphenols and to determine the polyphenolic content of black currant qualitatively. In addition the oxidation of polyphenols and L-ascorbic acid with hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) was investigated. The experiment was performed with an in-house built reactor coupled to a capillary electrophoresis system.

**Key words:** capillary electrophoresis, polyphenols, antioxidative activity, free radical scavenging assay.

### INTRODUCTION

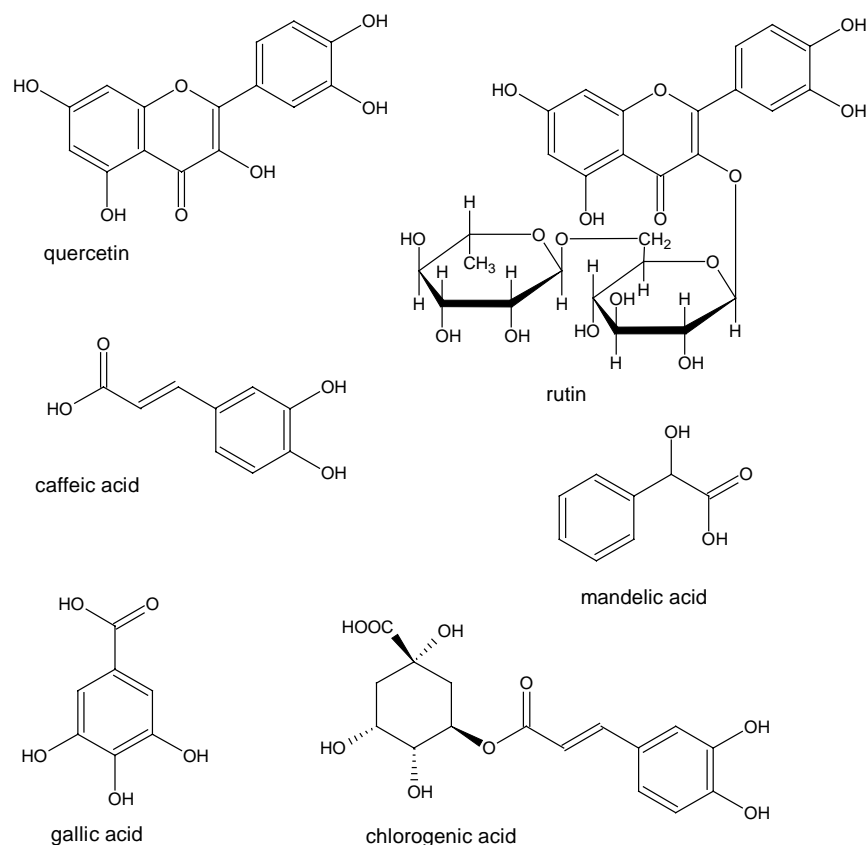
Fruits and vegetables are one of the main sources of antioxidants in our diets. Many clinical researches suggest that consuming fruits and vegetables is beneficial for preventing age-related diseases, cancers, heart diseases, etc. These protective effects are considered to be related in large part to the various antioxidants fruits and vegetables contain. There is overwhelming evidence to indicate that free radicals cause oxidative damage to lipids, proteins, and nucleic acids [1, 2]. Reactive oxygen species such as superoxide anion ( $\text{O}_2^{\bullet-}$ ), hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), and hydroxyl radical ( $\bullet\text{OH}$ ) are inevitably produced as by-products of normal aerobic metabolism and their concentrations increase under

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stress conditions. Living organisms have developed complex antioxidant systems consisting of multiple defence enzymes such as superoxide dismutase, glutathione peroxidase, ascorbate peroxidase and catalase as well as non-enzymatic antioxidants, such as albumin, glutathione, ascorbic acid (vitamin C),  $\alpha$ -tocopherol (vitamin E),  $\beta$ -carotene (provitamin A), uric acid, bilirubin, flavonoids, and many phenolic compounds [3].

Polyphenolic compounds (Fig. 1) are usually referred to as a diverse group of naturally occurring compounds containing multiple phenolic functionalities. These compounds are commonly found in higher plants. Naturally occurring polyphenols are known to have numerous biological activities. From a chemical point of view, polyphenols can react with one-electron oxidants, which prevents free radical formation in biological systems. The ability of these compounds to interact with metal ions such as  $\text{Fe}^{2+}$ , which is capable of generating free radicals, has significance in their bioactivity [4]. Several analytical methods have been proposed for determining antioxidant activity of various plant polyphenols, such



**Fig. 1.** Chemical formulas of polyphenols.

as scavenging of 1,1-diphenyl-2-hydrazyl (DPPH) radical [5, 6] or 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation [7, 8] or reactive oxygen species [3] and the inhibition activity against lipid peroxidation [9] or protein oxidation.

Traditional methods (Folin & Ciocalteu, Folin & Denis, Price & Butler, reviewed in [10]) for the determination of the phenolic component relied on colorimetric measurement of total phenols using one of a number of reagents of varying selectivity. The diversity of phenolic compounds means that selection of a reagent and/or absorbing wavelength will be a compromise although this is a smaller problem where a single class of phenols predominates. Furthermore, all spectrophotometric measurements lack specificity and give an over-estimation of "phenolic" content. Specificity can be enhanced by derivative spectrometry or by preliminary separation. Thin-layer chromatography (TLC) methods have been devised and exploited for the preliminary separation and clean-up of sample extracts [11]. The need for identifying individual phenolic compounds has caused the traditional methods to be replaced by high-performance chromatographic analyses. The limited volatility of many phenols has restricted the application of gas chromatography (GC) to their separation. However, with suitable derivatization (e.g. trimethylsilylation) they are amenable to GC and GC-mass spectrometry (MS). Nevertheless, high-performance liquid chromatography (HPLC) currently represents the most popular and reliable technique for analysis of phenols [12–14]. The typical system involves reversed phase liquid chromatography comprising a C<sub>18</sub> stationary phase or other alkyl chemistry. Routine detection in HPLC is typically based on the measurement of UV absorption or, less commonly, visible radiation in the case of anthocyanins. Identification of the eluted phenols in GC and HPLC is usually based on correspondence of retention data with an appropriate standard. Recently MS combined either on-line or off-line with chromatographic [1, 15] or electrophoretic techniques [16, 17] has been successfully used for separation and determination of phenols.

