UNIVERSITY OF PARDUBICE

FACULTY OF CHEMICAL TECHNOLOGY

Analytical chemistry

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Analysis of antioxidants in natural matrices using liquid chromatography coupled with mass spectrometry

Theses of the Doctoral Dissertation

Pardubice 2018

Study program: **Analytical chemistry**

Study field: **Analytical chemistry**

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Year of the defence: 2018

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List of published articles related to presented dissertation

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Abstract

This dissertation thesis is focused on analysis of antioxidants in natural matrices using liquid chromatography. The catechin degradation in green tea was monitored for 6 hours of the same infusion, as well as the influence of long-term storage on green tea catechins was monitored. Further, the influence of sample preparation of elderberry (dried and frozen) on the anthocyanin content was monitored. Then the influence of sample preparation of barley on the content of free and bounded polyphenols in barley were investigated. Last but not least, the influence of heat sample preparation of eggplant on content of chlorogenic acid was studied.

Abstrakt

Tato dizertační práce se zabývá analýzou látek s antioxidačními účinky v přírodních matricích pomocí kapalinové chromatografie. Je sledována degradace katechinů v zeleném čaji po dobu 6 hodin v jednom nálevu a také vliv dlouhodobého skladovaní na obsah zdraví prospěšných látek. Dále je zkoumán vliv úpravy vzorku černého bezu (sušení a mražení) na obsah antokyanů a vliv úpravy vzorku ječmene na obsah volných a vázaných látek v ječmeni. V neposlední řadě je studován vliv tepelné úpravy lilku na obsah kyseliny chlorogenové.

Keywords

Green tea, elderberry, barley, eggplant, catechins, anthocyanins, polyphenols, liquid chromatography

Klíčová slova

Zelený čaj, černý bez, ječmen, lilek, katechiny, antokyany, fenolické látky, kapalinová chromatografie

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1. Antioxidants

The interest of food containing phenolic substances has increased significantly due to their antioxidant activity. Attention has increased mostly in finding naturally occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants that, in some cases, have been reported to be carcinogenic [1].

Antioxidants are substances that prevent oxidative damage of biomolecules even at low content. Energy imbalances and excess of nutrients can lead to the production of free radicals and, consequently, to oxidative stress. These radicals act on biologically important compounds, especially lipids, proteins and nucleic acids; they transform their structure and modify their function. Oxidative stress is associated with several diseases such as type 2 diabetes, cardiovascular disease, cancer, high blood pressure or osteoarthritis [2-4].

Phenolic compounds belong to the group of substances with antioxidant properties which are used in biological and industrial processes. They represent a large group of secondary metabolites in plants and can react with free radicals [3, 5]. Phenolic compounds contain an aromatic ring with one or more hydroxyl groups; therefore they are referred as polyphenols. In nature, phenolics occur as conjugates with mono or polysaccharides and also as functional derivatives (esters or methyl esters) [6]. Approximately 8000 polyphenols have been described and they can be divided into several subgroups, from simple molecules (phenolic acids) to polymerized compounds (tannins). The properties of phenolic compounds influence the extraction of phenolics as well as the analytical method used for determination [7].

Methods which are used for analysis of phenolics are summarized in several studies [8, 9]. The most used method is high performance liquid chromatography (HPLC). HPLC separation is generally carried out C18 stationary phase columns with a binary gradient. The mobile phase usually consists with an aqueous solution of an acid and an organic solvent (acetonitrile or methanol). Traditional HPLC is most commonly associated with a spectrophotometric detector [10] or a diode array detector [11]. However, HPLC coupled with mass spectrometry (MS) is the most advantageous method for identification of individual phenolic compounds [12-15].

2. Extraction processes used for the determination of phenolic substances

The extraction of phenolic compounds from food samples can be influenced by many factors such as the chemical structure of phenolic compounds, extraction method, the composition of the food and the time of storage. The extraction efficiency depends on the solvent polarity, time, temperature, ratio of sample and solvent and also sample properties. The sample is milled and homogenized before extraction of phenolic compounds. These samples may be fresh, frozen or dried (by air or lyophilisation) [16].

3. Current trends in liquid chromatography

Traditional liquid chromatography methods with standard sizes of column and conventional stationary phases require flood of organic solvents. For example using a traditional column with an internal diameter of 4.6 mm, a length of 25 cm and flow rate of mobile phase about 1to 1.5 ml/min produced more than 1 litter of waste per day and this waste has to be disposed of [17]. Conventional volatile organic solvents, which are used in liquid chromatography, dissolve in the environment [18]. The current trend is green chromatography, which involves reducing solvent consumption by miniaturization od device, shortening the analysis time or replacing acetonitrile or methanol in the mobile phase for less harmful alternatives [19, 20].

Many strategies have been developed to reduce solvent consumption and analysis time. These procedures usually include factors that they are optimized individually or in combination with shortening the length of column, increasing the flow rate of the mobile phase, reducing the particle size, increasing the pressure, or raising the temperature [18]. All of these factors are usually optimized step-by-step to reduce analysis time without loss of resolution in case to develop a fast LC method.

