

## Evaluation of phenolic compound composition of *Sambucus nigra* berries grown in Latvia

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**Abstract.** Phenolic compounds in agricultural raw materials can vary within a species and can be affected by a combination of such factors as growing region, weather conditions and fruit ripeness. Climatic differences between the southern, central and northern parts of Europe can cause differences in the phenolic compounds present in the plant. The research was aimed to investigate the phenolic compounds of berries of *Sambucus nigra* varieties grown for commercial production in Latvia. During the study four elderberry varieties were analysed - ‘Haidegg 17’, ‘Korsör’, ‘Haschberg’ and ‘Emma’. All samples were analysed for total phenolic compound content (TPC), DPPH free radical scavenging activity and individual phenolic compounds. In addition, sample pH and total soluble solids (TSS) were measured. Results showed that ‘Emma’ berry samples had the highest value of TSS content - 10.5% (°Bx) and the lowest pH value - 3.65. The variety ‘Korsör’ showed the lowest TSS content - 8.1% (°Bx) as well as the lowest TPC and DPPH free radical scavenging activity, the variety ‘Haschberg’ showed the highest pH value. The variety ‘Haidegg 17’ stood out with a high TPC. In total six phenolic compounds were identified and quantified in the analysed samples - gallic acid, catechin, chlorogenic acid, p-coumaric acid, sinapic acid and 3,5-dihydroxybenzoic acid. According to the obtained results, it was evident that the indicators for some parameters differ from the information available in the literature about the composition of berries of crops grown in other regions. This suggests that it is worth further researching elder tree varieties grown in northern climate.

**Keywords:** DPPH, elderberry, HPLC-PDA, polyphenols, soluble solids.

### INTRODUCTION

The elder tree is considered a promising crop. It is predicted that the demand for elderberries will have an increasing trend in the period from 2021–2025 due to increasing awareness of their health benefits and an expansion in elderberry food products development and introduction into the market (Elderberry, 2023).

*Sambucus nigra* plants are widely distributed and known throughout Europe. In the wild, it can be found in open fields and forest edges. Plants can form very wide stands. The tree or shrub can reach a height of 4–12 meters. Its bark is light brown as if cracked and covered with fine warts, and the core of the trunk is cork-like (Atkinson & Atkinson, 2002; Stępień et al., 2023).

In general, there are many elderberry varieties, grown in different areas of the world. For this research, the varieties selected for analysis, including 'Haidegg 17', 'Korsör', 'Haschberg', and 'Emma', were chosen due to their high productivity and winter hardiness in the climatic conditions of Latvia, which is crucial for their suitability in commercial gardens in the northern region of Europe. 'Emma' is an open pollination seedling which has been selected from the wild population of the *Sambucus nigra* growing in Latvia. It has been observed that 'Emma' has relatively high winter hardiness, which indicates the suitability of this variety in the commercial gardens of the northern region of Europe. In addition, the berries of this variety have not been studied before. 'Emma' variety shares some characteristics with 'Korsör', a cultivar originating in Denmark. 'Korsör' is commercially grown throughout Europe and is recognized for its exceptional winter hardiness, capable of withstanding temperatures well below 0°F, and is characterized by its vigorous growth (Elderberry West Virginia University, 2022).

The 'Haidegg 17' variety is a less common Austrian variety with long and robust growing stems. Forms large umbels of flowers that bloom in late spring (Csorba et al., 2018).

The 'Haschberg' variety is one of the most popular and widely grown black elderberry varieties in Europe. However, it is important to expand the range of existing varieties to reduce susceptibility to diseases (Csorba et al., 2020), to create the most suitable varieties for the climatic conditions of each region, as well as to extend the flowering and fruiting period of the plant.

The elder tree is known as a plant with wide medicinal properties. *Sambucus nigra* fruit and flower extracts have antiviral and anti-inflammatory properties (Boroduške et al., 2022). The presence of flavonoids like quercetin and rutin, as well as phenolic acids such as caffeic acid and chlorogenic acid in elderberries, and their contribution to the health benefits are discussed in research by Boroduške et al. (2022). These compounds are known for their anti-inflammatory and antiviral effects and antioxidant properties. Some research suggests beneficial effect in the prevention of severe diseases such as cancer due to *Sambucus nigra* berries being a rich source of phenolic compounds which have been shown to have strong antioxidant activity in *in vitro* studies (Stępień et al., 2023). Berry extracts have also been shown to be potent inhibitors of  $\alpha$ -amylase and  $\alpha$ -glucosidase, so they would be useful for lowering blood glucose levels. This effect is attributed to the direct interaction of specific compounds, such as proanthocyanidins, with the enzymes and the synergistic interactions with inhibitors (Terzi et al., 2023). Berries are a rich source of anthocyanins and other polyphenols, which are used industrially as a source of food colorants (Banach et al., 2021) and bioactive substances (Ferreira et al., 2019). Extracts obtained from *Sambucus nigra* can be used as a source of biologically active compounds for the development of new biological preparations, including pharmaceuticals and functional foods (Ferreira-Santos et al., 2022; Terzi et al., 2023).