Capillary electrophoresis (CE) is a relatively new separation technique and represents an alternative method for the analysis of a variety of compounds, including antioxidants in food matrices [8]. CE has unique advantages that make it an excellent candidate for the analysis of these compounds including a small sample size requirement and high separation efficiency and speed. The most widely used buffers for the separation of polyphenols in CE are phosphate (pH 7) and borate buffers (pH 8–9.5) [18], sometimes also organic modifiers have been added to the buffer solution [19]. In addition to capillary zone electrophoresis (CZE) also micellar electrokinetic chromatography (MEKC), a technique able to separate both charged and uncharged analytes, has been used for the analysis of phenolic acids [20]. Lately various researches have applied also non-aqueous capillary electrophoresis (NACE) for the analysis of polyphenols, where either sodium acetate or various ionic liquids in acetonitrile and/or methanol were used as background electrolytes [21–23].

The objectives of this study were first to determine the phenolic content of selected Estonian berries. For that purpose CZE, a high-performing analytical technique, was used. Secondly we evaluate whether CE could be used for determining H<sub>2</sub>O<sub>2</sub> scavenging activity as a measure of antioxidant properties of vitamin C and various polyphenols.

## MATERIALS AND METHODS

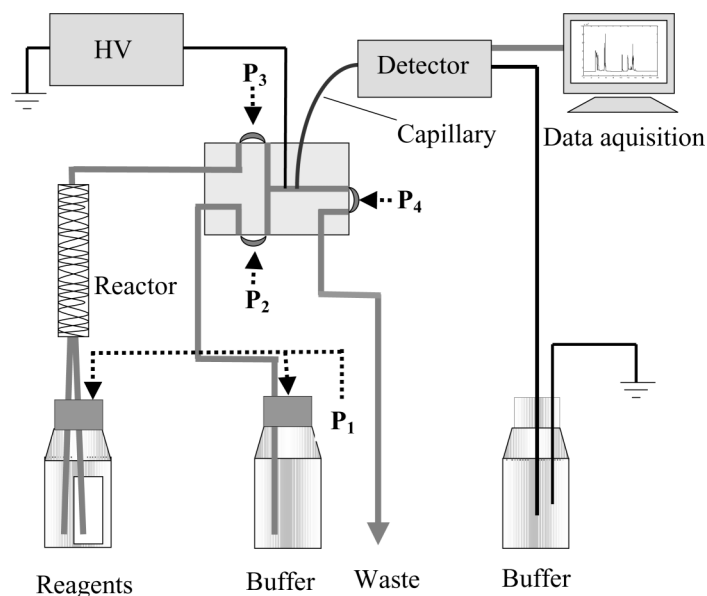
### Apparatus

#### *Capillary electrophoresis system*

The CE system, built in-house, consisted of a pneumatic sampler, a small reactor, a high-voltage power supply, and a UV detector (Prince Technologies) coupled to a personal computer and controlled by software written in-house using the Labview program (National Instruments Austin, TX, USA). The software controlled the sampling and recorded the detector signal via ADAM 4018/4060 interface blocks (Advantech Inc., Taipei, Taiwan). All experiments were conducted with an applied voltage of +20 kV. The separation was monitored at various wavelengths (210, 265, and 328 nm). An uncoated capillary (Polymicro Technologies, Phoenix, AZ, USA) with dimensions of 60 cm × 50 μm (41 cm to the detector) was used through the studies. Before use the capillary was rinsed with 1 M sodium hydroxide, water, and separation medium. Between analysis the capillary was washed with background electrolyte.

All samples were injected using a pneumatic sampler built in-house (Fig. 2). The sampler has three input channels and four solenoid valves (Type 6012 Miniature Solenoid Valve, Brüker, Helsinki, Finland), which control the pressure pulse sequences applied to the sample and buffer vessels. Three valves were used to activate the rinse of the channel where the capillary end is immersed by the buffer and the sample (from the reactor). The fourth valve was used to close the inlet channel waste end to perform the hydrodynamic sampling and capillary flushing. The pressure applied to the membranes that close the channels (P<sub>2</sub>, P<sub>3</sub>, P<sub>4</sub>) was 1.5 atm and that applied to the vessel (P<sub>1</sub>) was somewhat lower, about 0.5 atm.

The vessels of ascorbic acid and H<sub>2</sub>O<sub>2</sub> are placed so that equal amounts of both solutions are injected into the reactor at the same time. The small reactor consists of a Teflon tubing (1 m × 0.7 mm), where the H<sub>2</sub>O<sub>2</sub> and L-ascorbic acid solutions are mixed and the reaction is performed. It has been set to a “serpentine” form, which has 48 turns, to achieve good mixing of the reactants. After selected time intervals the sample from the reactor is injected hydrodynamically by flushing it before the inlet of the capillary and applying pressure to the waste end of the sampler.



**Fig. 2.** The scheme of the pneumatic sampler instrument. P<sub>1</sub>–P<sub>4</sub> – pressures applied.

### *UV analysis*

UV spectra of black currant extract and pure reference compounds (in MeOH) were run by a Jasco V-530 UV-vis spectrophotometer and spectra were acquired over the absorption range of 200–600 nm.

