



## European elderberry (*Sambucus nigra* L.) rich in sugars, organic acids, anthocyanins and selected polyphenols

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### ABSTRACT

Sugars and organic acids in the fruit of two cultivars and three selections of black elderberry (*Sambucus nigra* L.): 'Haschberg', 'Rubini', 'Selection 13', 'Selection 14' and 'Selection 25' were quantified. The anthocyanin as well as quercetin profiles of this plant material were also established by the use of HPLC/MS. Significant differences in the concentration of sugars and organic acids were detected between the widely spread cultivar 'Haschberg' and all other cultivars/selections; 'Haschberg' was the richest in organic acids (6.38 g kg<sup>-1</sup> FW), and it contained the least sugar (68.5 g kg<sup>-1</sup> FW). The following major cyanidin based anthocyanins were identified in the fruit of black elderberry: cyanidin 3-sambubioside-5-glucoside, cyanidin 3,5-diglucoside, cyanidin 3-sambubioside, cyanidin 3-glucoside and cyanidin 3-rutinoside. The most abundant anthocyanin in elderberry fruit was cyanidin 3-sambubioside, which accounted for more than half of all anthocyanins identified in the berries. The 'Rubini' cultivar had the highest amount of the anthocyanins identified (1265 mg/100 g FW) and the lowest amount was measured in berries of the 'Selection 14' (603 mg/100 g FW). The 'Haschberg' cultivar contained a relatively low amount of anthocyanins in ripe berries (737 mg/100 g FW). From the quercetin group, quercetin, quercetin 3-rutinoside and quercetin 3-glucoside were identified; the latter prevailing in black elderberry fruit. The cultivar with the highest amount of total quercetins was 'Selection 25' (73.4 mg/100 g FW), while the 'Haschberg' cultivar contained average amounts of quercetins (61.3 mg/100 g FW). The chemical composition of the 'Haschberg' cultivar, the most commonly planted, conforms to the standards for sugars, anthocyanins and quercetins and exceeds them in the content levels of organic acids, the most important parameter in fruit processing.

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### 1. Introduction

Black or common elder (*Sambucus nigra* L.), also called elderberry, is a widespread species, that grows on sunlight-exposed locations in most parts of Europe, Asia, North Africa and the USA. It is a deciduous shrub reaching up to 6 m in height, developing small, white hermaphrodite flowers in large corymbs, and flowering in early summer. Umbels consist of dark purple individual berries, with a diameter up to 6 mm; the fruit ripen in late summer. Elderberry cultivars are planted for ornamental purposes, but elderflower extracts are used as beverage and food flavourings (Christensen, Knaack, & Frette, 2007), and elderberry berries have been globally utilised as a medicine or a source of dietary supplements (Dawidowicz, Wianowska, & Baraniak, 2006; Lee & Finn, 2007). Many cultivars of *S. nigra* L. are planted in Europe, the most common being the 'Haschberg' cultivar with several selections, which are mainly harvested for fruit. This cultivar is seldom used for fresh consumption; mostly it is processed to concentrates and

juices. The juice pressed from black elderberry fruit contains many primary metabolites including various sugars and organic acids. High concentrations of organic acids are important in processing, since, unlike sugars, they cannot be added to the final product. Among secondary metabolites, elderberry juice is predominantly characterised by high amounts of the anthocyanins.

Anthocyanins are a class of flavonoid compounds responsible for the attractive orange to blue colour of flowers, as well as an important fruit quality indicator, since they influence fruit appearance and flavour (Del Caro & Piga, 2008; Lee & Finn, 2007). They have gained interest as functional compounds in food colorants and as potent agents against oxidative stress (Stintzing, Stintzing, Carle, Frei, & Wrolstad, 2002), reducing oxidative damage to the human body (Dawidowicz et al., 2006). Anthocyanins, as well as other flavonoids (e.g. quercetins), exhibit antioxidant, anticarcinogenic, immune-stimulating, antibacterial, antiallergic and antiviral properties; therefore, their consumption may contribute to prevention of several degenerative diseases such as cardiovascular disease, cancer, inflammatory disease and diabetes (Bonerz, Würth, & Dietrich, 2006; Brambilla, Lo Scalzo, Bertolo, & Torreggiani, 2008; Dawidowicz et al., 2006; Lata & Tomala, 2007; Nakajima, Tanaka, Seo, Yamazaki, & Saito, 2004). These compounds are well known

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free radical scavengers, reported as potential chemo-preventive agents. Therefore, elderberry cultivars containing higher concentrations of anthocyanins are considered most important.

