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Different extraction processes affect the metabolites in blue honeysuckle (*Lonicera caerulea* L. subsp. *edulis*) food products

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Abstract: Honeysuckle berries are becoming an attractive organically grown fruit with many aspects as a new functional food. They have the benefit of containing health-promoting compounds, such as saponins, ascorbic acid, iridoids, flavonoids, and anthocyanins. Fruit berries can be processed into many products with various contents of selected bioactive compounds. The contents of individual compounds were identified with the aid of high-performance liquid chromatography coupled with mass spectrophotometry. Their concentrations significantly differed among the various food products. Ascorbic acid had the highest values in blue honeysuckle spread (302.02 mg/100 g of dry weight), while infusion (119.17 mg/100 g) and juice (118.17 mg/100 g) had the lowest values. All the food products had low sugar contents. Of the health beneficial phenolic compounds honeysuckle spread had the highest content (1753.54 mg/100 g) and honeysuckle infusion (196.61 mg/100 g) had the lowest content. Honeysuckle liqueur and smoothie also had high total analyzed phenolic contents (1138.75 mg/100 g and 1108.25 mg/100 g, respectively).

Key words: Ascorbic acid, extract, heating, food product, *Lonicera*, phenolics

1. Introduction

Berry fruits follow bananas, citruses, and apples in terms of world fruit production (FAO, 2017). Short shelf-life in fresh form is a weakness of berry fruits. However, their availability in the market can be prolonged through processing into various products, such as juices, juice concentrates, jams, and jellies (Skrede et al., 2010; Šavikin et al., 2014). In the last few years, the berry of a subvariety of honeysuckle, blue honeysuckle, has become more and more popular; it belongs to the family *Caprifoliaceae*, which includes species mainly known as ornamental bushes (Thompson, 2008). For this fruit plant, the possibility of organic plantation is a great advantage. Wetland spaces along rivers, marshes, or forest clearings in northeastern Russia, China, Japan, and Canada are its natural habitats (Miyashita et al., 2009). Only blue honeysuckle berries of the species of the genus *Lonicera* have an elongated shape of berry. The berries are dark purple with a waxy coating, and generally a 2-cm elongated elliptical or cylindrical shape (Thompson, 2008; Hummer et al., 2012). The taste is bitter to tart-sweet as a mixture of known berry flavors (Hummer et al., 2012). The berries have become popular because of their nutraceutical and health-promoting properties due to their numerous and various beneficial ingredients, such as vitamins, minerals, polyphenolics, iridoids, and saponins (Jurikova et al., 2009, 2012; Becker et al., 2017; Oszmiański and Kucharska, 2018). Additionally, they have a low sugar content compared to some other fruits and may thus be a good source for people with diabetes (Palikova et al., 2009).

Blue honeysuckle berries are currently used for making jams, wine, candies, jelly, puffed snacks (Liu et al., 2009, 2010), juice, juice concentrate, tea, canned and frozen fruit, and for medical products as an antioxidant and healthy food (Skupień et al., 2007). Processing procedures might change the levels of some bioactive compounds (Dai and Mumper, 2010; Senica et al., 2016). Heat-processed foods are considered to possess lower health promoting properties than the corresponding fresh analogue (Choi et al., 2006). Some compounds change after a pressing or freeze-drying process (Oszmiański and Kucharska, 2018). Processing fruit, including heating or an increase in temperature, is reflected in water loss and changes in the concentration of selected compounds in fruit samples, thus increasing the contents of some compounds (Yadav and Singh, 2014; Çavuşoğlu, 2018).

The aim of the present study was to measure the contents of various primary and secondary metabolites in

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five different honeysuckle berry products: juice, infusion, spread, liqueur, and smoothie. The chemical composition of berries and their products have been well documented (Liu et al., 2009, 2010; Yadav and Singh, 2014; Senica et al., 2016) but to the best of our knowledge there have been no studies regarding organically grown fruit.

In addition, the current study is the first detailed research with respect to the impact of processing on the chemical composition of honeysuckle berries.

2. Materials and methods

2.1. Fruit material

Organically grown honeysuckle berries of the 'Aurora' cultivar were harvested at the optimal stage of ripeness (11 June 2017) from a location in Šmartno pri Litiji (46°2′38.7″N; 14°50′47″E; 250 m altitude). All samples were collected at the same location from several shrubs. For the determination of dry matter, ten berries in triplicate for each cultivar were dried at 105 °C for 12 h in a drying oven.

2.2. Chemicals

The following standards were purchased from Sigma Aldrich (Steinheim, Germany): ellagic acid, chlorogenic acid (5-caffeoylquinic acid), neochlorogenic acid (3-caffeoylqinic acid), luteolin-3-*O*-rutinoside, cyanidin-3-*O*-glucoside, quinic acid, shikimic acid, and ascorbic acid. *Meta*-phosphoric acid and methanol for vitamin C and phenolics extraction, respectively, were also obtained from Sigma Aldrich Chemie. We obtained epicatechin, quercetin-3-*O*-galactoside, quercetin-3-*O*-glucoside, quercetin-3-*O*-rutinoside, *p*-coumaric acid, procyanidin B2, kaempferol-3-*O*-glucoside, fructose, glucose, sucrose, citric acid, malic acid, fumaric acid, tartaric acid, fumaric acid, and acetonitrile for the mobile phase from Fluka Chemie (Buchs, Switzerland). Phenolic standards caffeic acid and catechin were purchased from Roth (Karlsruhe, Germany); quercetin-3-*O*-xyloside and quercetin-3-*O*-arabinofuranoside from Apin Chemicals (Abingdon, UK); and isorhamnetin-3-*O*-rutinoside, petunidin, and peonidin-3-glucoside from Extrasynthese (Genay, France). Water for extraction and the mobile phase was double distilled and purified using the Milli-Q system (Millipore, Bedford, MA, USA).