In a study by Česlová et al. (2023) on the effect of sample pretreatment on anthocyanin content in Czech wild black elderberry berries, it was found that the anthocyanin content in berries is influenced by geographical aspects such as the altitude of the plant's growth region above sea level.

Several research articles are available on the study of European black elderberry (*Sambucus nigra*) fruits growing in the central and southern regions of Europe, however, so far, no extensive research has been conducted on the chemical parameters of the fruits

of black elderberry varieties growing in northern Europe - Latvia. The aim of the research was to investigate the phenolic compounds of berries of *Sambucus nigra* varieties grown for commercial production in Latvia.

## **MATERIALS AND METHODS**

The research was carried out in the facilities of the Institute of Food of Latvia University of Life Sciences and Technologies. Tested plant material was obtained in September of 2023 and the analyses of samples were done from December of 2023 to the end of January of 2024.

For the purpose of this research four black elderberry varieties were analysed: 'Haidegg 17', 'Korsör', 'Haschberg' and 'Emma'.

Plant materials were collected on 13<sup>th</sup> September 2023 in Rancēni parish Valmiera county, Latvia. Berry samples were picked with the stalks and each berry variety was individually packed in PP (polypropylene) freezable bags, frozen ( $-20 \pm 2$  °C) immediately after collection and stored till further testing. Before the analysis, the berries were thawed at room temperature for one hour, separated from the stems and crushed.

### **Preparation of extracts for determination of individual phenolic compounds, total phenolic content and antiradical activity**

1 g of sample is added to 40 mL ethanol-water mixture (80:20). The resulting solution is treated in an ultrasonic bath device Sonorex digitec DT 100H (BANDELIN electronic GmbH & Co, Germany) for 15 min. at a temperature of 60 °C. Device processing settings - 35 kHz. In a 50 mL measuring flask, the solution is filtered through filter paper and an ethanol-water mixture (80:20) is added up to the mark (Tomsone, 2015).

### **Total phenolic compound content (TPC)**

Total phenolic content was determined by a modified Folin-Ciocalteu reagent method (Singleton et al., 1999) with some modifications described by Ozola & Kampuse, (2017): 2.5 mL of Folin-Ciocalteu reagent, diluted tenfold with distilled water, was added to 0.5 mL of the prepared sample. After 5 minutes, 2 mL of 7.5% Na<sub>2</sub>CO<sub>3</sub> was added, mixed and kept for 30 minutes. The result was determined with a spectrophotometer Jenway 6300, ('Baroworld Scientifid', Great Britain) at a wavelength of 765 nm.

The total phenolic compound content in the analysed samples was expressed in fresh weight (FW) as milligrams of Gallic acid equivalent per 100 grams of sample (mg GAE 100 g<sup>-1</sup>).

Measurements of each berry variety were performed in six replicates.

### **DPPH free radical scavenging activity**

The antiradical activity was determined by 2,2 diphenyl-1-picrylhydrazyl-(DPPH) reagent method (Yu et al., 2003).

3.5 mL of freshly prepared DPPH ethanol solution is added to 0.5 mL of the prepared extract, mixed and kept in the dark for 30 minutes. Absorbance is read in a Jenway 6300 spectrophotometer (Baroworld Scientifid, Great Britain) at a wavelength of 517 nm (Tomsone et al., 2013).

The antiradical activity in the analyzed samples were expressed in FW as milligrams of Trolox equivalent per 1 g<sup>-1</sup> sample (mg TE g<sup>-1</sup>).

Measurements of each berry variety were performed in six replicates.