Several studies were made on anthocyanin and other polyphenolic content levels of various *S. nigra* L. cultivars with little information on the content of various classes of primary metabolites (Nakajima et al., 2004; Wu, Gu, Prior, & McKay, 2004; Zakay-Rones, Thom, Wollan, & Wadstein, 2004; Lee & Finn, 2007); however, research papers on the overall chemical composition of elderberry fruit are scarce.

The objective of this study was to compare the content levels of three sugars (sucrose, fructose and glucose) and four organic acids (malic acid, citric acid, fumaric acid and shikimic acid) in two cultivars and three selections of *S. nigra* L. Moreover, the anthocyanin as well as quercetin profiles of the selected plant material were established. Since common elderberry is an important fruit species for processing, internal quality parameters were compared to other fruit cultivated for processing as well as those for fresh consumption.

## 2. Material and methods

### 2.1. Plant material

The fruit of two elderberry cultivars ('Rubini', 'Haschberg') and three elderberry selections ('Selection 13', 'Selection 14' and 'Selection 25') were picked at their optimum fruit maturity at the end of August 2006 in Austria, on a laboratory research field near Graz. All plants were mature and growing in similar, sunny conditions. Umbels were harvested from several representative plants of each cultivar/selection, and approximately 1 kg of elderberry berries (with stalks) were packed in plastic bags and stored at  $-18\text{ }^{\circ}\text{C}$  until further analysis. Various compounds (sugars, organic acids, anthocyanins and quercetins) were analysed from whole umbels (berries, including stalks). For each cultivar/selection five replicates were carried out ( $n = 5$ ).

### 2.2. Analysis of individual carbohydrates and organic acids

Elderberry fruit samples were analysed for their content levels of carbohydrates (sucrose, glucose and fructose) and organic acids (malic, citric, fumaric and shikimic). In the laboratory, umbels of each cultivar/selection were mashed to a pulp and 10 g of the fresh mass was immersed in 50 ml of twice distilled water and homogenised with the T-25 Ultra-Turrax (Ika-Labor Technik). The fruit samples were left for extraction for half an hour at room temperature, with frequent stirring, and the extracted samples were afterwards centrifuged at 10,000g for 7 min at  $10\text{ }^{\circ}\text{C}$  (Eppendorf Centrifuge 5810R, Hamburg, Germany). The supernatants were filtered through a  $0.45\text{ }\mu\text{m}$  filter (Macherey-Nagel), transferred to a vial, and analysed according to the method described by Sturm, Koron, and Stampar (2003) using high-performance liquid chromatography (HPLC; Thermo Scientific, Finnigan Spectra System, Waltham, MA, USA). For each analysis,  $20\text{ }\mu\text{l}$  of sample was used. Analysis of sugars was carried out using a Rezex-RCM-monosaccharide column ( $300 \times 7.8\text{ mm}$ ; Phenomenex, Torrance, CA) with a flow rate of  $0.6\text{ ml min}^{-1}$  and with column temperature maintained at  $65\text{ }^{\circ}\text{C}$ . For the mobile phase, twice distilled water was used, and an RI detector for identification. Organic acids were analysed in the Amniex-HPX-87 H column ( $300 \times 7.8\text{ mm}$ ; Bio-Rad, USA), and a UV detector set at  $210\text{ nm}$  with a flow rate of  $0.6\text{ ml min}^{-1}$  maintaining the column temperature at  $65\text{ }^{\circ}\text{C}$ . For the mobile phase,  $4\text{ mM}$  sulphuric acid was used. The concentrations of carbohydrates and organic acids were calculated with the help of corresponding external standards.