2.3. Preparation of five different honeysuckle berry products (infusion, juice, liqueur, smoothie, and fruit spread)

Various honeysuckle berry products were prepared based on the most commonly used recipes for different fruit products (Senica et al., 2016). Honeysuckle infusion was prepared from 2 g (1 teaspoon) of dried berries infused in 200 mL of boiling water for 10 min for each repetition (7 repetitions). Honeysuckle berry juice was pressed from 50 g of ripe berries for each repetition (7). Liqueur was prepared in a glass flask, which was filled with 100 mL of 45% ethanol and in which 20 g of whole ripe berries was steeped. The berries were allowed to steep for 3 weeks in a dark place at room temperature. For the preparation of blue honeysuckle spread, 30 g of berries in each laboratory glass was heated to boiling point. The heat was then reduced to 60 °C and the spread was heated for a further 30 min. The honeysuckle smoothie was prepared from 10 g of honeysuckle berries for each repetition, mixed with an Ultra-Turrax T-25 macerator (Ika-Labortechnik, Stauden, Germany) (Senica et al., 2016).

2.4. Determination of ascorbic acid in honeysuckle infusion, juice, spread, and smoothie

For the control, 5 g of berries was mashed in a mortar and extracted with 10 mL of 2% *meta*-phosphoric acid. One gram of mixed berries for the smoothie, 1 g for the honeysuckle spread, 1 g for the infusion, and 1 g for the juice were extracted with 2 mL of 2% *meta*-phosphoric acid. The control, honeysuckle berry smoothie, spread, infusion, and juice samples were then left at room temperature for 1 h on a shaker (Grant-Bio POS-300, Grant Instruments, Shepreth, UK). The samples were subsequently centrifuged at 4 °C at 10,000 rpm for 7 min and filtered through a Chromafil A-20/25 mixed ester filter (Macherey-Nagel, Düren, Germany) into vials until further analysis. Determination of ascorbic acid was carried out with a Thermo Finnigan Surveyor HPLC system (Thermo Scientific, San Jose, CA, USA). The conditions were previously described by Mikulic-Petkovsek et al. (2016). Ascorbic acid in the honeysuckle liqueur was not determined due to the presence of ethanol, which could be destructive for the HPLC system.

2.5. Determination of sugars and organic acids in honeysuckle infusion, juice, spread, and smoothie

Sugar and organic acids in various honeysuckle cultivars were estimated according to the method reported by Mikulic-Petkovsek et al. (2016). For the control, 5 g of fresh honeysuckle berries was blended using an Ultra-Turrax T-25 macerator and extracted with 25 mL of double-distilled water. One gram for the honeysuckle spread, 1 g of mixed berries for the smoothie, and 1 g for the juice were extracted with 5 mL of double-distilled water. Each sample was left at room temperature for 1 h on a shaker. The samples were then centrifuged at 4 °C at 10,000 rpm for 7 min and filtered through Chromafil A-20/25 cellulose ester filters into vials and stored for further analysis. The honeysuckle water infusion was directly filtered through cellulose ester filters into vials up to 2 mL. The determination of individual sugars and organic acids was carried out with a Thermo Finnigan Surveyor HPLC system, as previously described by Mikulic-Petkovsek et al. (2016). Organic acids and sugars were not determined in the honeysuckle liqueur because of the presence of

ethanol, which could be destructive for the HPLC system.

2.6. Determination of individual phenolics in honeysuckle infusion, juice, spread, and smoothie

The extraction of phenolic compounds for the 5 different honeysuckle berry products was carried out as described by Mikulic-Petkovsek et al. (2016), with some modifications. For the control treatment, honeysuckle berries were homogenized with an Ultra-Turrax T-25 and 5 g of fruit paste was extracted in 30-mL centrifuges with 15 mL of methanol containing 3% formic acid. For the other products, we used 1 g of each product, extracted with 3 mL of methanol containing 3% formic acid. Each sample was then placed in a cool ultrasonic bath for 1 h. The extracts were then centrifuged for 10 min at 12,000 rpm. Each supernatant was filtered through a Chromafil AO-20/25 polyamide filter (Macherey Nagel, Düren, Germany) and transferred into vials for further HPLC and MS analysis. Separation of phenolic compounds was performed on a mass spectrometer (LCQ Deca XP MAX, Thermo Scientific) with electrospray ionization (ESI) operated in negative and positive ion modes. The ESI parameters were described by Senica et al. (2016). Analyses were carried out using an Accela HPLC system (Thermo Scientific, San Jose, CA, USA), equipped with a diode array detector, controlled by CromQuest 4.0 chromatography workstation software with technical characteristics as described by Senica et al. (2016).

2.7. Statistical analysis

The statistical evaluation of primary and secondary metabolites among different blue honeysuckle berry products was conducted by one-way analysis of variance (ANOVA) with the statistical program R. Differences in the contents of the studied compounds were established with Duncan's test. P-values of less than 0.05 were considered statistically significant.

3. Results and discussion

The contents of the selected primary and secondary metabolites greatly varied among the different honeysuckle products. There are several factors that influence their contents (Gündüz and Özbay, 2018). In general, the type and time of extraction greatly affect the final contents in the food product and, additionally, heat treatment increases water loss and the chance of oxidation, which decreases or increases the content of phenolics and other compounds in extracts (Dai and Mumper, 2010; Yadav and Singh, 2014; Senica et al., 2016). The results were expressed in mg/g or mg/100 g (ascorbic acid; phenolics) of dry weight (Tables 1 and 2; Figures 1 and 2).