### **Individual phenolic compounds**

High performance liquid chromatography was used for the determination and measurement of individual phenolic compounds. Shimadzu LC-40 Nexera liquid chromatograph (Shimadzu Corporation, Japan) equipped with the Shimadzu Photodiode Array Detector SPD-M40 (Shimadzu Corporation, Japan) was used for triplicate sample analysis. For individual phenolic compound detection analytical column PerkinElmer C18 (4.6 mm × 250 mm I.D., particle size 5 µm) was used. The analysis was performed under gradient conditions as described previously (Keke & Cinkmanis, 2022). To determine individual phenolic compounds in the analysed samples, their retention times were compared to the retention times of the standards.

Preparation of standard solution: The mixture of 0.0068 ± 0.0001 g gallic acid, 0.0012 ± 0.0001 g catechin, 0.0131 ± 0.0001 g chlorogenic acid, 0.0121 ± 0.0001 g p-coumaric acid, 0.0881 ± 0.0001 g sinapic acid, 0.0074 ± 0.0001 g 3,5-dihydroxybenzoic acid, 0.0128 ± 0.0001 g 4-hydroxybenzoic acid, 0.00121 ± 0.0001 g homovanillic acid, 0.0145 ± 0.0001 g vanillic acid, 0.0138 ± 0.0001 g caffeic acid, 0.0188 ± 0.0001 g syringic acid, 0.0160 ± 0.0001 g epicatechin, 0.0098 ± 0.0001 g vanillin, 0.0092 ± 0.0001 g ferulic acid, 0.0112 ± 0.0001 g 2-hydroxycinnamic acid, 0.0061 ± 0.0001 g rutin, 0.0043 ± 0.0001 g quercetin, 0.0096 ± 0.0001 g kaempferol, 0.0091 ± 0.0001 g luteolin were weighted in 100 mL volumetric flask with a narrow neck, slowly dissolved in small portion of methanol and filled with methanol till the mark and mixed. The standards used for the analysis were HPLC grade and purchased from Fluka and Sigma-Aldrich.

### **Total soluble solids (TSS)**

Total soluble solids content was determined using a digital refractometer DR301-95 (A.KRÜSS Optronic GmbH, Germany). Analysis determination standard ISO 2173:2003. Results are expressed as percent soluble solids (°Bx). Sample measurements were performed in triplicate

### **pH**

The pH of the samples was determined using a Milwaukee MW102-FOOD digital pH meter (Milwaukee Electronics Kft., Hungary). Analysis determination standard ISO 5542:2010. Sample measurements were performed in triplicate.

### **Statistical analysis**

The standard deviation as well as the mean value was obtained from repeated measurements of the samples. One-way analysis of variance (*ANOVA*) was used to analyse these data. The obtained p-value shows whether there is a significant difference between the samples (significant at  $p < 0.05$ ). Significance of differences was determined using *Tukey's HSD test*.

LibreOffice Calc 7.1.6.2 for Linux program was used for data processing.

## RESULTS AND DISCUSSION

### Total soluble solids (TSS) and pH

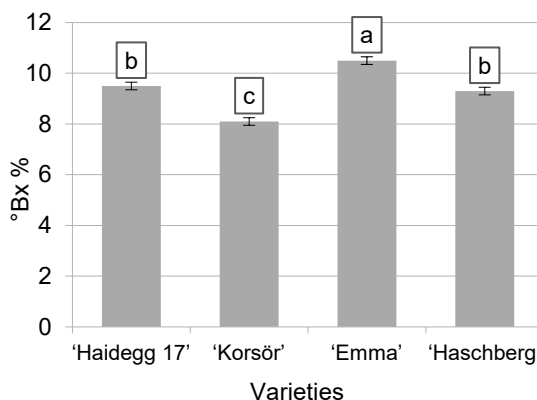
TSS, together with pH, are among the most important parameters characterizing the taste of a product. In addition, the pH value is essential for the stability of anthocyanins, as their stability is higher in an acidic environment (Skrede & Wrolstad, 2016).

The TSS composition are presented in Fig. 1. and pH measurements are presented in Fig. 2. The highest TSS content and the lowest pH value was for the 'Emma' variety -  $10.5\% \pm 0.15$  ( $^{\circ}\text{Bx}$ ) and  $3.65 \pm 0.01$  respectively. 'Korsör' variety had the lowest TSS content -  $8.1\% \pm 0.17$  ( $^{\circ}\text{Bx}$ ), while 'Haschberg' variety had the highest pH -  $3.93 \pm 0.02$ .