### 2.3. Extraction and HPLC analysis of anthocyanins and quercetins

For the analysis of individual anthocyanins and quercetins, 1 g of berries was extracted with 20 ml methanol containing 1% HCl and 1% 2,6-di-*tert*-butyl-4-methylphenol (BHT) in an ultrasonic bath for 30 min. After extraction, the treated samples were centrifuged for 7 min at 12,857 rcf and the supernatant was filtered through a Chromafil AO-45/25 polyamide filter produced by Macherey-Nagel (Düren, Germany) to a vial prior to injection into the HPLC system. HPLC analysis was performed using a Surveyor HPLC system with a diode array detector (DAD), controlled by CromQuest 4.0 software (Thermo Finnigan, San Jose, CA). The anthocyanins were analysed at 530 nm and the quercetins at 350 nm. The column used was a Gemini C<sub>18</sub> ( $150 \times 4.6\text{ mm } 3\text{ }\mu\text{m}$ ; Phenomenex) operated at  $25\text{ }^{\circ}\text{C}$ . The elution solvents were 1% formic acid in twice distilled water (A) and 100% acetonitrile (B). The samples were eluted according to the linear gradient described by Marks, Mullen, and Crozier (2007), with the injection amount of  $20\text{ }\mu\text{l}$  and a flow rate  $1\text{ ml min}^{-1}$ . The phenolics were identified by comparing their UV-Vis spectra from 220 to 550 nm and retention times. Quantification was achieved according to concentrations of the corresponding standard and was confirmed using the Thermo Scientific LCQ Deca XP mass spectrometer with an electrospray interface (ESI) operating in positive (anthocyanins) or negative (quercetins) ion mode. The concentrations of the identified anthocyanins cyanidin 3-sambubioside-5-glucoside, cyanidin 3,5-diglucoside, cyanidin 3-sambubioside, cyanidin 3-glucoside and cyanidin 3-rutinoside were assessed from peak areas and calculated as equivalents of cyanidin. The content of total anthocyanins was expressed in mg cyanidin equivalents (CGE) per 100 g of elderberry fruit. The concentrations of the identified quercetins were calculated from peak areas of the corresponding standards – quercetin-glucoside, quercetin-rutinoside and quercetin.

### 2.4. Chemicals

The following standards were used to determine the chemical compounds in elderberry fruit: sucrose, fructose, glucose; malic, citric, fumaric and shikimic acid; cyanidin chloride; rutin, Q-glucoside, Q-xyloside, phloretine-xylosylglucoside and quercetin.

The chemicals for mobile phases and sample preparation were methanol and acetonitrile from Sigma-Aldrich Chemie GmbH (Steinheim, Germany) and formic acid from Fluka Chemie GmbH (Buchs, Switzerland).

The water used in sample preparation, solutions and analysis was twice distilled and purified with a Milli-Q water purification system by Millipore (Bedford, MA).

### 2.5. Statistical analysis

Statistical analysis was conducted with the Statgraphics Plus 4.0 programme (Statgraphics, Herndon, VA). One-way analysis of variance (ANOVA) was used for the analysis of the effect of cultivar/selection on the content level of sugars, organic acids, anthocyanins and quercetins. Differences between cultivars/selections were estimated with the HSD test and were considered statistically significant when  $p < 0.05$ .

## 3. Results and discussion

### 3.1. Sugars

Two cultivars and three selections of black elderberry were examined in this study and the concentrations of individual sugars were assessed. The most abundant sugars in black elderberry fruit

**Table 1**Concentrations of individual sugars in fruits of two cultivars and three selections of black elderberry (*S. nigra* L.) in g kg<sup>-1</sup> FW

<i>S. nigra</i> L. cultivar/selection	Sucrose	Fructose	Glucose	Sum sugars
'Haschberg'	1.21 ± 0.06 b	33.99 ± 0.93 a	33.33 ± 0.67 a	68.53 ± 1.55 a
'Selection 13'	0.47 ± 0.04 a	44.14 ± 1.02 b	42.42 ± 0.84 b	87.03 ± 1.86 b
'Selection 14'	0.48 ± 0.05 a	45.30 ± 1.02 b	45.17 ± 1.13 c	90.96 ± 2.16 b
'Selection 25'	1.68 ± 0.16 c	52.25 ± 1.43 c	50.23 ± 0.53 d	104.16 ± 1.84 c
'Rubini'	1.38 ± 0.03 b	44.12 ± 1.63 b	41.93 ± 1.26 b	87.44 ± 2.88 b
Mean	1.04 ± 0.10	43.96 ± 1.29	42.62 ± 1.18	87.62 ± 2.48

Average values ± standard error are presented. Different letters (a–d) in rows mean statistically significant differences between individual sugar contents among cultivars/selections at  $\alpha < 0.05$ .