3.1. Ascorbic acid contents

Interestingly, the entire spread and smoothie processing caused an increase in the ascorbic acid concentration (Table 1). The spread contained 302.02 mg of ascorbic acid per 100 g of fruit dry weight, which was 100% higher content than the control (Table 1). Aerobic and anaerobic chemical reactions have the main influence on ascorbic acid content. Munyaka et al. (2010) reported that the crushing of plant material and time and degree of heating influence the stability of vitamin C. Plant material heated before crushing retained more vitamin C than plant material in which the plant cells were disrupted before heating (Munyaka et al., 2010). Heat treatment at temperatures above 70 °C completely inactivates ascorbic acid oxidative enzymes, which degrade the ascorbic acid

Table 1. Ascorbic acid, sugar, and organic acid contents (mean ± standard deviation in mg/100 g for ascorbic acid and in mg/g for sugars and organic acids per dry weight) of five different blue honeysuckle berry products.

	Control	Infusion	Juice	Spread	Smoothie
Ascorbic acid	154.89 ± 2.99^{bc}	119.17 ± 5.32^{c}	118.70 ± 5.85^{c}	302.02 ± 50.22^{a}	172.88 ± 3.06^{b}
Fructose	245.01 ± 44.54^{b}	155.25 ± 9.71^{c}	225.20 ± 12.37^{b}	543.51 ± 36.55^{a}	245.99 ± 2.37^{b}
Glucose	192.32 ± 33.88^{b}	131.39 ± 7.88^{c}	177.11 ± 9.03^{b}	454.72 ± 50.54^{a}	190.71 ± 2.12^{b}
Sucrose	17.30 ± 3.71^{b}	1.31 ± 0.22^{d}	13.86 ± 1.97^{bc}	65.20 ± 17.53^{a}	4.08 ± 2.44^{cd}
Total sugars	454.63 ± 80.70^{b}	287.96 ± 17.74^{c}	416.17 ± 22.13^{b}	1063.42 ± 96.73^{a}	440.78 ± 1.93^{b}
Citric acid	205.13 ± 31.38^{b}	62.29 ± 2.83^{e}	160.28 ± 6.35^{c}	312.18 ± 31.36^{a}	115.56 ± 7.79^{d}
Fumaric acid	0.06 ± 0.03^{b}	0.03 ± 0.00^{b}	0.02 ± 0.00^{b}	0.23 ± 0.01^{a}	0.03 ± 0.00^{b}
Malic acid	61.08 ± 5.56^{b}	28.47 ± 1.99^{c}	53.95 ± 3.40^{b}	126.72 ± 19.33^{a}	45.34 ± 4.23^{b}
Quinic acid	54.39 ± 3.43^{b}	44.70 ± 3.26^{b}	55.34 ± 3.29^{b}	93.26 ± 8.41^{a}	36.71 ± 4.32^{b}
Shikimic acid	0.11 ± 0.01^{ab}	0.08 ± 0.00^{b}	0.04 ± 0.00^{b}	0.21 ± 0.01^{a}	0.06 ± 0.00^{b}
Tartaric acid	17.03 ± 0.57^{b}	17.18 ± 0.11^{b}	8.49 ± 0.07^{b}	33.39 ± 0.99^{a}	8.37 ± 0.67^{b}
Total acids	337.79 ± 78.76^{b}	152.84 ± 30.46^{c}	278.11 ± 7.96^{b}	566.00 ± 78.75^{a}	206.07 ± 16.01^{c}

Table 2. Individual phenolic contents (mean ± standard deviation in mg/100 g per dry weight) of six different blue honeysuckle berry products.