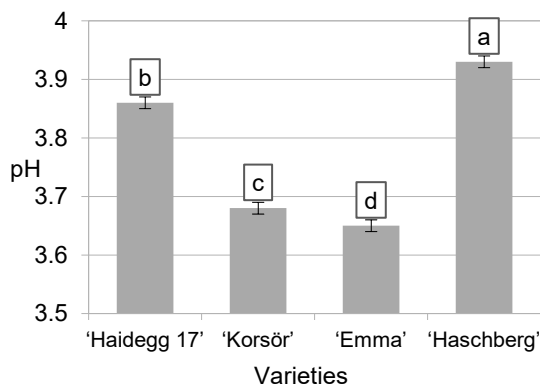
In Kolarov et al. (2021) study was reported that pH level of Serbian wild *Sambucus nigra* berries was  $3.84 \pm 0.03$ . The results are similar to those obtained in this study.

TSS results obtained in this study differ from those in the literature. Ferreira et al. (2019) studied *Sambucus nigra* berries harvested in Portugal, TSS content depending on the variety and harvest year fluctuated within limits 13.3–18.1% ( $^{\circ}\text{Bx}$ ). However Csorba et al. (2020) reported that berries harvested in Hungary ranged in TSS from 10.8–14.6% ( $^{\circ}\text{Bx}$ ).

TSS content of berries is influenced by various factors, including genotype (cultivar), maturation stage, environmental conditions, and harvesting year. These factors can affect the TSS content in different ways, leading to variations in the sweetness, flavour, and overall quality of the berries (Ferreira et al., 2019; Ferreira et al., 2022; Pedrosa Costa et al., 2021). Pedrosa Costa et al., (2021) reported that climatic conditions, especially water status, strongly impact elderberry chemical



**Figure 1.** Total soluble solids of different black elderberry varieties. Results are expressed as mean value  $\pm$  standard deviation ( $n = 3$ ). Significant differences among elderberry varieties are marked with different letters ( $p < 0.05$ , Tukey HSD test).



**Figure 2.** pH of different black elderberry varieties. Results are expressed as mean value  $\pm$  standard deviation ( $n = 3$ ). Significant differences among elderberry varieties are marked with different letters ( $p < 0.05$ , Tukey HSD test).

composition, including TSS content. High temperatures and sufficient sunlight can enhance sugar accumulation in berries, increasing their TSS content. Conversely, excessive rainfall or lack of sunlight can lower TSS levels. Ferreira et al. (2022) observed significant differences in TSS content among different elderberry cultivars. The maturation stage of the berries significantly influences the TSS content of elderberries. As berries mature, their TSS content generally increases. This is due to the accumulation of sugars as the fruit develops. Ferreira et al. (2022) noted a significant increase in TSS content with advancing maturation stages for elderberries harvested in different years.

Since Latvia is situated at a higher latitude than Portugal and Hungary, the differences in sunlight exposure and temperature due to latitude could result in variations in the TSS of elderberries as Latvia receives less sunlight and experiences colder summers (Climate Change Knowledge Portal, 2021), which may lead to elderberries with lower TSS values compared to those grown in the warmer and sunnier climates of Portugal and Hungary.

### Total phenolic compound content (TPC)

TPC in the analyzed black elderberry varieties are presented in Table 1.

The highest content of TPC of fresh weight (FW) was observed in ‘Haidegg 17’ variety -  $1,344.7 \pm 33.7$  mg GAE 100 g<sup>-1</sup> FW, while the lowest content was in the berries of the variety ‘Korsör’ -  $982.3 \pm 25.4$  mg GAE 100 g<sup>-1</sup> FW. The difference between TPC of ‘Haidegg 17’ and ‘Korsör’ is 27%. There was no significant difference ( $p = 0.10$ ) between the samples of ‘Haschberg’ and ‘Emma’, TPC measurements were -  $1,293.2 \pm 14.0$  mg GAE 100 g<sup>-1</sup> FW and  $1,258.9 \pm 18.1$  mg GAE 100 g<sup>-1</sup> FW respectively.

The results obtained in this study are similar to those reported by Ferreira et al. (2019), where TPC was 820–1,476 mg GAE 100 g<sup>-1</sup> FW. However, Csorba et al. (2020) reported higher values and wider variation in TPC between analysed cultivars 852.6–2,541.5 mg GAE 100 g<sup>-1</sup> FW. Interestingly Csorba et al. (2020) also reported the lowest TPC for the variety ‘Haidegg 17’ -  $852.6 \pm 93.8$  mg GAE 100 g<sup>-1</sup> FW but for variety ‘Korsör’ results was  $1,894.8 \pm 464.7$  mg GAE 100 g<sup>-1</sup> FW, which is contrary to the results obtained in this study. Ferreira et al. (2022) report on the effect of variety, ripening stage, and year on the sugar and phenolic composition of elderberry indicates that different varieties of the same fruit species can have different biochemical compositions, including differences in phenolic compounds due to genetic differences and their interaction with environmental conditions and agricultural practices.