2. Catechins degradation in green tea

2.1. Introduction

Tea is consumed throughout the world and is, after water, the most popular beverage. It has been shown that a fresh tea leaf is unusually rich in the flavanoid group of polyphenols which are known as catechins [21]. The main catechins in green tea are epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG) and epicatechin (EC). Tea catechins undergo many chemical changes during the course of manufacturing and brewing processes [22].

Individual catechins undergo epimerization at high temperatures. This epimerization takes place more easily in tap water than in distilled water. The complexity of the ions in tap water and the different a pH between tap and distilled water are thought to be the main reasons for the different conversion rates of individual catechins. Stability studies of catechins in green tea strong infusion have shown that epimerization can be observed during the prolonged storage. Therefore, it is thought that, not only temperature, but also the leaching time influences the epimerisation of catechins in green tea infusion [22].

The analysis of catechins in biological matrices is typically performed using liquid chromatography coupled to a spectrophotometric detector or to a mass spectrometer (MS). The major drawback of most published HPLC methods is a long chromatographic run of about 20 – 45 min per analysis [11, 23-29]. An alternative to improve HPLC separation efficiency and speed without reducing particle size is the use of superficially porous particles, also called as core–shells [30]. These are typically composed of a 1.9 μm solid core enclosed by a 0.35 – 0.5 μm porous shell (dp = 2.6 – 2.7 μm), providing reduced band broadening and outstanding efficiency, while preserving sufficient particle size to allow acceptable operation pressure [30, 31]. For these reasons, the new technology columns have been already successfully applied to the analysis of various compounds in several foods [32-34].

This chapter focused on the study of degradation of catechins and other phenolic compounds in green tea using fast gradient HPLC/MS analysis. The degradation was studied during 6 hours of tea infusion after leaching. Further, the degradation of

compounds with health benefits in green tea in terms of longer time storage in domestic conditions (the teabags were stored in a cupboard in the original bags for 16 weeks) was monitored. The degradation of phenolic compounds was monitored approximately every 3 weeks. The results were compared with antioxidant capacity and total phenolic content measured spectrophotometrically. Multivariate data analysis was applied to classify substances in green tea.

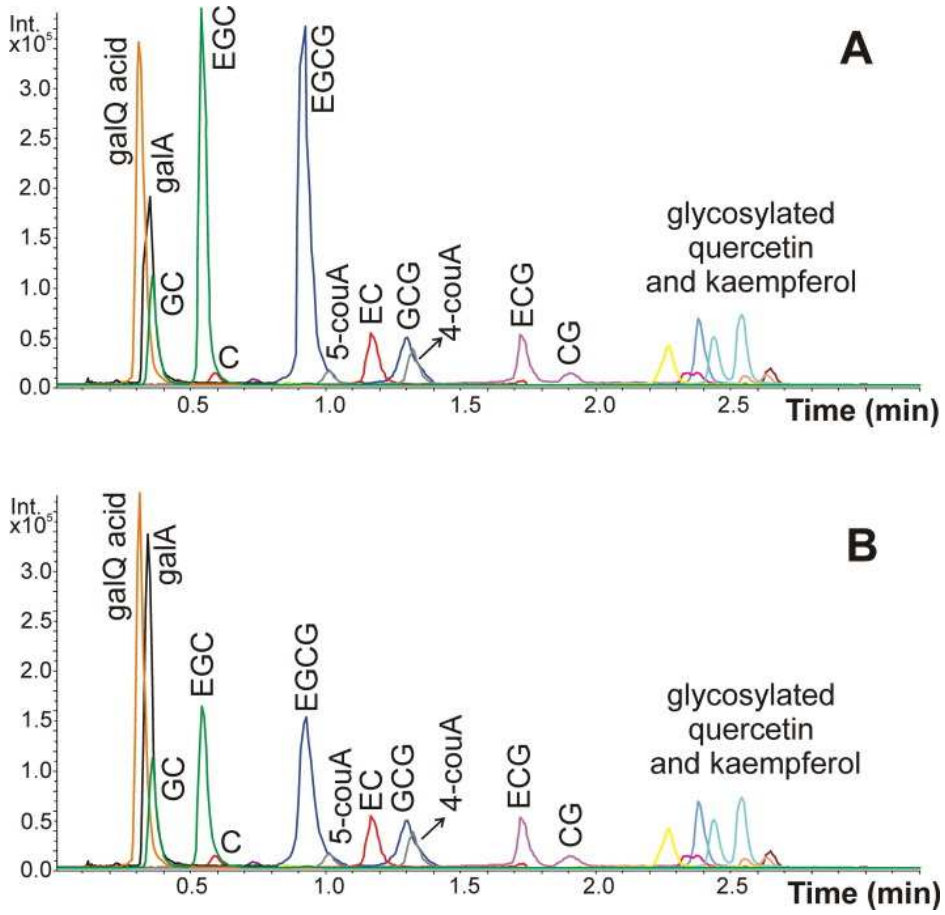

Figure 1: Optimized chromatographic separation of studied phenolic compounds (A). The degradation of the EGC and EGCG monitored after 6 hours of infusion (B), sample 5 (Dukát). Ascentis Express C18 (5 cm × 2.1 mm × 2 μm), 20 % - 70 % of methanol in water in 2.5 minutes (+ 0.1 % of formic acid in both solvents), t = 40 °C, F_m = 0.5 ml/min, injection 0.5 μl.