	Control	Infusion	Juice	Liqueur	Spread	Smoothie
Neochlorogenic acid	19.69 ± 0.34^{a}	3.20 ± 0.54^{e}	6.39 ± 0.82^{d}	17.11 ± 0.11^{b}	6.96 ± 0.12^{d}	8.41 ± 0.39^{c}
4-Caffeoylquinic acid	0.57 ± 0.01^{b}	0.14 ± 0.03^{d}	0.07 ± 0.02^{e}	0.83 ± 0.01^{a}	0.42 ± 0.01^{c}	/
Chlorogenic acid	539.20 ± 40.48^{a}	53.84 ± 6.88^{d}	60.62 ± 22.95^{d}	186.97 ± 1.25^{c}	342.10 ± 20.35^{b}	192.49 ± 11.32^{c}
Coumaroylquinic acid	1.84 ± 0.01^{a}	/	/	0.78 ± 0.00^{b}	/	/
p-Coumaric acid hexoside	0.26 ± 0.01^{a}	0.003 ± 0.000^{f}	0.016 ± 0.002^{e}	0.105 ± 0.001^{c}	0.12 ± 0.01^{b}	0.09 ± 0.02^{d}
Dicaffeoylquinic acid	10.91 ± 0.61^{b}	0.14 ± 0.03^{c}	1.95 ± 0.02^{c}	3.73 ± 0.24^{c}	31.16 ± 7.77^{a}	11.60 ± 0.19^{b}
Ellagic acid hexoside	16.09 ± 1.75^{a}	3.16 ± 2.37^{c}	1.19 ± 0.08^{c}	1.58 ± 0.10^{c}	11.36 ± 2.54^{b}	6.41 ± 0.08^{c}
(+)Catechin	20.04 ± 2.76^{b}	2.06 ± 0.07^{e}	0.07 ± 0.00^{e}	13.84 ± 0.09^{c}	24.46 ± 3.38^{a}	5.02 ± 0.02^{d}
(-)Epicatechin	232.83 ± 23.50^{a}	0.28 ± 0.00^{c}	1.23 ± 0.08^{c}	242.39 ± 1.62^{a}	20.85 ± 2.57^{b}	11.49 ± 0.73^{bc}
Procy dimer	2.99 ± 0.27	0.09 ± 0.01^{f}	0.29 ± 0.06^{e}	2.77 ± 0.02^{b}	1.02 ± 0.06^{c}	0.68 ± 0.05^{d}
Procy trimer	47.28 ± 2.60^{a}	/	/	19.99 ± 0.13^{b}	/	/
Procy tetramer	0.55 ± 0.05^{c}	0.08 ± 0.01^{d}	1.55 ± 0.42^{a}	1.13 ± 0.01^{b}	1.69 ± 0.09^{a}	1.40 ± 0.08^{ab}
Luteolin hexoside	3.01 ± 0.15^{b}	0.04 ± 0.00^{e}	0.22 ± 0.02^{de}	0.56 ± 0.03^{d}	3.62 ± 0.09^{a}	1.28 ± 0.08^{c}
Luteolin-3-rutinoside	4.85 ± 0.21^{ab}	0.20 ± 0.01^{d}	3.82 ±0.08^{b}	0.73 ± 0.10^{cd}	5.45 ± 0.33^{a}	1.82 ± 0.08^{c}
Genistein hydroxyhexoside	1.91 ± 0.07^{b}	0.14 ± 0.01^{d}	0.45 ± 0.02^{c}	0.04 ± 0.00^{d}	3.21 ± 0.39^{a}	0.04 ± 0.00^{d}
Isorhamnetin acetylhexoside	1.47 ± 0.16^{a}	0.11 ± 0.09^{de}	0.03 ± 0.00^{e}	0.69 ± 0.03^{b}	0.44 ± 0.04^{c}	0.16 ± 0.04^{d}
Isorhamnetin acetylrhamnosyl hex	1.36 ± 0.04^{b}	0.05 ± 0.01^{e}	0.03 ± 0.00^{e}	0.69 ± 0.03^{d}	2.01 ± 0.03^{a}	0.95 ± 0.01^{c}
Isorhamnetin hexosyl pentoside	1.95 ± 0.11^{b}	0.03 ± 0.00^{d}	0.24 ± 0.10^{d}	0.77 ± 0.03^{c}	5.98 ± 0.61^{a}	2.02 ± 0.03^{b}
Isorhamnetin-3-rutinoside	14.08 ± 0.92^{b}	0.05 ± 0.00^{d}	0.03 ± 0.00^{d}	6.53 ± 0.02^{c}	20.72 ± 3.42^{a}	8.05 ± 0.50^{c}
Kaempferol acetylhexoside	0.51 ± 0.00^{c}	0.12 ± 0.00^{de}	0.05 ± 0.00^{e}	0.31 ± 0.00^{cd}	1.79 ± 0.05^{a}	0.83 ± 0.15^{b}
Kaempferol hexosyl pentoside	3.55 ± 0.08^{a}	0.15 ± 0.01^{d}	0.13 ± 0.04^{d}	0.14 ± 0.04^{d}	1.39 ± 0.74^{b}	0.92 ± 0.04^{c}
Kaempferol-3-glucoside	1.95 ± 0.02^{b}	0.05 ± 0.01^{c}	0.10 ± 0.01^{c}	0.48 ± 0.05^{c}	3.16 ± 0.17^{a}	0.44 ± 0.08^{c}
Q-3-acetyl hexoside	14.13 ± 0.08^{b}	0.08 ± 0.02^{c}	2.24 ± 0.38^{c}	4.76 ± 0.24^{c}	40.06 ± 0.99^{a}	15.22 ± 2.42^{b}
Q-3-arabinofuranoside	0.06 ± 0.00^{a}	0.001 ± 0.000^{c}	0.003 ± 0.000^{c}	0.02 ± 0.00^{b}	0.06 ± 0.00^{a}	0.01 ± 0.00^{bc}
Q-3-galactoside	30.73 ± 1.90 ^{b}	0.44 ± 0.09^{d}	3.09 ± 0.30^{d}	12.45 ± 0.46^{c}	103.81 ± 15.74^{a}	32.53 ± 4.91^{b}
Q-3-glucoside	2.20 ± 0.24^{c}	0.87 ± 0.01^{c}	0.70 ± 0.01^{c}	1.19 ± 0.07^{c}	6.48 ± 0.25^{a}	5.00 ± 0.03^{b}
Q-3-hexoside	2.33 ± 0.19^{a}	0.14 ± 0.01^{e}	0.20 ± 0.05^{e}	1.78 ± 0.08^{b}	0.96 ± 0.06^{c}	0.39 ± 0.04^{d}
Q-3-hexosylpentoside	6.60 ± 0.05^{b}	0.07 ± 0.01^{e}	0.16 ± 0.03^{e}	3.80 ± 0.06^{c}	9.10 ± 0.05^{a}	2.86 ± 0.05^{d}
Q-3-rutinoside	173.44 ± 15.19^{b}	24.03±1.88 ^{e}	33.91± 0.57^{e}	94.57 ± 4.91^{d}	248.92 ± 33.42^{a}	127.01 ± 7.74^{c}
Q-3-vicianoside	16.24 ± 0.55^{b}	4.23 ± 0.37^{d}	2.79 ± 0.37^{d}	0.03 ± 0.00^{d}	31.44 ± 7.61^{a}	11.40 ± 0.29^{c}
Q-3-xyloside	0.40 ± 0.03^{b}	0.001 ± 0.000^{d}	0.003 ± 0.000^{d}	0.22 ± 0.01^{c}	0.72 ± 0.07^{a}	0.26 ± 0.04^{c}
Naringenin hexoside	18.90 ± 0.96^{b}	0.06 ± 0.00^{e}	1.22 ± 0.05^{de}	3.06 ± 0.10^{d}	23.75 ± 3.50^{a}	9.97 ± 0.55^{c}
Loganin-pentoside	3.28 ± 0.38^{b}	0.22 ± 0.06^{c}	2.14 ± 0.07^{d}	0.05 ± 0.00^{d}	5.06 ± 0.09^{a}	4.71 ± 0.27^{a}
Cyanidin-3.5- diglucoside	29.53 ± 0.36^{a}	0.99 ± 0.12^{d}	0.97 ± 0.07^{d}	15.29 ± 0.51^{b}	11.18 ± 0.55^{c}	10.01 ± 0.25^{c}
Cyanidin-3-glucoside	268.46 ±17.30^{b}	54.32 ± 9.01^{d}	139.91 ± 6.03^{c}	285.14 ± 9.03^{ab}	321.03 ± 47.65^{a}	309.69 ± 18.32^{ab}
Cyanidin-3-rutinoside	44.19 ± 2.52^{c}	31.08 ± 2.35^{d}	29.02 ± 2.49^{d}	69.74 ± 1.11^{b}	110.36 ± 7.06^{a}	106.34 ± 9.45^{a}
Pelargonidin-dihexoside	7.21 ± 0.45^{bc}	0.04 ± 0.01^{d}	3.92 ± 0.05^{cd}	11.03 ± 0.40^{b}	16.24 ± 0.75^{a}	8.55 ± 0.62^{b}
Pelargonidin-3-glucoside	10.76 ± 0.43^{c}	0.41 ± 0.08^{e}	6.82 ± 0.06^{d}	17.55 ± 1.21^{b}	24.75 ±2.39^{a}	18.19 ± 1.98^{b}
Peonidin-3,5-dihexoside	12.39 ± 0.90^{d}	1.59 ± 0.12^{d}	37.70 ± 5.44^{c}	44.74 ± 9.72^{c}	173.06 ± 19.76^{a}	106.73 ± 14.55^{b}
Peonidin-3-glucoside	40.07 ± 1.52^{d}	12.39 ± 2.20^{f}	29.22 ±1.93^{e}	69.31 ± 1.79^{c}	134.21 ±12.39^{a}	83.42 ± 4.05^{b}
TOTAL	1612.61 ± 68.14^{b}	196.61 ± 22.90^{d}	373.45 ± 34.48^{d}	1138.75 ± 18.85^{c}	1753.54 ± 82.93^{a}	1108.25 ± 88.4^{c}