## **Chemicals**

### *Standards*

Quercetin (3,3',4',5,7-pentahydroxyflavone), rutin (3,3',4',5,7-pentahydroxyflavone-3-rutinoside), caffeic acid (3,4-dihydroxycinnamic acid), mandelic acid, gallic acid (3,4,5-trihydroxybenzoic acid), chlorogenic acid (1,3,4,5-tetrahydroxycyclohexanecarboxylic acid 3-(3,4-dihydroxycinnamate)), and L-ascorbic acid as well as sodium tetraborate, disodiumhydrogen phosphate, and sodium hydroxide were form Merck (Darmstadt, Germany). For the preparation of buffers and solutions of analytes, ultra pure Milli Q water (Millipore S.A. Molsheim, France) was used throughout the experiments.

### *Sample preparation*

Black currants (*Ribes nigrum*) were collected near Tallinn, Estonia, at the end of August when they were ripe, and stored in a freezer (–18°C). The weighed portion (50 g) of raw material was squeezed and extracted for 24 h with 80% methanol. Then the sample was filtered and stored at +4°C until analysed.

### **Determination of hydrogen peroxide scavenging activity**

The measurement was started with releasing pressure  $P_3$  in the sampler for 1–2 s. This yielded equal amounts of 7.5% (2.45 M)  $H_2O_2$  in 15 mM disodium-hydrogen phosphate (pH 7.4) and 5 mM L-ascorbic acid or selected polyphenol (1 mM) solutions to be mixed in the reactor. The reaction mixture was let to incubate at room temperature for different periods of time. Then pressure  $P_3$  was released again for 1–2 s and the mixture of the reactants and products was transported before the inlet end of the capillary for sampling. The separation of analytes was started when the inlet channel was filled with the buffer solution (by releasing pressure  $P_2$ ). When separation was completed a new reaction and analysis cycle was started but with a different incubation time. By increasing the time of incubation from experiment to experiment, the decrease of L-ascorbic acid concentration was recorded. To make sure that L-ascorbic acid was not degrading because of alkaline pH (pH > 7) another experiment was performed where no  $H_2O_2$  was added to the vitamin solution. The obtained results (not shown) proved that the L-ascorbic acid concentration remained constant for the time needed to perform the reaction.

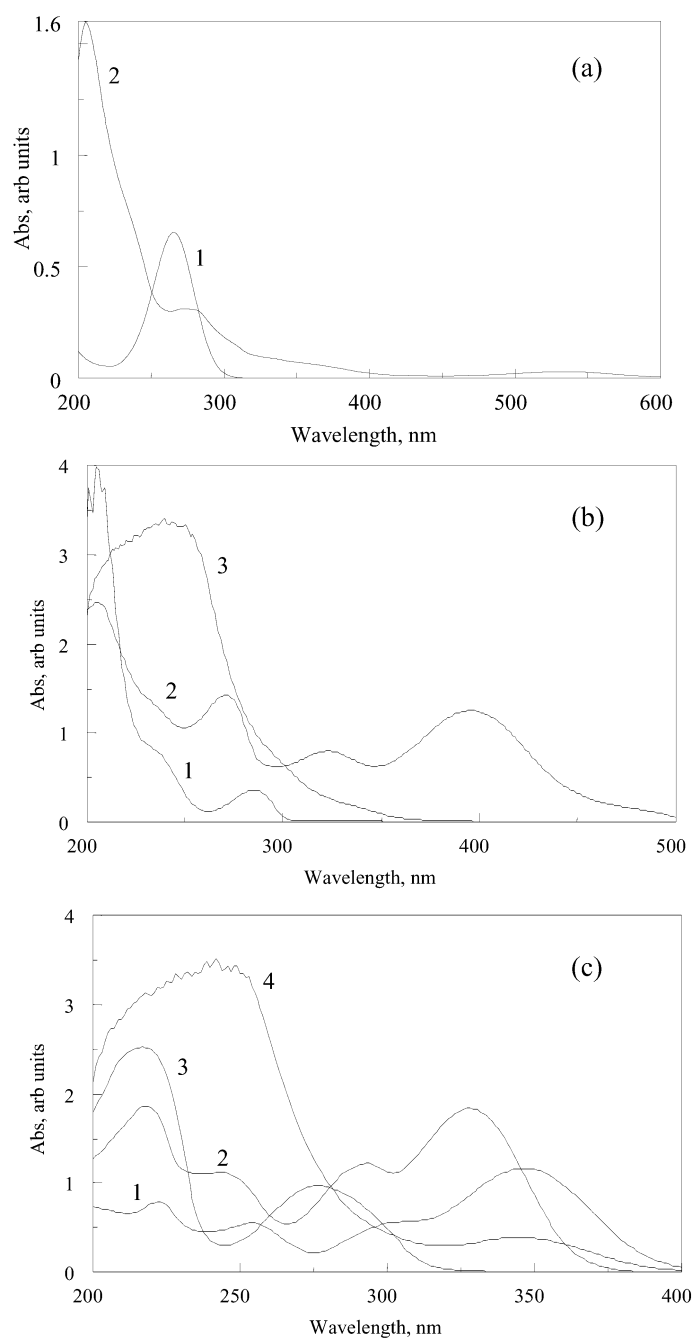
## **RESULTS AND DISCUSSION**

### **The UV spectra of vitamin C and various polyphenols**

Spectrophotometry was used to determine the absorption maximums of L-ascorbic acid and black currant methanolic extract (Fig. 3a) and also of various polyphenols (Figs. 3b, 3c). Water or water–methanol mixture was used as blank. For that  $H_2O_2$  (in 2 × excess) was mixed with solutions of various polyphenols. After letting polyphenol to react with  $H_2O_2$  for 15 min, the spectra of the reaction mixture were measured. An example of spectra of quercetin and products of quercetin oxidation is presented in Fig. 3b, and chlorogenic acid and its oxidation products in Fig. 3c. Sodium borate buffer (pH 9.6) was added to the reaction media to enhance the speed of the reaction as it is generally known that  $H_2O_2$  degrades more easily in an alkaline environment. The strong bleaching effect of  $H_2O_2$  is evident from Figs. 3b and 3c, where long wavelength adsorption bands (in visible region between 300 and 500 nm), which are characteristic of quercetin and chlorogenic acid, disappear completely in the reaction mixtures.