2.2. Monitoring of catechin degradation in green tea

Ten green tea samples were used for quantitative analysis and for monitoring of the catechins degradation and other phenolic compounds in green tea. The degradation was monitored every hour for six hours of infusion prepared by pouring 70 °C, 80 °C and 90 °C hot water over a tea bag. The representation of phenolic compounds was similar in all samples, but they are distinguished in amount of individual phenolics. The main difference is in catechin contents. The significant degrees of EGC and EGCG content including their epiforms and conversely significant increase of gallic and galloylquinic acid (galQ acid) was observed in infusions measured after 6 hours of infusion (see figure 1).

2.3. Conclusion

The fast gradient separation of green tea phenolic compounds taking less than 3 minutes was optimized in this work. The separation of catechins was carried out only in 2 minutes. This fast chromatographic separation was used to monitor degradation of catechins and other phenolics in green tea infusions over the course of their long duration. Duration of infusion for 6 hours causes the degradation of some catechins, probably to gallic acid. The influence of the storage of tea bags on catechin content was also monitored. During the few weeks of tea bag storage, the content of important catechins decreased. Together with an HPLC/MS analysis, the antioxidant capacity and total phenolic content were measured using spectrophotometric techniques.

It was found, that the samples of green tea prepared at 70 °C contain a lower concentration of health beneficial substances in comparison with the samples prepared at 90°C. Though there appears a degradation of these healthy beneficial substances over the course of six hourse, for samples prepared at a lower temperature this degradation is not so significant. The HPLC-MS method with multivariate statistical methods recognized the green teas, which were stable within the storage time and so they bring useful information for common customers.

3. Analyses of anthocyanins in elderberry

European elderberry (*Sambucus nigra L.*) is a deciduous, tree-like shrub, widespread in almost every continent of the world. It usually blooms from May to July and the berries ripen from August to late September. The creamy-white and shiny flowers that produces violet-black drupes, grow in clusters, holding hundreds of purplish-black berries. They have been used for centuries for medicinal purposes and are traditionally consumed to prevent or diminish the effects of several diseases [35-37]. Several bioactive compounds are reported on elderberries, namely phenolic compounds as anthocyanin derivatives, including cyanidin 3-glucoside, cyanidin 3-sambubioside, cyanidin 3-sambubioside-5-glucoside and cyanidin 3,5-diglucoside [38-40]; as well as triterpenic compounds such as ursolic and oleanolic acids, and sterols, as β-sitosterol, were reported as elderberry bioactive components [37, 41, 42]. The colour of anthocyanins is dependent on pH due to the existence of four different structures in aqueous solutions. The four structures have different colours and, since each of them vary in concentration throughout the pH-scale with the red flavylium cation as the dominant structure in acidic environment, the exact colour of the solution depends on the pH-value [43].

This work is focused on the evolution of new sample preparation (dried and frozen samples) and its effect on the composition of anthocyanins in elderberry fruit. The optimization of fast and simple HPLC separation of anthocyanins was managed. All results obtained by HPLC method were compared with results obtained spectrophotometrically.

3.1. Identification and quantification of anthocyanins in elderberry

Cyanidin-3,5-O-diglucoside (2) was use for quantification of cyaniding-3-Osambubiosid-5-O-glucoside (1) and both compounds were evaluated together. Also Cyanidin-3-*O*-sambubioside (3) and cyanidin-3-*O*-glucoside (4) were evaluated together; because they were not separated properly due to the pH of mobile phase. The extract of elderberry was measured three times and the content of anthocyanins was related to 1 g of sample. The results are shown in figure 2 and 3. Cyanidin-3-*O*-sambubioside (3) and cyanidin-3-*O*-glucoside (4) are major compounds in frozen

sample (F) of elderberry with 81 % (w/w) of all compounds. Cyanidin-3-*O*-sambubioside (3) and cyanidin-3-*O*-glukoside (4) in dried samples (D) are in lower content (54 %, w/w of all compounds), which is caused probably by temperature during sample preparation.