*Different letters (a–e) in rows denote statistically significant differences in individual phenolic levels among blue honeysuckle berry products by Duncan's multiple range test ($P < 0.05$); n=7. Procy…procyanidin, Q…quercetin, hex…hexoside

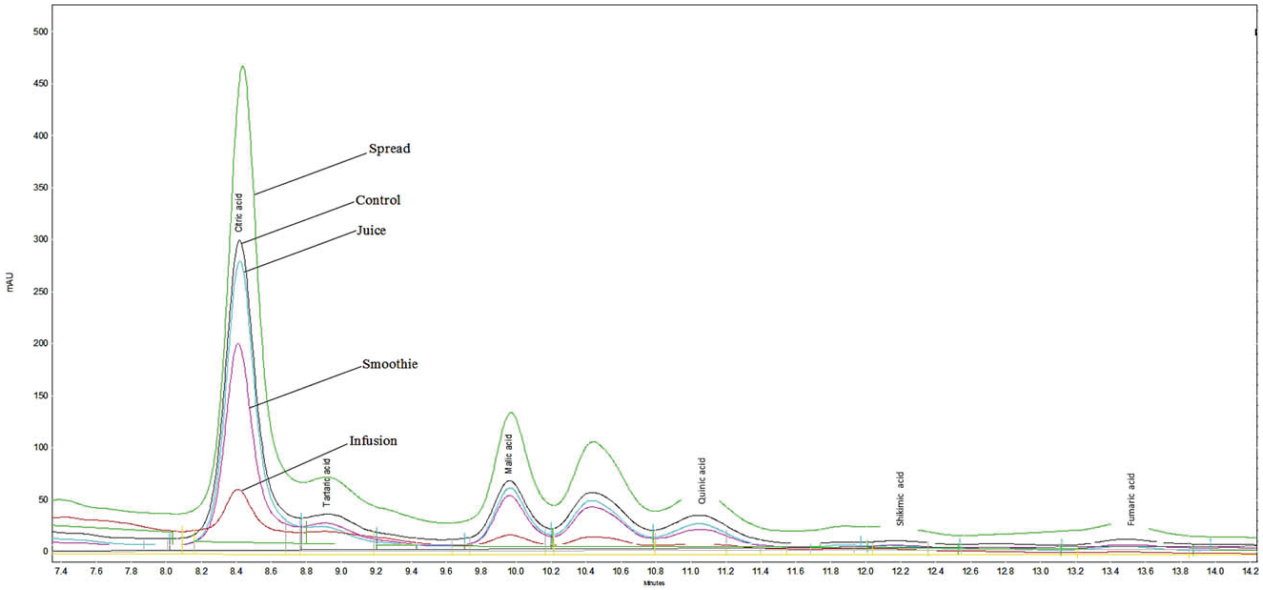

Figure 1. Chromatogram for organic acids of five different blue honeysuckle berry products (control, spread, juice, smoothie, and infusion).