were fructose and glucose, sucrose was detected only in small amounts. The individual and total sugar content in elderberry fruit was highest in 'Selection 25', with average values of 104.2 g kg<sup>-1</sup> fresh weight (FW); significant differences were present between this selection and all other cultivars/selections. Lowest concentrations of total carbohydrates were detected in the fruit pulp of the 'Haschberg' standard cultivar, with average values of 68.53 g kg<sup>-1</sup> (Table 1). Elderberry fruit contains moderate amounts of sugars compared to apple, which contains 115–183 g kg<sup>-1</sup> total analysed sugars (Hofer et al., 2005) and significantly lower amounts of total sugars than sweet cherry, which averagely contains 150–230 g kg<sup>-1</sup> (Usenik, Fabčić, & Stampar, 2008). The content level of sugars in elderberry fruit is comparable to sour cherry fruit (*Prunus cerasus* L.), which contain approximately 90 g l<sup>-1</sup> of total sugars and are also mainly used in processing (Bonerz et al., 2006; Füzfa, Katona, Kovács, & Molnár-Perl, 2004). The amount of sugar in elderberry fruit, however, is not the focal chemical compound for technological processing, as fructose and sucrose can easily be added to the final products (i.e. juices, concentrates, beverages).

### 3.2. Organic acids

Four organic acids were identified in the elderberry berries: citric acid, malic acid, shikimic acid and fumaric acid (Table 2). Citric acid was the most abundant organic acid in all cultivars/selections, followed by malic acid and smaller concentrations of shikimic and fumaric acid. The content level of citric acid in the fruit of black elderberry ranged from 3.11 g kg<sup>-1</sup> FW in 'Selection 13' to 4.81 g kg<sup>-1</sup> FW in cultivar 'Haschberg'. Compared to apple, which contains between 0.07 and 0.52 g kg<sup>-1</sup> FW citric acid (Hofer et al., 2005), sweet cherry, which contains between 0.11 and 0.54 g kg<sup>-1</sup> FW citric acid (Usenik et al., 2008) and sour cherry, which contains between 0.08 and 0.14 g kg<sup>-1</sup> FW citric acid (Bonerz et al., 2006), elderberry fruit is exceptionally rich in this organic acid. This is an important parameter for processing purposes and justifies the leading role of the 'Haschberg' elderberry cultivar in European plantations, since it is particularly rich in citric acid. Moreover, the most significant statistical differences were observed in the concentration of shikimic acid among different cultivars/selections with values ranging from as low as 0.14 g kg<sup>-1</sup> FW

in 'Selection 14' to 0.93 g kg<sup>-1</sup> FW in 'Selection 25'. The lowest amount of total organic acid was detected in fruits of 'Selection 13', which contained 4.52 g kg<sup>-1</sup> FW and the highest in the cultivar 'Haschberg' with 6.38 g kg<sup>-1</sup> FW. The total organic acid concentration of all cultivars/selection of elderberry is lower than in apple, which contains, on average, between 6.00 and 14.00 g<sup>-1</sup> FW organic acids (Hofer et al., 2005) and sweet cherry, which contains, on average, between 3.50 and 8.20 g kg<sup>-1</sup> FW organic acids (Usenik et al., 2008).

### 3.3. Anthocyanins

High performance liquid chromatography analysis of black elderberry (*S. nigra* L.) revealed the presence of five anthocyanin peaks: cyanidin 3-sambubioside-5-glucoside ([M+H]<sup>+</sup> at *m/z* 743), cyanidin 3,5-diglucoside ([M+H]<sup>+</sup> at *m/z* 611), cyanidin 3-sambubioside ([M+H]<sup>+</sup> at *m/z* 581), cyanidin 3-glucoside ([M+H]<sup>+</sup> at *m/z* 449) and cyanidin 3-rutinoside ([M+H]<sup>+</sup> at *m/z* 595). Other anthocyanins occurred in lower amounts. According to Inami, Tamura, Kikuzaki, and Nakatani (1996), Wu et al. (2004) and Kaack, Frettté, Christensen, Landbo, and Meyer (2008) the two major anthocyanins in *S. nigra* L. are cyanidin 3-glucoside and cyanidin 3-sambubioside, and this is consistent with our results (Table 3). The major anthocyanin in all berries from these cultivars, cyanidin 3-sambubioside, accounted for up to half of all determined anthocyanins, approximately. The only exception was 'Selection 13', where the major anthocyanin was cyanidin 3-glucoside, accounting for more than 60% of all analysed anthocyanins. The lowest concentration of cyanidin 3-sambubioside was detected in 'Selection 13' (271 mg CGE/100 g FW) and the highest in cultivar 'Rubini' (631 mg CGE/100 g FW). Significant differences in concentration of cyanidin 3-sambubioside were present between 'Selection 13' and all cultivars/selections analyzed. The lowest amount of second major anthocyanin, cyanidin 3-glucoside, was measured in the berries of 'Selection 14' (221 mg CGE/100 g FW) and the highest in 'Selection 13' (456 mg CGE/100 g FW). Significant differences were detected between each cultivar/selection, with the exception of 'Haschberg' cultivar and 'Selection 25', which contained similar amounts of cyanidin 3-glucoside. Separation and peak identification of cyanidin 3-sambubioside and cyanidin 3-glucoside were