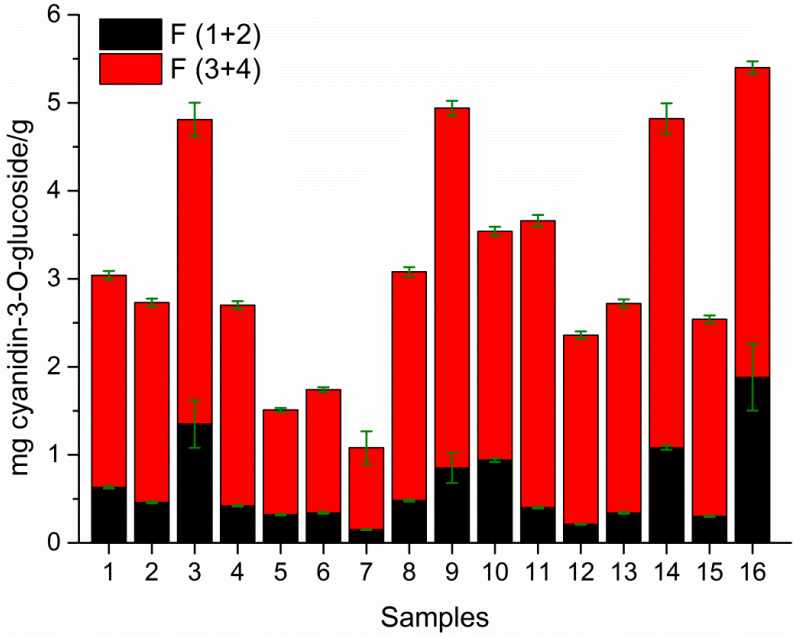

Figure 2: The content of anthocyanins in frozen samples. (1) cyanidin-3-*O*-sambubioside-5-*O*-glucoside, (2) cyanidin-3,5-*O*-diglucoside, (3) cyanidin-3-*O*-sambubioside, (4) cyanidin-3-*O*-glucoside.

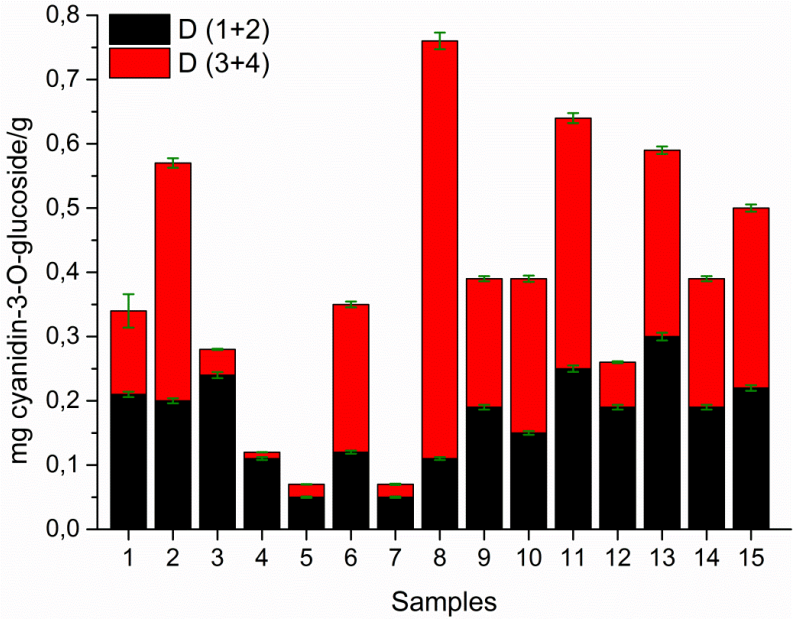

Figure 3: The content of anthocyanins in dried samples. (1) cyanidin-3-*O*-sambubioside-5-*O*-glucoside, (2) cyanidin-3,5-*O*-diglucoside, (3) cyanidin-3-*O*-sambubioside, (4) cyanidin-3-*O*-glucoside.

3.2. Conclusion

This chapter is focused on analysing of anthocyanins in elderberry using HPLC method. The optimization was performed with column Ascentis Express C18 (250 mm x 4,6 mm, 5 μm) and isocratic elution. Two major anthocyanins cyanidin-3-O-sambubioside and cyanidin-3-O-glucoside were not separated because the mobile phase with low concentration of formic acid was used for the protection of the column against low pH. The significant influence of pH of mobile phase on separation of these anthocyanins was observed, therefore the anthocyanins were not separated properly and they were evaluated together.

Spectrophotometric methods were used for determination of antioxidant capacity and total phenolic content and total anthocyanins content. The antioxidant capacity was measured by ABTS method, total phenolic content using Folin-Ciocalteauo reagent and total anthocyanins content using pH differential method. The spectrophotometric data and chromatographic data were evaluated using multivariable data analysis. The principal component analysis and factor analysis were used. All samples were divided into individual groups according to their antioxidant capacity and sample preparation (frozen and dried sample). The outliers were found and they were located out of group because of high antioxidant capacity due to the addition of ascorbic acid as preservative. The frozen sample reached higher content of anthocyanins, antioxidant capacity and total phenolic content in comparison with dried sample. The lower values of dried samples were caused by degradation of the compounds during drying process.

4. Influence of sample preparation on free and bonded polyphenols in barley (*Hordeum vulgare L.*) using HPLC/MS

4.1. Introduction

The phenolic compounds identified in a barley hull are mainly benzoic, gallic, ferulic and coumaric acids (with lower amounts of 4-hydroxybenzaldehyde, 3,4-dihydroxybenzaldehyde, vanillic acid and vanillin) [44]. Moreover, more than 50 proanthocyanidins were reported in barley, including oligomeric and polymeric flavan-3-ol, catechin (c) and gallocatechin (gc). The most abundant proanthocyanidins are dimeric proanthocyanin B3 and procyanidin B3; major trimers include T1 (gc-gc-c), T2 (gc-c-c), T3 (c-gc-c), and T4 or procyanidins C2 (c-c-c) [45].