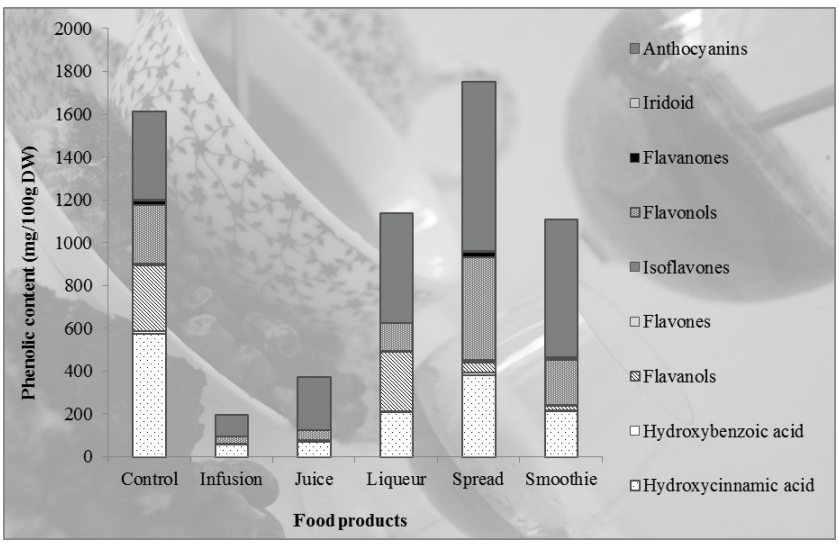

Figure 2. The content of phenolic groups in six different blue honeysuckle berry products; n = 7.

content in the processed product (Munyaka et al., 2010). In our study, the honeysuckle berries used for the spread were not crushed before heating and had the highest ascorbic acid content. Whole berries were placed in glass pots and heated to boiling, with the temperature then reduced to 60 °C. The berries lost their structure, cell walls were disrupted, and ascorbic acid oxidase was extracted and inactivated at temperatures above 70 °C. It was the opposite with the honeysuckle smoothie, for which the berries were mashed and ascorbic acid oxidase extracted from the cell walls and the reaction with ascorbic acid facilitated. Immediately thereafter, the smoothie samples were extracted with *meta*-phosphoric acid to obtain the vitamin C contents, similar to the control. Accordingly to that, the control and smoothie contain similar values of ascorbic acid: 155.89 mg/100 g and 172.88 mg/100 g, respectively (Table 1). The honeysuckle water infusion (119.17 mg/100 g) and juice (118.17 mg/100 g), with the lowest ascorbic acid contents, were processed without added acid and oxidative enzymes degraded most of the ascorbic acid. Furthermore, the stability of ascorbic acid may be connected with antioxidant active polyphenolics.

Phenolic antioxidants protect vitamin C against oxidative degradation in various food products (Gutzeit et al., 2008). The results of our study were in accordance with this. Honeysuckle berries products that had the highest anthocyanin contents also had high ascorbic acid contents. The honeysuckle spread and smoothie had high anthocyanin contents and they also had the highest ascorbic acid contents. Similarly, the honeysuckle juice and infusion, with the lowest anthocyanin contents, also had the lowest ascorbic acid contents (Table 1; Figure 2).

3.2. Sugars and organic acid contents

Three sugars (fructose, glucose, and sucrose) and six organic acids (citric, fumaric, malic, quinic, shikimic, and tartaric acid) were determined in the honeysuckle berries (Figure 1). Citric acid was predominant among the organic acids and fructose among the sugars in all honeysuckle berry products. Their contents differed between some fruit products but the honeysuckle berry spread had the highest total organic acid (566.00 mg/g) and sugar (1063.2 mg/g) contents (Table 1; Figure 1). The spread was boiled for 30 min. Water loss occurred with high temperature, causing a higher concentration of soluble solids (Yadav and Singh, 2014). This was caused by the water evaporated from the berries, leaving higher concentrations of organic acids and sugars. The honeysuckle berry infusion had the lowest sugar content (287.96 mg/g) and organic acid (152.84 mg/g) content (Table 1). This was caused by the extraction time and the permeability of the berry skin. Blue honeysuckle berries have very thick peel, which impedes the osmotic process (Oszmiański et al., 2016). Berries used for the infusion were not crushed and were barely steeped in water, and so there was poor transfer from berries to the beverage in such a short time. This resulted in a very low concentration of acids and sugars in the extract. Juice and smoothie products made from blue honeysuckle berries were not significantly distinguished from the control. Their sugar and organic acid contents were similar (Figure 1).