Similar to TSS, environmental factors have a major effect on polyphenol content. Even environmental stress affects polyphenols (Kondakova et al., 2009). Plants often produce more phenolic compounds in response to environmental stress, such as drought or extreme temperatures (Skrovankova et al., 2015). Also exposure to sunlight and UV radiation can impact the phenolic content in berries. Brandt et al. (2019) reported that exclusion of solar

**Table 1.** Total phenolic compounds in different varieties of *Sambucus nigra* fruit

<i>Sambucus nigra</i> varietie	TPC mg GAE 100 g <sup>-1</sup> (FW)
‘Korsör’	$982.3 \pm 24.5^c$
‘Emma’	$1,258.9 \pm 18.1^b$
‘Haschberg’	$1,293.2 \pm 14.0^b$
‘Haidegg 17’	$1,344.7 \pm 33.7^a$

Results are expressed as mean value  $\pm$  standard deviation ( $n = 6$ ). Significant differences among elderberry varieties are marked with different letters ( $p < 0.05$ , Tukey HSD test).

UV radiation decreased the flavonol concentration in grape berries. This suggests that UV radiation plays a significant role in the synthesis of certain phenolic compounds, such as flavonols.

### Individual phenolic compounds

In this study, gallic acid, catechin, chlorogenic acid, p-coumaric acid, sinapic acid and 3,5-dihydroxybenzoic acid were identified and quantified. Variety ‘Emma’ showed a higher content of catechin -  $4.97 \pm 0.22 \text{ mg g}^{-1}$ , ‘Haidegg 17’ variety had a higher content of chlorogenic acid -  $100.67 \pm 2.96 \text{ mg g}^{-1}$ , p-Coumaric acid -  $41.95 \pm 2.97 \text{ mg g}^{-1}$  and sinapic acid -  $0.99 \pm 0.09 \text{ mg g}^{-1}$ , but the highest content of 3,5-dihydroxybenzoic acid was in the berries of the ‘Haschberg’ variety -  $3.89 \pm 0.12 \text{ mg g}^{-1}$ .

**Table 2.** Phenolic compounds in different varieties of *Sambucus nigra* fruit

Phenolic compound mg g <sup>-1</sup> (FW)	‘Korsör’	‘Emma’	‘Haschberg’	‘Haidegg 17’
Gallic acid	$0.07 \pm 0.02^b$	$0.16 \pm 0.01^a$	$0.13 \pm 0.02^a$	$0.13 \pm 0.02^a$
3,5-dihydroxobenzoic acid	$2.99 \pm 0.26^b$	$2.71 \pm 0.15^b$	$3.89 \pm 0.12^a$	$1.84 \pm 0.19^c$
Catechin	$2.67 \pm 0.09^c$	$4.97 \pm 0.22^a$	$2.92 \pm 0.15^c$	$4.00 \pm 0.14^b$
Chlorogenic acid	$61.52 \pm 3.94^b$	$66.65 \pm 2.83^b$	$64.19 \pm 4.64^b$	$100.67 \pm 2.96^a$
p-Coumaric acid	$17.34 \pm 1.26^c$	$19.95 \pm 1.10^{bc}$	$23.83 \pm 1.77^b$	$41.95 \pm 2.97^a$
Sinapic acid	$0.81 \pm 0.05^b$	$0.71 \pm 0.03^b$	$0.73 \pm 0.06^b$	$0.99 \pm 0.09^a$

Results are expressed as mean value  $\pm$  standard deviation ( $n = 3$ ). Significant differences among elderberry varieties are marked with different letters ( $p < 0.05$ , Tukey HSD test).

Przybylska-Balcerek et al. (2021) in the study of *Sambucus nigra* extracts reported Gallic acid in the range  $0.34\text{--}8.32 \text{ mg g}^{-1}$ . They found sinapic acid content to be higher, ranging from  $18.45\text{--}164.75 \text{ mg g}^{-1}$ . However, the content of chlorogenic acids content are consistent with that observed in this study and falls within the reported range  $25.50\text{--}254.07 \text{ mg g}^{-1}$ . While lower amounts of chlorogenic acid is reported by Ochmian et al. (2009) -  $0.15 \text{ mg g}^{-1}$ . In this study higher p-Coumaric acid results were observed compared to Przybylska-Balcerek et al. (2021) where the reported range is  $0.16\text{--}1.21 \text{ mg g}^{-1}$ .