### **The separation of polyphenols in the standard mixture and analysis of black currant extract**

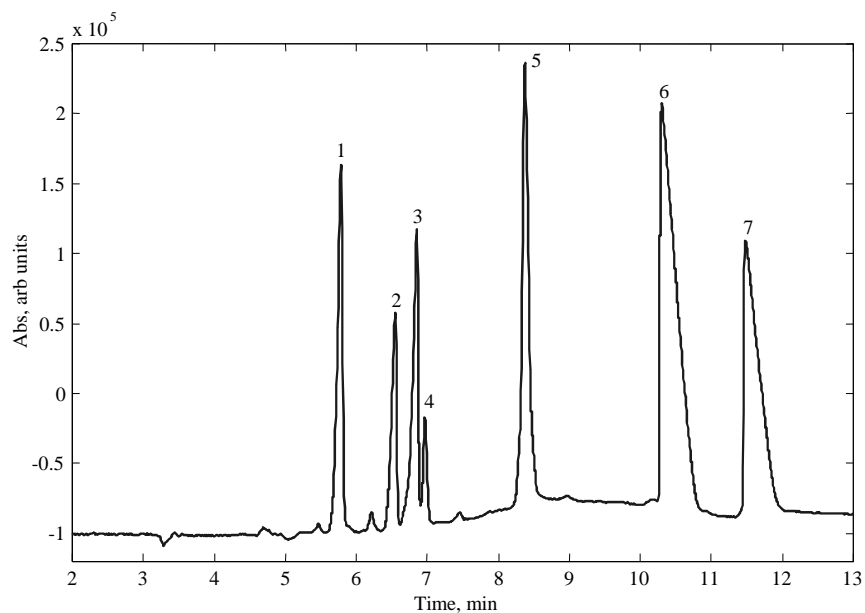
The best separation of the standard mixture of polyphenols was obtained with a 20 mM disodium tetraborate buffer at pH 9.6. In the borate buffer a borate ion and certain analytes containing vicinal hydroxyl groups form a negatively charged borate complex. Therefore, under alkaline conditions when the poly-



**Fig. 3.** Spectra of L-ascorbic acid and polyphenols: a – spectra of L-ascorbic acid (1) and black currant extract (2); b – spectra of catechin (1), quercetin (2), and reaction mixture of quercetin and  $H_2O_2$  after 15 min of incubation; c – spectra of chlorogenic acid (1), caffeic acid (2), gallic acid (3), and reaction mixture of chlorogenic acid and  $H_2O_2$  after 15 min of incubation.

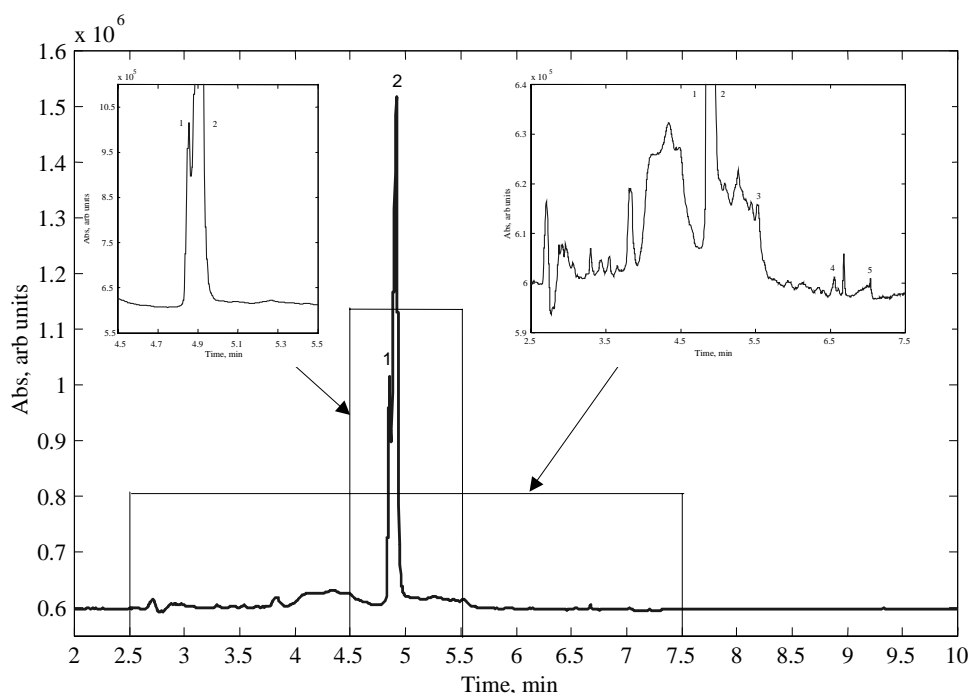
phenols are negatively charged, the separation of the borate buffer under basic experimental conditions in which the polyphenols are negatively charged, could effect the separation either based on charge-to-mass ratios of the deprotonated polyphenols or through borate–phenol association. An example of the separation of quercetin, rutin, caffeic acid, gallic acid, chlorogenic acid, and mandelic acid is presented in Fig. 4.