**Table 2**Concentrations of organic acids in fruits of two cultivars and three selections of black elderberry (*S. nigra* L.) in g kg<sup>-1</sup> FW

<i>S. nigra</i> L. cultivar/selection	Citric acid	Malic acid	Shikimic acid	Fumaric acid	Sum organic acids
'Haschberg'	4.81 ± 0.19 b	1.10 ± 0.04 b	0.18 ± 0.04 ab	0.29 ± 0.001 d	6.38 ± 0.26 d
'Selection 13'	3.11 ± 0.10 a	1.10 ± 0.03 b	0.16 ± 0.03 a	0.14 ± 0.001 b	4.52 ± 0.13 ab
'Selection 14'	3.39 ± 0.09 a	1.31 ± 0.03 c	0.14 ± 0.02 a	0.10 ± 0.001 a	4.95 ± 0.12 bc
'Selection 25'	3.09 ± 0.05 a	0.97 ± 0.01 a	0.93 ± 0.02 c	0.18 ± 0.01 c	5.17 ± 0.07 c
'Rubini'	3.08 ± 0.07 a	1.02 ± 0.04 ab	0.24 ± 0.01 b	0.13 ± 0.004 b	4.48 ± 0.12 a
Mean	3.50 ± 0.14	1.10 ± 0.03	0.33 ± 0.06	0.17 ± 0.01	5.10 ± 0.15

Average values ± standard error are presented. Different letters (a–d) in rows mean statistically significant differences between individual organic acids among cultivars/selections at  $\alpha < 0.05$ .

**Table 3**Concentrations of five anthocyanins in fruits of two cultivars and three selections of black elderberry (*S. nigra* L.) in mg CGE/100 g FW

<i>S. nigra</i> L. cultivar/selection	Cy 3-sam-5-glu	Cy 3,5-diglu	Cy 3-sam	Cy 3-glu	Cy 3-rut	Sum anthocyanins
'Haschberg'	33.29 ± 1.30 c	9.47 ± 1.03 ab	352.7 ± 25.6 b	331.7 ± 13.0 b	9.63 ± 0.45 c	736.7 ± 40.2 b
'Selection 13'	19.52 ± 0.98 a	7.41 ± 0.31 a	270.8 ± 18.6 a	456.2 ± 26.4 c	2.98 ± 0.52 b	756.9 ± 45.3 b
'Selection 14'	21.91 ± 0.64 ab	11.35 ± 0.23 b	346.7 ± 10.5 b	221.4 ± 6.0 a	1.49 ± 0.59 a	602.9 ± 15.5 a
'Selection 25'	53.49 ± 3.55 d	23.29 ± 1.63 d	592.8 ± 42.0 c	285.1 ± 15.8 b	2.52 ± 0.21 b	957.3 ± 63.0 c
'Rubini'	25.63 ± 0.73 b	20.18 ± 0.84 c	630.8 ± 9.00 c	586.4 ± 12.1 d	2.25 ± 0.30 ab	1265.3 ± 21.0 d
Mean	30.77 ± 2.61	14.34 ± 1.33	438.8 ± 31.2	376.2 ± 27.4	3.77 ± 0.62	863.8 ± 49.9