Due to their well-known antioxidant and anticarcinogenic properties and other positive effects on human health [46-48], phenolic compounds have been thoroughly investigated for a long time, however, there is still lack of a widely applicable method for their isolation and determination. In recent years, various methods have been developed for the extraction of bioactive compounds from cereals [49] and plants in general [50]. The methods utilized ultrasound-assisted extraction [51, 52], microwave-assisted extraction [53, 54] and extraction using supercritical fluid [55]; however, solvent extraction is still among the most simple and the most common approaches used for the isolation of phenolic compounds [56].

In recent years, the researchers focused also on the application of alkaline [57, 58] or acidic [59], or both hydrolysis processes [60, 61], for recovering of bonded polyphenols. Phenolic compounds, in fact, occur in plants in a soluble form (free or conjugated to soluble carbohydrates by ester/ether bonds) or in an insoluble form (bound by ester/ether bonds to the cell wall constituents such as cutin, lignin, suberin). Insoluble polyphenols are considered as the major contributors to the total antioxidant capacity of cereals [62-64].

Several analytical methods were used for the determination of polyphenols including high performance liquid chromatography (HPLC), high speed counter current chromatography, paper chromatography, thin-layer chromatography, capillary electrophoresis and gas chromatography (GC). GC methods developed for the analysis

of polyphenols require the derivatisation step (e.g. methylation, trifluoroacetylation or silylation) volatile derivatives [56].

Based on this knowledge, the aim of current research was to develop an extraction procedure for the complete recovery of free and bonded phenolic compounds from barley samples. The target polyphenols present in barley extracts were analysed using optimized RP-HPLC-MS/MS method in multiple reaction monitoring mode. The developed methods were applied for characterization of four variety of barley harvested in two region of Czechia.

4.2. Isolation of bound polyphenols using hydrolytic processes

Using the first part of the developed procedure, only free soluble polyphenols can be recovered from barley samples. Insoluble polyphenols are considered as the major contributors to the total antioxidant capacity of cereals, so the exhaustive recovery of both forms is necessary to estimate their total and real content in food. Soluble bonded phenolic compounds or insoluble polyphenols have to be liberated from ether or ester bonds by acidic or alkaline hydrolysis, respectively, to achieve an exhaustive recovery of all phenolic compounds. The hydrolytic processes are important for precise and correct determination of the content of phenolic compounds, as demonstrated e.g. [60] on the extraction of polyphenols from millet grains. The alkaline hydrolysis is performed with addition of ascorbic acid and EDTA to prevent eventual degradation phenomena of several phenolic compounds [65]. The hydrolytic processes were carried out with solid residue and water residue after extraction of soluble phenolic compounds. Using whole extraction procedure, four extracts were acquired. Extraction of soluble phenolic compounds was carried out by 70% (v/v) acetone. This extract was subjected to further extraction step to separate the soluble compounds to free and bonded derivatives. Free soluble phenolic compounds (sample α) were extracted to ethyl acetate. Bonded soluble phenolic compounds presented in water residue were release from ester and ether bonds by alkaline (sample β) and acidic (sample γ) hydrolysis, respectively. Insoluble phenolic compounds (sample δ) in solid residue were liberated by alkaline hydrolysis.

4.3. Determination of polyfenols in barley

In the first part of the research, a rapid HPLC-MS/MS method for the determination of polyphenols in food matrices was developed. For this purpose, the mixture of 20 standards was analysed in reversed-phase system using four different columns packed with porous shell alkyl silica particles. For each column, four different gradient profiles were tested with the aim of obtaining the retention times and peak widths of each standard. The best average of resolution in the shortest time of analysis was obtained with the Ascentis Express C18 (150 mm × 3.0 mm, 2.7 μm) column. The optimized gradient was: 0 min – 12 % B, 4 min – 12 % B, 8 min – 30 % B, 12 min – 70 % B and the separation of standards is shown in figure 4.