The most widely used method for the analysis of polyphenols in plant extracts is HPLC, where the identification of compounds of interest is done according to the correspondence of retention times with appropriate standards. However, CE is known to have a rather poor reproducibility of migration times and therefore the standard addition method was used to identify the target analytes in the berry extract. When the selected standard was added to the berry extract, an increase of the target peak was observed in the pherogram (not presented). An example of the separation of black currant extract components is shown in Fig. 5. The separation was performed with 15 mM borate buffer at pH 9.2. We found the black currant methanolic extract to contain L-ascorbic, caffeic, and gallic acids, and quercetin. Häkkinen et al. determined various polyphenols in 19 berries. In black currant they found *p*-coumaric acid, myricetin, quercetin, gallic acid, caffeic acid, ferulic acid, kaempferol, and *p*-hydroxybenzoic acid [24], which is in accordance with our results. In addition we assume that the extract may contain anthocyanin, which is characteristic of black currant, but this was not proven because of lack of anthocyanin standard.



**Fig. 4.** Separation of standard mixture of polyphenols. Separation buffer: 20 mM borate buffer, pH 9.6. The applied voltage was 20 kV, and detection was performed at 210 nm. Peaks: 1 – rutin; 2 – mandelic acid; 3 – chlorogenic acid; 4 – adduct of chlorogenic acid; 5 – quercetin; 6 – caffeic acid; 7 – gallic acid.



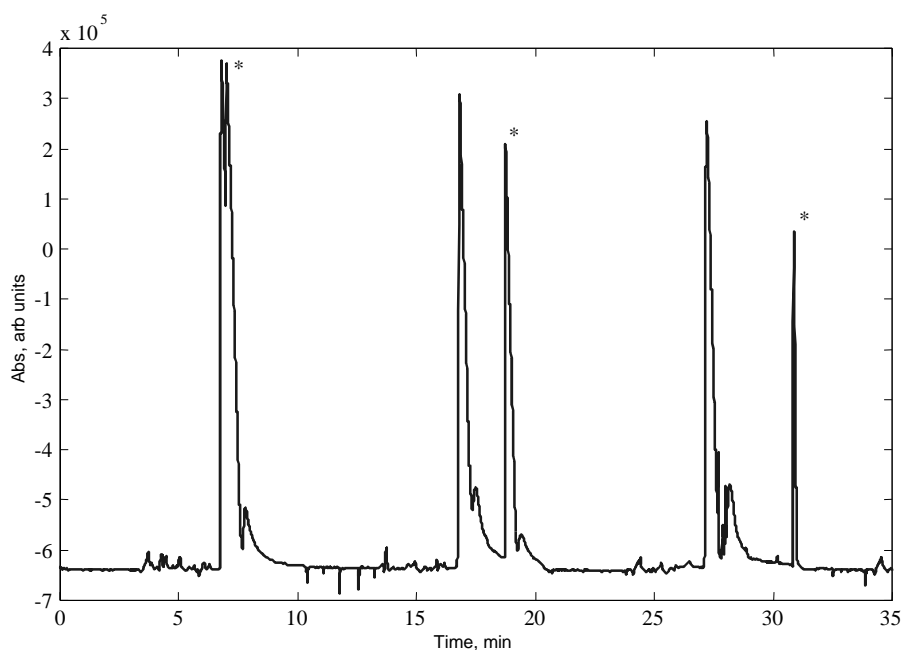


**Fig. 5.** CE separation of black currant extract. Separation buffer: 15 mM borate buffer, pH 9.2. The detection was performed at 265 nm. Other conditions as in Fig. 4. Peaks: 1 – L-ascorbic acid, 2 – anthocyanin, 3 – quercetin, 4 – caffeic acid, 5 – gallic acid.

### **Antioxidant potentials of vitamin C and polyphenols against radicals generated in the aqueous phase**

Several methods have been developed to evaluate the free radical scavenging ability of antioxidants. These are based on a large variety of radical generating systems and various methods have been used for oxidation end-point determination. The results of different evaluation tests depend on the specificity and test parameters of the method employed to analyse the progress of oxidation, including the degree of oxidation chosen as end-point. Generally, it is preferable to confirm the general trend of measured activities by one or more additional methods operating in the same antioxidant content (i.e. an environment containing hydrophilic or lipophilic radicals).

In this study the antioxidant properties of polyphenols (caffeic acid) and L-ascorbic acid were determined by the  $H_2O_2$  assay. The decrease of their concentration in the reaction mixture was monitored by CE (Figs. 6 and 7). The  $H_2O_2$  test enables to evaluate the abilities of molecules to scavenge hydroxyl radical ( $OH^\bullet$ ) and superoxide radical anions ( $O_2^{\bullet-}$ ). Hydrogen peroxide is substantially more acidic than water, with a  $pK_a$  of 11.6. The perhydroxyl anion is unstable and the decrease in the stability of  $H_2O_2$  is caused by the instability of

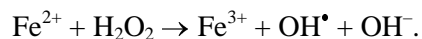


**Fig. 6.** Monitoring of the oxidation of caffeic acid. Reaction mixture: 0.5 mM caffeic acid and 1.2 M H<sub>2</sub>O<sub>2</sub> in phosphate buffer (pH 7.4). Running buffer: 20 mM sodium tetraborate, pH 9.6. The applied voltage was 20 kV, and detection was performed at 328 nm. \* – caffeic acid peak (used to monitor the oxidation process).