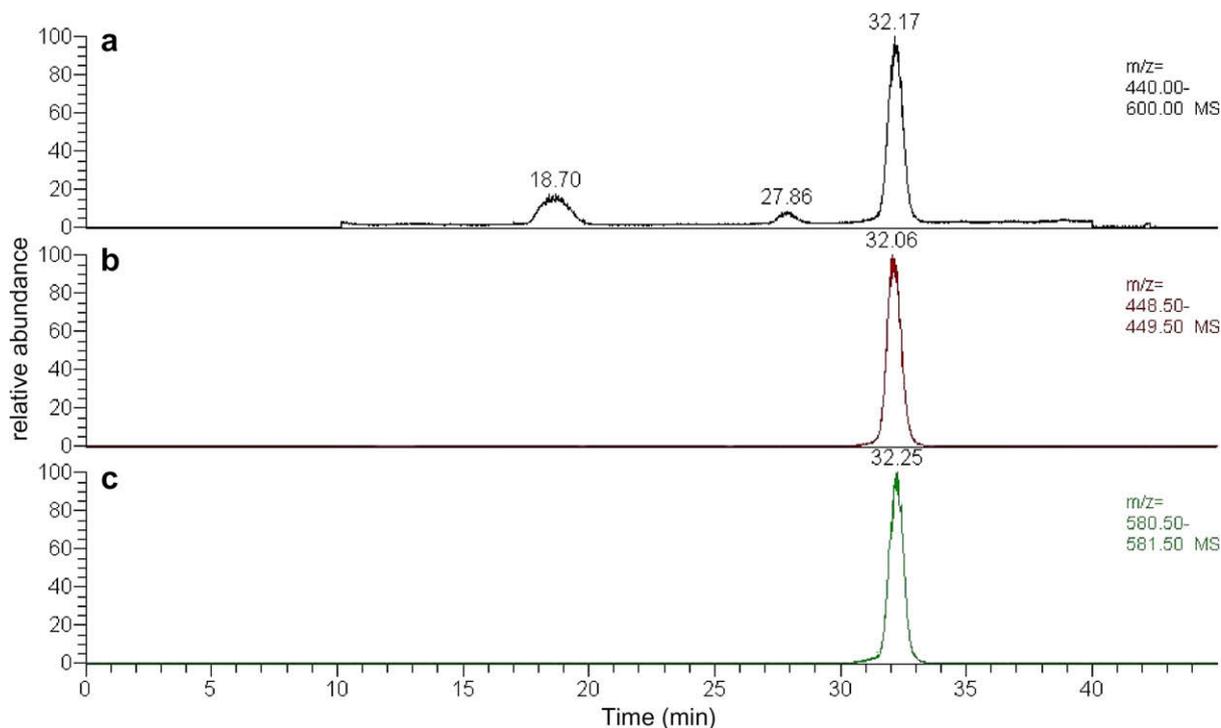
Anthocyanins in black elderberry fruit: cy 3-sam-5-glu: cyanidin 3-sambubioside-5-glucoside; cy 3,5-diglu: cyanidin 3,5-diglucoside; cy 3-sam: cyanidin 3-sambubioside; cy 3-glu: cyanidin 3-glucoside; cy 3-rut: cyanidin 3-rutinoside. Average values ± standard error are presented. Different letters (a–d) in rows mean statistically significant differences between individual anthocyanins among cultivars/selections at  $\alpha < 0.05$ .

not possible given the chosen method because of identical retention times. The identification was therefore done with the help of mass analysis (Fig. 1). Three minor peaks were also identified in the berries of analysed cultivars/selections of elderberry; cyanidin 3-sambubioside-5-glucoside was lowest in 'Selection 13' (195 mg kg<sup>-1</sup> FW) and highest in 'Selection 25' (53.5 mg CGE/100 g FW), cyanidin 3,5-diglucoside was lowest in 'Selection 13' (7.41 mg CGE/100 g FW) and highest in 'Selection 25' (23.3 mg CGE/100 g FW) and cyanidin 3-rutinoside was lowest in 'Selection 14' (1.49 mg CGE/100 g FW) and highest in cultivar 'Haschberg' (9.60 mg CGE/100 g FW). The concentration of total anthocyanins ranged from 603 mg CGE/100 g FW in 'Selection 14' to 1265 mg CGE/100 g FW in berries of cultivar 'Rubini', which was similar to the results of Kaack et al. (2008). The standard 'Haschberg' cultivar contained moderate amounts of total anthocyanins (737 mg CGE/100 g FW); however, we must take into account that a higher content of anthocyanins does not automatically mean a darker colour of the fruit, and 'Haschberg' is, therefore also suitable for processing. In comparison to black figs, which contain approximately 95 mg CGE/100 g FW total anthocyanins (Del Caro & Piga, 2008) and sweet cherry, which contains, on average, 100–120 mg CGE/100 g

FW (Usenik et al., 2008), elderberry fruit has significantly higher anthocyanin content levels (see Table 4).