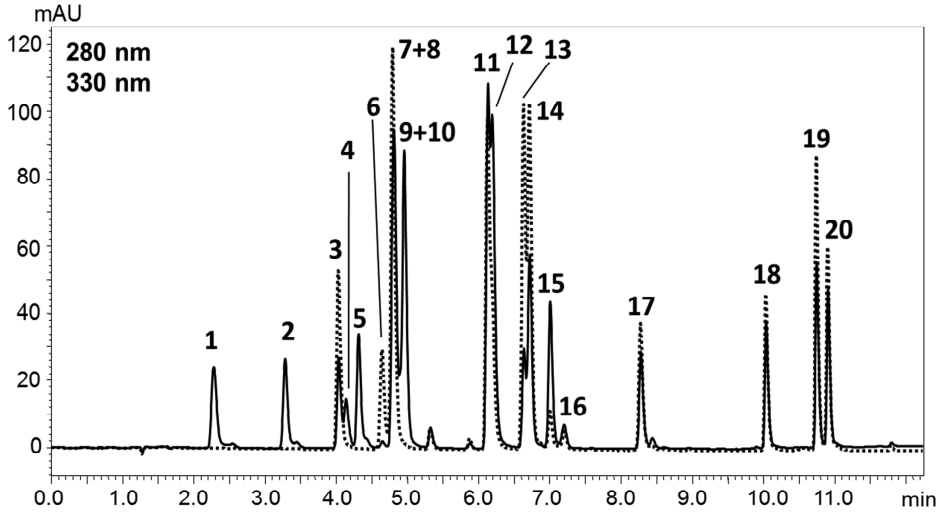

Figure 4: Separation of the mixture of standards at 280 (solid line) nm and 330 nm (dotted line). Column Ascentis Express C18 (150 x 3.0 mm, 2.7 μm); mobile phase containing solution of 0.4 % formic acid in water (A) and acetonitrile (B); gradient profile 0 min – 17 % B, 4 min – 17 % B, 12 min – 70 % B; flow rate 0.5 mL/min; temperature 30 °C. Identity of the compounds: 1. Gallic acid, 2. Protocatechuic acid, 3. Chlorogenic acid, 4. Catechin, 5. *p*-hydroxybenzoic acid, 6. Gentisic acid, 7. Vanillic acid, 8. Caffeic acid, 9. Epicatechin, 10. Syringic acid, 11. *p*-coumaric acid, 12. Vanillin, 13. Sinapic acid, 14. Ferulic acid, 15. Taxifolin, 16. Rutin, 17. Myricetin, 18. Quercetin, 19. Apigenin and 20. Kaempferol

4.4. Conclusion

The aim of this research was the optimization of an extraction procedure and a rapid LC-MS/MS method for characterization of polyphenols in barley samples.

Five different cultivars of barley were analysed and showed a very similar profile, with only some differences in the composition of flavonols, mainly in insoluble bonded form to the cell wall structures. All cultivars were rich in *p*-hydroxybenzoic and ferulic acids. The highest amount of *p*-hydroxybenzoic acid was found in almost all samples in soluble fraction after alkaline hydrolysis, while ferulic acid was revealed mainly in the solid residue after alkaline hydrolysis. Many other compounds were revealed only after the hydrolysis processes, demonstrating their need for the exhaustive extraction and the correct estimation of polyphenols content. Both the developed extraction procedure and the developed LC-MS/MS method can be considered useful strategies for the correct and rapid determination of phenolic compounds in plants and food matrices in general.

5. Influence of head sample preparation on eggplant

Eggplant (*Solanum melongena L.*) is native in Southeast Asia and it was first domesticated more than 4000 years ago. The colour, size, and shape significantly depend on the variety of eggplant [66]. World production of eggplants in 2016 was around 83.3 million tonnes, where China was main the producer with 32 million tonnes followed by India (12.6 million tonnes), Egypt (1.19 million tonnes), Turkey (0.85 million tonnes) and Iran (0.68 million tonnes), see table 1. Production of eggplant in Europe was around 0.97 million tonnes, where Italy was main producer with 0.32 million tonnes followed by Spain (0.23 million tonnes), Romania (0.11 million tonnes), Ukraine (84.2 thousand tonnes) and Greece (64.1 thousand tonnes) [67]. Eggplant is usually considered to be vegetable, but botanically it belongs to the fruit. Eggplant also belongs among the top ten vegetable due to its beneficial antioxidant properties caused by phenolic substances [68], where the major phenolic substance is chlorogenic acid [69]. But isomers of chlorogenic acid such as cryptochloroic acid (4-caffeoylchinic acid) or neochlorogenic acid (3-caffenoylchinic acid), are minor compounds in Eggplant [70, 71].

Table 1: The world production of eggplant in 2016

Continent	Production [tunnes]
Asia	4,82E+07
Africa	1,71E+06
Europe	9,71E+05
America	3,85E+05
Oceania	1,31E+03
World total	5,13E+07

Polyphenols and also chlorogenic acid have biological and pharmacological effects as antihypertensive, antimutagenic, anticarciogenic, prevention against besity and diabetes, hypolipidemic, anti-inflammatoric. They are also antioxidants, and they influence the activity of trypsin, amylase and other enzymes [72, 73]. Other studies are focused on comparison of antioxidant capacity of different eggplant varieties [66, 74]. Whitaker and Stommel in 2003 published a detailed study of the determination of phenolic acids in different eggplant varieties [69].

Heat treatment before consumption significant affects vegetable and it can influence polyphenols profile and antioxidant capacity. Conversely, in some cases, the concentration of phenolic substances may increase in comparison to raw vegetables [75, 76]. An effect of cooking techniques depends on the polarity of media where it is carried out. The effect of hydrothermal processes is negative on the content of soluble antioxidants (including phenolic substances). Conversely, a dramatic decrease of phenolic substances is not caused in non-polar media which are used during frying process [77]. However, decrease of phenolic substances during cooking is also caused by the ratio of water and vegetables, cooking time and surface size [78].

Analysis of phenolics in eggplant is performed using liquid chromatography with C18 reversed-phase column and gradient elution of methanol or acetonitrile. Detection of chlorogenic acid is performed using spectrophotometric detector at a wavelength in the range of 210-400 nm or a mass spectrometry [69].

This study is focused on cooking techniques of eggplant and its effect to content of chlorogenic acid.