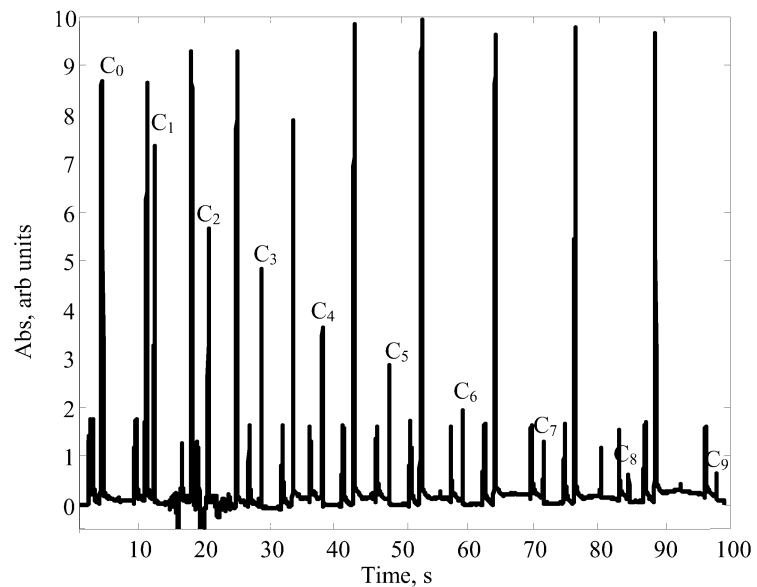
HOO<sup>-</sup>. However, heat and base increase the decomposition of H<sub>2</sub>O<sub>2</sub>. The highly reactive hydroxyl radicals (HO<sup>•</sup>) are formed when the perhydroxyl anion reacts with H<sub>2</sub>O<sub>2</sub> [25]:



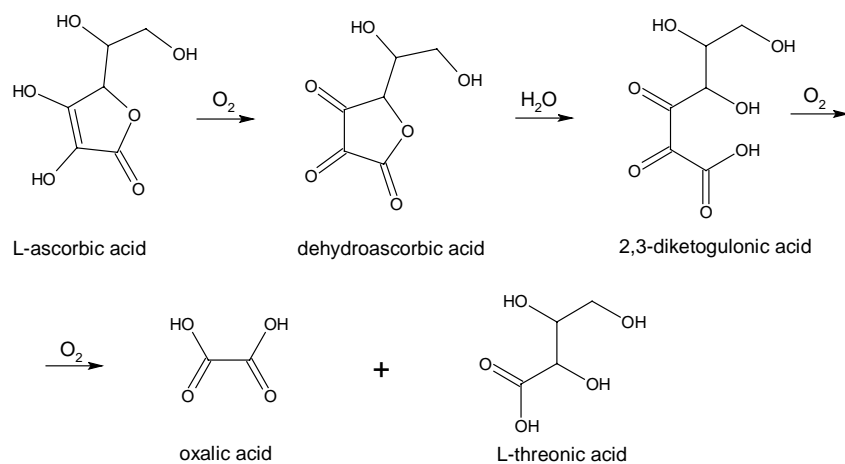
The oxidation of vitamin C (L-ascorbic acid) to oxalic acid in aqueous solution is generally known to be catalysed by metal ions to increase the rate of radical formation. The Fenton reaction is often performed to generate hydroxyl radicals (OH<sup>•</sup>), which are formed through one-electron reduction of H<sub>2</sub>O<sub>2</sub> and catalysed by Fe<sup>2+</sup> [26]:



This process involves oxidation of ascorbic acid to dehydroascorbic acid, followed by hydrolysis that leads to the opening of the lactone ring to produce 2,3-diketogulonic acid. Cleavage and further oxidation produce oxalic acid and L-threonic acid (Scheme 1) [27].



**Fig. 7.** Monitoring of the oxidation of L-ascorbic acid. Reaction mixture: 2.5 mM L-ascorbic acid and 1.2 M H<sub>2</sub>O<sub>2</sub> in phosphate buffer (pH 7.4). The detection was performed at 265 nm, other conditions as in Fig. 6. C<sub>0</sub>–C<sub>9</sub> – L-ascorbic acid peak (used to monitor the oxidation process).



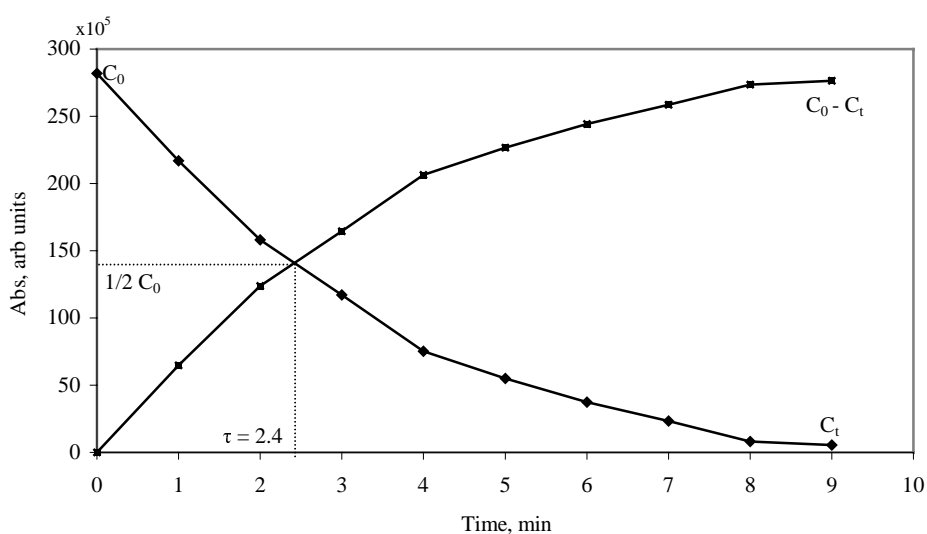
**Scheme 1.** Hydrolysis and oxidation of L-ascorbic acid.

In addition to iron(III) various other metal ions such as copper(II), cobalt(II), nickel(II), manganese(II), and zinc(II) have been used as catalysts in the oxidation of ascorbic acid. It has been stated that unless the metal ion has a stable lower valence form, it will not participate in the electron transfer scheme and will thus not function as a catalyst [27].