### 3.4. Quercetins

In the group of quercetins, we detected the following: quercetin ([M–H]<sup>-</sup> at *m/z* 301), quercetin 3-rutinoside ([M–H]<sup>-</sup> at *m/z* 609) and quercetin 3-glucoside ([M–H]<sup>-</sup> at *m/z* 463). The main quercetin in these elderberry cultivars/selections was quercetin 3-rutinoside (rutin), with values ranging from 35.6 mg CGE/100 g FW in 'Selection 14' to 52.0 mg CGE/100 g FW in the 'Haschberg' cultivar. These results are comparable to the concentration of quercetin 3-rutinoside in fruit of black figs, which according to Del Caro and Piga (2008) contain approximately 53 mg CGE/100 g FW of this compound. The other two quercetins were in considerably lower amounts, especially the aglicon quercetin; the concentration of this was approximately 1/10 of the amount of quercetin 3-rutinoside. Total quercetins were lowest in 'Selection 14' (51.9 mg CGE/100 g FW) and highest in 'Selection 25' (73.4 mg CGE/100 g FW), the 'Haschberg' cultivar containing moderate amounts of total quercetins (61.3 mg CGE/100 g FW).



**Fig. 1.** Mass chromatogram of elderberry extract selected by *m/z*. Mass chromatogram revealed that elderberry extract in the peak at 32.17 min (a) contains two anthocyanins, namely cyanidin 3-glucoside (b) and cyanidin 3-sambubioside (c).

**Table 4**Concentrations of quercetins in fruits of two cultivars and three selections of black elderberry (*S. nigra* L.) in mg CGE/100 g FW

<i>S. nigra</i> L. cultivar/selection	Q	q-rut	q-glu	Sum quercetins
'Haschberg'	2.89 ± 0.46 a	52.02 ± 2.48 b	6.38 ± 0.37 a	61.29 ± 2.79 bc
'Selection 13'	3.22 ± 0.21 ab	35.82 ± 2.24 a	17.42 ± 1.33 c	56.44 ± 3.52 ab
'Selection 14'	2.70 ± 0.22 a	35.59 ± 1.37 a	13.64 ± 0.57 b	51.94 ± 1.92 a
'Selection 25'	4.06 ± 0.46 bc	50.04 ± 0.65 b	19.32 ± 0.41 c	73.43 ± 1.20 d
'Rubini'	4.50 ± 0.13 c	35.88 ± 0.89 a	26.52 ± 0.57 d	66.90 ± 1.49 cd
Mean	3.47 ± 0.19	41.87 ± 1.68	16.67 ± 1.37	62.00 ± 1.82

Quercetins in fruit of black elderberry: Q: quercetin; q-rut: quercetin 3-rutinoside; q-glu: quercetin 3-glucoside. Average values ± standard error are presented. Different letters (a–d) in rows mean statistically significant differences between individual quercetins among cultivars/selections at  $\alpha < 0.05$ .

#### 4. Conclusions

Elderberry fruit is predominately used for processing; therefore cultivars with higher concentrations of organic acids, anthocyanins and other phenolics are particularly suitable for commercial growing. The leading European cultivar 'Haschberg' has significantly higher amounts of total organic acids than other cultivars/selections analysed but had lower concentrations of secondary metabolites than some selections. All cultivars of black elderberry contained high amounts of anthocyanins especially of cyanidin 3-sambubioside and cyanidin 3-glucoside compared to other fruit varieties (sweet cherry, fig). The cultivar with the highest amounts of total analysed anthocyanins was 'Rubini'; it also contained high amounts of quercetins, especially quercetin 3-rutinoside, the predominant quercetin in our study. Analysis of the combined data of primary and secondary metabolites can be utilised for further selection of *S. nigra* L. cultivars/selections, used for processing elderberry juices and extracts with health-promoting properties.

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