Chlorogenic acid was the major individual phenolic compound among all black elder varieties ( $61.52\text{--}100.67 \text{ mg g}^{-1}$ ), followed by p-coumaric acid ( $7.34\text{--}41.95 \text{ mg g}^{-1}$ ). Chlorogenic acid has many health-promoting abilities, including anti-cancer properties (Gil & Wianowska, 2017). Both chlorogenic acid and p-coumaric acid possess antioxidant properties (Skrede & Wrolstad, 2016). In a study on the influence of light quality, photoperiod, CO<sub>2</sub> concentration and air temperature on the accumulation of chlorogenic acid and rutin in young lettuce plants, it was reported that the concentration of chlorogenic acid in the plant increases under unfavourable growing conditions (Naoya et al., 2022). Mudge et al., 2016 in a study on elderberry, concluded that the variability of chlorogenic acid profiles in berries is complex and likely influenced by multiple factors, including genetic and environmentally mediated variation.

### DPPH free radical scavenging activity

DPPH in the analyzed black elderberry varieties are presented in Table 3.

DPPH measurements were similar ( $p < 0.05$ ) between varieties 'Haidegg 17', 'Haschberg' and 'Emma' which were  $95.59 \pm 2.0$  mg TE g<sup>-1</sup> FW;  $98.38 \pm 1.0$  mg TE g<sup>-1</sup> FW and  $94.82 \pm 3.6$  mg TE g<sup>-1</sup> FW respectively. Significantly ( $p > 0.05$ ) lower DPPH measurement was for variety 'Korsör' -  $84.93 \pm 3.6$  mg TE g<sup>-1</sup> FW. However, Kolarov et al. (2021), in a study on the antioxidant capacity of wild blueberries, elderberries and strawberries, reported DPPH measurements of  $125.03 \pm 9.6$  mg TE g<sup>-1</sup> FW in elderberries grown in the Carpathian region of Serbia. The measurements from Kolarov et al. (2021) study is higher than those obtained in this study.

**Table 3.** DPPH free radical scavenging activity in different varieties of *Sambucus nigra* fruit

<i>Sambucus nigra</i> varietie	DPPH mg TE g <sup>-1</sup> (FW)
'Korsör'	$84.93 \pm 3.6^b$
'Emma'	$94.82 \pm 3.6^a$
'Haschberg'	$98.38 \pm 1.0^a$
'Haidegg 17'	$95.59 \pm 2.0^a$

Results are expressed as mean value  $\pm$  standard deviation ( $n = 6$ ). Significant differences among elderberry varieties are marked with different letters ( $p < 0.05$ , Tukey HSD test).

## CONCLUSIONS

The results obtained in this study show lower DPPH free radical scavenging activity and TSS compared to black elderberries grown in the southern and eastern regions of Europe.

The results indicated that the 'Emma' variety had the most intense taste characteristics, such as acidity and sweetness. Compared to the other varieties, it had the highest TSS (10.5% °Bx) and the lowest pH (3.65) content, which could indicate a higher amount of sugars and acids.

The 'Korsör' variety showed the lowest TPC content and the lowest DPPH free radical scavenging activity. In addition, the TPC content of the 'Korsör' variety grown in Latvia is lower than that of the same variety grown in Hungary. This indicates that the growth region of the plant affects the TPC content of the berries. In the climatic conditions of Latvia, the variety 'Haidegg 17' showed the highest TPC content ( $1,344.7 \pm 33.7$  mg GAE 100g<sup>-1</sup> FW) compared to the other analysed varieties.

Differences in the content of identified individual phenolic compounds were observed between the analysed varieties. In general, the variety with the highest content of identified different individual phenolic compounds was 'Haidegg 17', which had the highest content of chlorogenic acid -  $100.67 \pm 2.96$  mg g<sup>-1</sup>, p-Coumaric acid -  $41.95 \pm 2.97$  mg g<sup>-1</sup> and sinapic acid -  $0.99 \pm 0.09$  mg g<sup>-1</sup>. Chlorogenic acid was observed to be predominant among the individual phenols.

The results obtained in the study show that it is worth investigating other biologically active compounds in black elderberries grown in Latvia, as their values may also differ from elderberries grown in other regions.

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