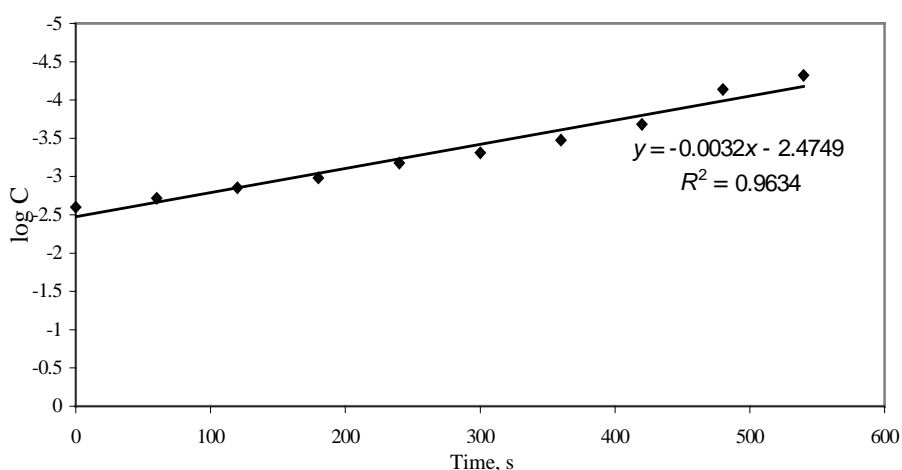
### Kinetics of the oxidation of ascorbic acid

Figure 8 illustrates the calculation of half-life for the oxidation of ascorbic acid. The time needed for 50% degradation of the L-ascorbic acid was 2.4 min. In Fig. 9  $\log[C]$  was plotted against time, which resulted in a straight line with a correlation coefficient ( $R^2$ ) of 0.963, indicating that it is the first-order reaction. Thus the rate of the reaction is only dependent on the concentration of L-ascorbic acid:

$$\text{Rate} = k[\text{vitamin C}].$$



**Fig. 8.** Calculation of the half-life of L-ascorbic acid.  $C_0 - C_t$  – L-ascorbic acid peak.



**Fig. 9.** Determination of the rate constant.

The rate constant from Fig. 9 was found to be equal to  $32 \times 10^{-4} \text{ s}^{-1}$ , which is about one order of magnitude higher compared to the literature where the rate constant for L-ascorbic acid oxidation reaction was found to be  $48.98 \times 10^{-5} \text{ s}^{-1}$  [28]. The explanation can be that in our case the reaction was performed in weakly alkaline conditions, where hydroxyl radicals are more easily formed.

There have also been studies about the oxidation of anthocyanins by hydrogen peroxide. It has been reported to be a first-order reaction, and the rate constants brought in the literature are comparable with our results [29].

### CONCLUDING REMARKS

Several polyphenolic compounds were qualitatively determined in black currant methanolic extract. The antioxidant activity of L-ascorbic acid and polyphenols was studied by following their  $\text{H}_2\text{O}_2$  scavenging ability. Two methods were used for that purpose. First spectrophotometry was applied to determine the absorption maximums of analytes and to obtain preliminary results about the oxidation of polyphenols. Secondly capillary electrophoresis was used to investigate the kinetics of oxidation reactions of vitamin C and caffeic acid by monitoring the decrease of their concentration in the reaction mixture. Spectrophotometric methods are widely used for the investigation of the antioxidant activities of various plant extracts capillary electrophoresis; however, earlier these methods have not been used for studying the oxidation kinetics of these compounds. Further, kinetic parameters, such as the half-life and order of reaction were determined. The present method may be useful for the evaluation of the antioxidant activity of phenolic compounds as well as natural antioxidants in biological material.

### REFERENCES

1. Cheng, F.-C., Jen, J.-F. & Tsai, T.-H. Hydroxyl radical in living systems and its separation methods. *J. Chromatogr. B*, 2002, **781**, 481–496.
2. Shui, G. & Leong, L. P. Analysis of polyphenolic antioxidants in star fruit using liquid chromatography and mass spectrometry. *J. Chromatogr. A*, 2004, **1022**, 67–75.
3. Paździoch-Czochra, M. & Wideńska, A. Spectrofluorimetric determination of hydrogen peroxide scavenging activity. *Anal. Chim. Acta*, 2002, **452**, 177–184.
4. Handique, J. G. & Baruah, J. B. Polyphenolic compounds: an overview. *React. Funct. Polym.*, 2002, **52**, 163–188.
5. Bondet, V., Brabnd-Williams, W. & Berset, C. Kinetics and mechanisms of antioxidant activity using the DPPH<sup>•</sup> free radical method. *Lebensm.-Wiss. Technol.*, 1997, **30**, 609–615.
6. Gorinstein, S., Martin-Belloso, O., Katrich, E., Antonin, L., Číž, M., Gligelmo-Miguel, N., Haruenkit, R., Park, Y.-S., Jung, S.-T. & Trakhtenberg, S. Comparison of the contents of the main biochemical compounds and the antioxidant activity of some Spanish olive oils as determined by four different radical scavenging tests. *J. Nutr. Biochem.*, 2003, **14**, 154–159.

7. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. & Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biol. Med.*, 1999, **26**, 1231–1237.
8. Minussi, R. C., Rossi, M., Bologna, L., Cordi, L., Rotilio, D., Pastore, G. M. & Durán, N. Phenolic compounds and total antioxidant potential of commercial wines. *Food Chem.*, 2003, **82**, 409–416.
9. Sroka, Z. & Cisowski, W. Hydrogen peroxide scavenging, antioxidant and anti-radical activity of some phenolic acids. *Food Chem. Toxicol.*, 2003, **41**, 753–758.
10. Ragan, M. A. & Glombitza, K.-W. Progress in phycolological research. In *Round FE* (Chapman, D. J., ed.). Biopress, Bristol, 1986, 129–214.
11. Wollgast, J. & Anklam, E. Review on polyphenols in *Theobroma cacao*: changes in composition during the manufacture of chocolate and methodology for identification and quantification. *Food Res. Int.*, 2000, **33**, 423–447.
12. Robards, K. Strategies for the determination of bioactive phenols in plants, fruit and vegetables. *J. Chromatogr. A*, 2003, **1000**, 657–691.
13. Makris, D. P., Psarra, E., Kallithraka, S. & Kefalas, P. The effect of polyphenolic composition as related to antioxidant capacity in white wines. *Food Res. Int.*, 2003, **36**, 805–814.
14. De Souza, T. P., Holzschuh, M. H., Lionço, M. I., González Ortega, G. & Petrovick, P. R. Validation of a LC method for the analysis of phenolic compounds from aqueous extract of *Phyllanthus niruri* aerial parts. *J. Pharm. Biomed. Anal.*, 2002, **30**, 351–356.
15. Alonso Borbalán, Á. M., Zorro, L., Guillén, D. A. & Barroso, C. G. Study of the polyphenol content of red and white grape varieties by liquid chromatography–mass spectrometry and its relationship to antioxidant power. *J. Chromatogr. A*, 2003, **1012**, 31–38.
16. Yanes, E. G., Gratz, S. R., Baldwin, M. J., Robinson, S. E. & Stalcup, A. M. Capillary electrophoretic application of 1-alkyl-3-methylimidazolium-based ionic liquids. *Anal. Chem.*, 2001, **73**, 3838–3844.
17. Yanes, E. G., Gratz, S. R. & Stalcup, A. M. Tetraethylammonium tetrafluoroborate: a novel electrolyte with a unique role in the capillary electrophoretic separation of polyphenols found in grape seed extracts. *Analyst*, 2000, **125**, 1919–1923.
18. Gu, X., Chu, Q., O'Dwyer, M. & Zeece, M. Analysis of resveratrol in wine by capillary electrophoresis. *J. Chromatogr. A*, 2000, **881**, 471–481.
19. Li, X., Zhang, Y. & Yuan, Z. Separation and determination of rutin and quercetin in the flowers of *Sophora japonica* L. by capillary electrophoresis with electrochemical detection. *Chromatographia*, 2002, **55**, 243–246.
20. Pomponio, R., Gotti, R., Hudaib, M. & Cavrini, V. Analysis of phenolic acids by micellar electrokinetic chromatography: application to *Echinacea pupurea* plant extracts. *J. Chromatogr. A*, 2002, **945**, 239–247.
21. Demianová, Z., Sirén, H., Kuldvee, R. & Riekkola, M.-L. Nonaqueous capillary electrophoretic separation of polyphenolic compounds in wine using coated capillaries at high pH in methanol. *Electrophoresis*, 2003, **24**, 4264–4271.
22. Vaher, M. & Koel, M. The separation of polyphenolic compounds extracted from plant matrices using capillary electrophoresis. *J. Chromatogr. A*, 2003, **990**, 225–230.
23. Wright, L. P., Aucamp, J. P. & Apostolides, Z. Analysis of black tea theaflavins by non-aqueous capillary electrophoresis. *J. Chromatogr. A*, 2001, **919**, 205–213.
24. Häkkinen, S., Heinonen, M., Kärenlampi, S., Mykkänen, H., Ruuskanen, J. & Törrönen, R. Screening of selected flavonoids and phenolic acids in 19 berries. *Food Res. Int.*, 1999, **32**, 345–353.
25. Qin, C. Q., Du, Y. M. & Xiao, L. Effect of hydrogen peroxide treatment on the molecular weight and the structure of chitosan. *Polym. Degrad. Stab.*, 2002, **76**, 211–218.
26. Lo Scalzo, R., Iannocari, T., Summa, C., Morelli, R. & Rapisarda, P. Effect of thermal treatments on antioxidant and antiradical activity of blood orange juice. *Food Chem.*, 2004, **85**, 41–47.
27. Arendse, M., Anderson, G. K. & Rath, N. P. Oxidative degradation of the ascorbate anion in the presence of platinum and palladium oxalate complexes. *Polyhedron*, 2001, **20**, 2495–2503.

28. Sultan, S. M. & Desai, N. I. Mechanistic study and kinetic determination of vitamin C employing the sequential injection technique. *Talanta*, 1998, **45**, 1061–1071.
29. Özkan, M. Degradation of anthocyanins in sour cherry and pomegranate juices by hydrogen peroxide in the presence of added ascorbic acid. *Food Chem.*, 2002, **78**, 499–504.

## **Polüfenoolide ja L-askorbiinhappe lahutamine ning antioksidatiivse aktiivsuse uurimine kapillaarelektroforeesi kasutades**

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Tänu üha uuenevatele teadmistele vabade radikaalide ja antioksidantide kohta on huvi nende ühendite vastu jätkuvalt kasvanud. Tugevate antioksidatiivsete omaduste poolest on tuntud vitamiinid C, E ning karotenoidid. Kuid viimastel aastatel on suurenenud huvi ka polüfenoolide kui antioksidantide vastu. Antud töös on kasutatud polüfenoolide standardsegu – kohvhape, gallushape, klorogeenhape, mandelhape, katehhiin ja kvartsetiin – lahutamisel efektiivset analüüsi-meetodit: kapillaarelektroforeesi. Lisaks on uuritud polüfenoolide ja L-askorbiinhappe antioksidatiivset aktiivsust, kusjuures vabade radikaalide allikana on kasutatud vesinikperoksiidi ( $H_2O_2$ ). Töös on kasutatud TTÜ matemaatika-loodus-teaduskonna analüütilise keemia õppetoolis konstrueeritud reaktor-kapillaarelektroforeesi süsteemi.