5.1. Determination of cholorogenic acid in eggplant

The samples of eggplant were purchased from local shops in Pardubice and one sample was obtained from private grower and two samples were obtained from Italy (Modena) and Sicily (Messina). The extraction of chlorogenic acid was optimized using Box, Hunter & Hunter (2^{k-p}) plan. The optimized parameters were: solvent (methanol, acetone), concentration of solvent, concentration of HCOOH. The extraction was performed with 40 ml of solvent in 3 minutes.

The optimization of separation was performed with C18 Ascentis Express C18 (150 mm × 3.0 mm, 2.7 μm) and optimized parameters were: mobile phase, concentration of HCOOH, gradient profile and temperature on column. The optimized separation is shown in figure 5.

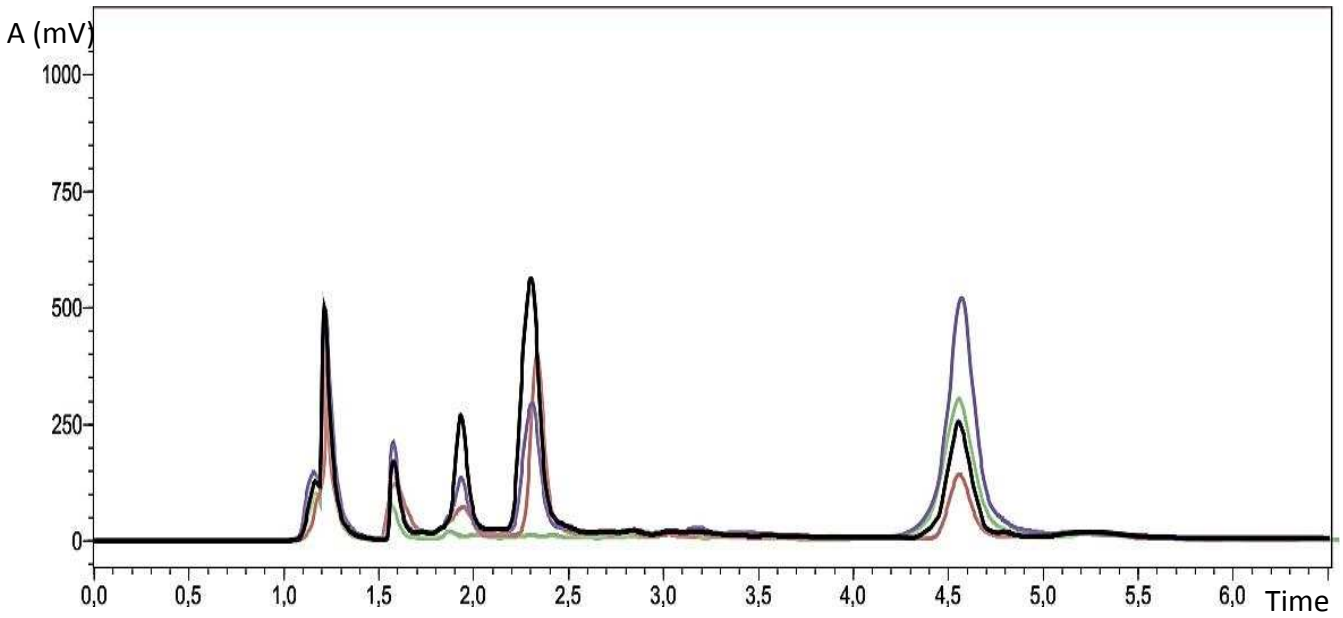

Figure 5: Optimized separation of eggplant after heat treatment: **green**: fresh, **black**: fried, **red**: baked, **purple**: grilled

5.2. Influence of sample preparation of eggplant

The major compound such as chlorogenic acid and its derivatives in eggplant were found. The heat treatment (frying, baking and grilling) caused changes in the content of chlorogenic acid and formation of other polar compounds with retention times: 1.6 min (λ_{max} = 225 nm, 266 nm), 1.91min (λ_{max} = 290 nm) and 2.35min (λ_{max} = 280 nm). Unfortunately, these compounds were not identified because they were not ionized by MS analysis.

The highest amount of chlorogenic acid was obtained in grilled and fried samples and the lowest amount of chlorogenic acid was obtained in fresh eggplant. Table 2 shows the content of chlorogenic acid in all eggplant samples. The significant increase (99 %, w/w) of chlorogenic acid was found in grilled eggplant from Sicily in comparison with raw sample. The highest content of chlorogenic acid in fresh sample was determined in eggplant from Spain which was 6 times more than the other samples (figure 6).