3.3. Individual phenolic contents

The contents regarding individual phenolic contents are presented in Table 2 and Figure 2. Based on high contents the major phenolic group in the honeysuckle berries were anthocyanins, followed by hydroxycinnamic acids and then flavanols. Selected phenolics differed among the various honeysuckle products used in the present study. The honeysuckle spread contained the highest phenolic contents of all the food products prepared from blue honeysuckle berries (1753.54 mg/100 g). Their levels were higher than those in the control treatment (1612.61 mg/100 g) (Table 1). The honeysuckle liqueur (1138.75 mg/100 g) and smoothie (1108.25 mg/100 g) also had lower but nevertheless high phenolic contents. The honeysuckle juice (373.45 mg/100 g) and infusion (196.61 mg/100 g) had poor phenolic contents (Table 2). Thermal treatment and the duration and the type of extraction solution are the main influences on the levels of the selected phenolic compounds during food processing (Senica et al., 2016). Kim et al. (2013) reported that some phenolic compounds increase with heat processing. Heat treatment destroys plant cell walls and releases the bound selected phenolic compounds. Higher temperatures can promote higher analyte solubility by increasing the solubility and mass transfer rate. Heat treatment additionally deactivates endogen oxidative enzymes, which prevent enzymatic oxidation and loss of selected antioxidant compounds (Choi et al., 2006). However, it is important that longer heating at lower temperature is better than higher temperatures for a short time (Kim et al., 2013). Additionally, heating disrupts certain oxygen enzymes, which accelerate the chemical reactions of the selected compounds. Heating of the spread thus destroyed certain oxygen enzymes and water evaporated from the berries. Thus selected phenolics were concentrated in the spread. In addition, Senica et al. (2016) reported the importance of the duration and type of extraction. The blue honeysuckle infusion had the lowest contents of total investigated phenolics. The berries were only left in the hot water for 10 min. The time was too short to transfer phenolics from the fruit into the beverage. It is important that almost all phenolics were better extracted in organic solvents than in water solvents (Senica et al., 2016). The water infusion therefore had low phenolic contents.

Anthocyanins, which form the major part of total analyzed phenolics, are responsible for fruit color and have beneficial effects on human health (Skupień et al., 2007; Mikulic-Petkovsek et al., 2015). Seven different anthocyanins were detected in all the honeysuckle berry products (Table 2). Cyanidin-3-glucoside was the most prevalent of all the studied anthocyanins as in mahonia berries (*Mahonia aquifolium* L.) (Coklar and Akbulut, 2017). The highest level of both cyanidin-3-glucoside and total anthocyanins was measured in the honeysuckle spread (321.03 mg/100 g), followed by the smoothie (309.69 mg/100 g) and the liqueur (285.14 mg/100 g) (Table 2). The lowest proportion of anthocyanins as the main contributor to total phenolic content was determined in the honeysuckle berry infusion (Figure 2). The stability of anthocyanins is affected by several factors (Symonowicz et al., 2012). Lambri et al. (2015) proposed that a short duration of high temperature is the most efficient combination of time/temperature for anthocyanin extraction. Another very important reason is anthocyanin extractability. They are better extracted in organic solvents than in water (Laleh et al., 2006; Wang and Xu, 2007; Dai and Mumper, 2010; Senica et al., 2016; Coklar and Akbulut, 2017). The juice and infusion are nonalcoholic beverages

and showed the lowest anthocyanin contents. Additionally, the juice is concentrated and reacting molecules, such as oxygen, become closer to anthocyanin structures and the rate of chemical reaction accelerates, which means lower anthocyanin contents (Wang and Xu, 2007). Khattab et al. (2016) found that different anthocyanins have different degradation kinetics in juice, which is reflected in their content in beverages. Pelargonidin-3-glucoside, cyanidin-3-glucoside, and cyanidin-3,5-diglucoside showed high reduction with processing into juice, while peonidin-3-glucoside was more stable and did not decrease much. These results are in accordance with Khattab et al.'s (2016) study.

After anthocyanins, hydroxycinnamic acids were the second major phenolic group in almost all honeysuckle berry products. A similar phenolic composition was found in mahonia berries in the study by Coklar and Akbulut (2017). The major hydroxycinnamic acid in all studied food products was chlorogenic acid (*trans*-5-caffeoylquinic acid). The spread had the highest amount of chlorogenic acid, as well as total hydroxycinnamic acids (342.10 mg/100 g), followed by the smoothie (192.49 mg/100 g) and the liqueur (186.10 mg/100 g) (Table 2). The honeysuckle infusion and juice had the lowest content of chlorogenic acid and total hydroxycinnamic acid derivatives. The selected hydroxycinnamic acids were greatly affected by heat processing. Zorić et al. (2014) reported in their study that temperatures above 100 °C for longer than 20 min decreased the contents of chlorogenic acid. Chlorogenic acid in the honeysuckle berry spread was significantly lower than in the control, as reported in Senica et al.'s (2016) study. The honeysuckle berry juice, infusion, smoothie, and liqueur had both lower chlorogenic acid and total hydroxycinnamic acid levels than the control. They are better extracted in a mixture of organic/water solution than separately in organic or water solution (Senica et al., 2016). An additional factor that affects the contents of hydroxycinnamic acids is the duration of extraction (Mikulic-Petkovsek et al., 2015). Berries in the water infusion were steeped for the shortest time and this treatment contained the lowest hydroxycinnamic acids levels.

The group of flavonols was numerous, of which quercetin-3-rutinoside was top, with approximately 67% of total flavonols. The spread made from honeysuckle berries contained the highest flavonol levels, as well as quercetin-3-rutinoside contents, of all the food products (248.92 mg/100 g) (Table 2). The smoothie also had high quercetin-3-rutinoside contents (127.01 mg/100 g). The honeysuckle infusion (24.03 mg/100 g) and juice (33.91 mg/100 g) had the lowest quercetin-3-rutinoside and total flavonols levels. The contents mostly changed with a combination of heating and extraction solution during processing into food products (Reis et al. 2012; Mikulic-Petkovsek et al., 2015; Senica et al., 2016). Organic solvent seems the best for extraction of quercetin glycosides and water the poorest. The juice and infusion are nonalcoholic beverages and they contained the lowest flavonol contents compared to products in which extraction was performed with organic solvents.

Of phenolics, the group of flavanols, which represented 20% of total phenolics in fresh berries, dropped to 3%, 2%, and 1% of total phenolics in the honeysuckle spread, infusion, and juice, respectively. They were better extracted in water and poorly in organic solvents (Senica et al., 2016). There should therefore be high flavanol levels in the infusion and juice, but there was too short a time of extraction to transfer the compounds from the berry into the beverage. Epicatechin, which is known to be sensitive to oxidation (Wang et al., 2000), composed 75% of total flavanols. The juice in our study thus contained only 1% of total flavanols. Additionally, this group showed an extremely high drop during the processing of the honeysuckle spread, which suggests that flavanols are more sensitive to heat treatment than other phenolic groups. Their heat sensitivity depends on the structural solidity of the particular compound (Chaaban et al., 2017). They contain fewer double bonds in their general structure than some other flavonoids and so only a little energy is sufficient to degrade the structure.

The group of hydroxybenzoic acids, flavanones, flavones, and isoflavones represented less than 5% of total analyzed phenolics. All of them are more soluble in organic solvents than in water. The spread and smoothie therefore had the highest reported contents, in contrast to the infusion and juice with the lowest contents, for which water was the main solvent (Figure 2).

Loganin pentoside, of the iridoids group, has important nutraceutical and health-promoting properties (Oszmiański and Kucharska, 2018). Oszmiański and Kucharska (2018) reported that they change with pressing into juice and freeze drying. Its content was present only in traces and its values significantly differed among the various products. The honeysuckle berry spread (5.06 mg/100 g) and smoothie (4.71 mg/100 g) had the highest contents, while the infusion (0.22 mg/100 g) and liqueur (0.05 mg/100 g) had very low contents of loganin pentoside (Table 2). The significant factor was the crushing of the berries (Oszmiański and Kucharska, 2018). The berries were crushed and mashed in the spread, smoothie, and juice preparation, while with the infusion and liqueur the whole berries were just soaked. In principle, berry cells were not crushed for the infusion and liqueur, and the compound did not transfer from cells into the beverages.

Dendrogram clustering showed some dissimilarity among the different blue honeysuckle products (Figure 3). It was characterized by 3 distinct branches, with the blue honeysuckle spread in one cluster, the infusion and juice

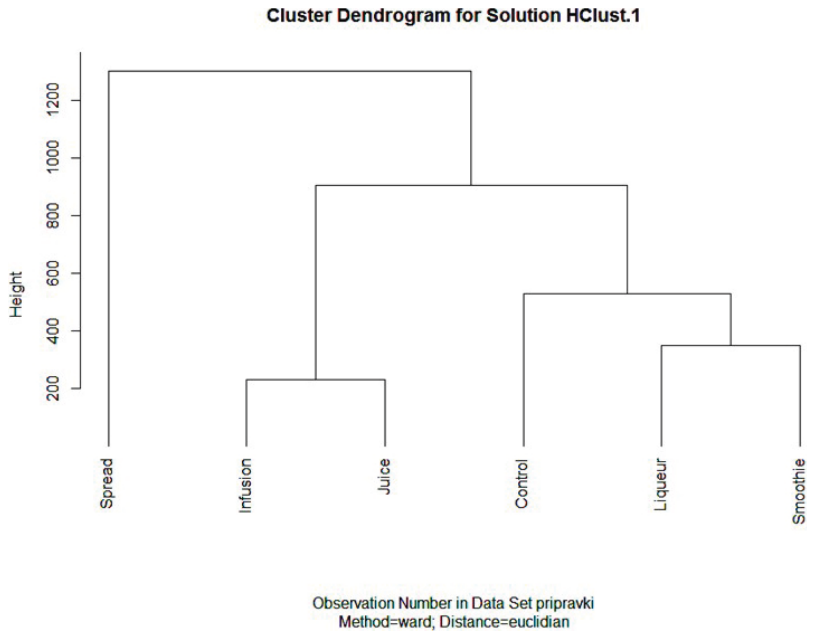

Figure 3. Dendrogram of total analyzed primary and secondary metabolites of different blue honeysuckle berry products, using Ward's method, based on square Euclidean distance.

in the second, and the smoothie and liqueur in the third. The spread made from blue honeysuckle berries had the highest contents of all analyzed compounds, while the infusion and juice from the second cluster had the lowest contents of both primary and secondary metabolites. The smoothie and liqueur from the third cluster had contents of phenolics similar to those of the control treatment.

4. Conclusion

Tea is the most consumed beverage in the world after water. In our study, the water infusion of berries (fruit tea) was not a rich source of the selected primary or secondary metabolites compared to the other studied products. The mentioned compounds poorly transferred from fruit into the beverage due to low berry skin permeability and also because of the short time of extraction. In comparison with other commonly consumed herbal teas and fruit tea bags, honeysuckle berry fruit teas are a good source of flavonoids and anthocyanins. Juice is also a high consumption product and has a low content of primary (sugars, organic acids) and secondary compounds (phenolics) compared to other food products. Its preparation is based on pressing berries, which causes cell wall disruption and enzyme oxidation. The honeysuckle liqueur made with organic solvent and the spread or smoothie with a longer time of extraction had higher contents of the studied compounds. The blue honeysuckle spread made by cooking had significantly higher levels of all analyzed compounds. Heating destroyed enzymes and evaporated water from the products; this was reflected in higher levels of the studied compounds than in the control. All products made from blue honeysuckle berries had low sugar contents, which mean they are a good food additive for diabetics. Furthermore, products such as the smoothie and spread contained high levels of phenolics, which have antioxidative properties, as did the liqueur, but because of the alcohol it is not recommended to be drunk in large amounts. All the blue honeysuckle berry products can contribute to human health.

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