Table 2: The content of chlorogenic acid (mg/g in raw) in sample of eggplant after head treatment

Sample	Czechia	Nederland	Italy	Spain	Sicily
Raw	0.299 ± 0.058	0.536 ± 0.021	0.250 ± 0.0814	1.855 ± 0.230	0.094 ± 0.070
Baked	0.341 ± 0.067	0.659 ± 0.056	0.500 ± 0.090	3.003 ± 0.128	1.037 ± 0.100
Fried	5.187 ± 0.250	1.740 ± 0.180	4.025 ± 0.390	6.726 ± 0.100	10.112 ± 0.130
Grilled	5.226 ± 0.325	3.089 ± 0.095	3.084 ± 0.071	3.740 ± 0.270	10.908 ± 0.150

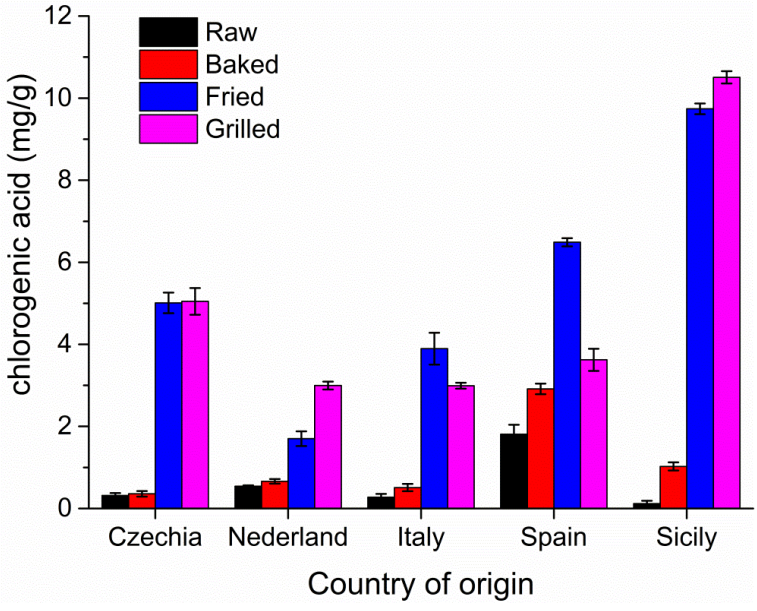

Figure 6: The content of chlorogenic acid divided according to the country of origin

5.3. Conclusion

A simple and fast extraction and HPLC analysis of eggplants were optimized. The influence of heat treatment the composition of phenolic substances in eggplant was monitored. HPLC data was evaluated using multidimensional statistical analysis. The discriminant analysis divided the all eggplant samples according to the heat treatment (frying, grilling and baking) and subsequently to the country of origin. The influence of storage to content of chlorogenic acid was observed. The significant decrease of chlorogenic acid in fresh samples (5 times) and significant increase of chlorogenic acid in grilled sample (91 %, w/w) within 4 weeks were observed. The grilling of eggplant can be recommended for heat treatment due to the enrichment of antioxidants (chlorogenic acid).

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7. List of Students' Published Works

Presentations

1. P. Šilarová, D. Havlová, L. Česlová, Optimalizace HPLC/MS separace pro studium degradace katechinů, Monitorování cizorodých látek v životním prostředí, Ovčárna pod Pradědem, 8.-10. duben 2015, ISBN 978-80-7395-926-5. Plnotextový sborník: ISBN 978-80-7395-926-5
2. P. Šilarová, D. Havlová, L. Česlová, Study of Catechin Degradation in green tea by HPLC/MS, Studentská konference Chemie je Život, VUT Brno, 3.12. 2015, sborník: ISBN: 978-80-214-5290-6
3. P. Hofmeister, P. Šilarová, L. Česlová, Stanovení fenolických látek s antioxidačními vlastnostmi v obilovinách. Studentská vědecká odborná činnost 2015/2016 (SVOČ-FCHT), sborník příspěvků str. 57-62, Pardubice 14.6. 2016. ISBN 978-80-7560-004-2

Posters

1. P. Šilarová, M. Adam, Analýza aromaprofilu bylinných čajů metodou HS-SPME/GC-MS s využitím více sorpčních teplot v jednom extrakčním kroku, Česká konference hmotnostní spektrometrie, Hradec Králové,15.-17. duben 2015
2. P. Šilarová, D. Havlová, L. Česlová, Study of Catechin Degradation in Green Tea by Fast Gradient HPLC/MS, 7th International Symposium on Recent Advances in Food Analysis, Book of Abstract p. 186, Prague, Czech Republic, 3.-6. 11. 2015
3. P. Šilarová, V. Brighenti, L. Boulekbache-Makhlouf, L. Česlová, F. Pellati, Study of termal processing of eggplant using liquid chromatography coupled mass spectrometry, 31st International symposium on chromatography, Cork, Ireland, 28.8-1.9.2016
4. L. Česlová, P. Šilarová, R. Mazáčová, A. Arigo, Influence of Malting on Distribution of Free and Bonded Barley Phenolic Compounds, 44th International Symposium on High Performance Liquid Phase Separations and Related

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5. L. Česlová, P. Hofmeister, P. Šilarová, Determination of phenolic compounds in cereals using HPLC/MS, 45th International Symposium on High Performance Liquid Phase Separations and Related Techniques (HPLC 2017), APP11-P30-We, str. 281., Prague, Czech Republic, 18.-22.6. 2017
6. P. Šilarová, L. Dubnová, L. Česlová. Determination of anthocyanins in elderberry fruit. ISSS 2017 23rd International Symposium on Separation Science. 19-22.9.2017 Vídeň, Univerzity of Technology, Rakousko, str. 228, ISBN: 978-3-9504017-7-6