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The impact of food processing on the phenolic content in products made from juneberry (*Amelanchier lamarckii*) fruits

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Short version of title: **Food processing of juneberry fruits...**

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ABSTRACT: Sugars, organic acids and phenolic compounds were analysed in the fruits of juneberry (*Amelanchier lamarckii)*. Different food products were made from fresh juneberry fruits: jam, liqueur, juice and tea. A detailed analysis of the phenolic component was performed using high pressure liquid chromatography coupled with mass spectrometry, and the content of phenolics in different products was compared with the control treatment (70% methanol). Four sugars and six organic acids were determined in the fruits. The main sugars were glucose (61 g/kg FW) and fructose (64 g/kg FW) and the acids: malic (5.85 g/kg FW) and citric acid (2.6 g/kg) were abundant. Hydroxycinnamic acids, anthocyanins and flavonol glycosides were the major phenolic groups in juneberry fruits. Fruit processing significantly affected the content of phenolic compounds in the different fruit products. Liqueur had 17% higher phenolic acid contents than the control and jam had 14% higher content than the control, calculated on the dry mass of fruit. Juneberry juice had the highest content of total analysed phenolics (298 mg/100 ml), followed by liqueur (108 mg/100ml) and tea (8 mg/100 ml). Fruits of juneberry are rich in bioactive compounds and a useful source for the food industry for making various health snacks, jellies, marmalades, alcoholic drinks, juices etc.

Keywords: *Amelanchier lamarckii*, food processing, sugars, organic acids, phenolic compounds, health products

Practical Application:

Juneberry is becoming a desirable organically grown fruit species with many views as new functional food. The content of phenolic compounds have been measured in different food products made from juneberries. The results could be useful for food and nutraceutical industry.

Introduction

Twenty-five species belong to the *Amelanchier* Medik. genus, including juneberry (*Amelanchier lamarckii* F. G. Schroed.) (Adhikari, Francis, Schutzki, Chandra, & Nair, 2005). It belongs to the Rosaceae family. This plant originated in the forests of north-eastern North America. It is also sometimes called snowy mespilus or apple serviceberry or, in Canada, serviceberry or saskatoon. The following species grow in Europe: *Amelanchier ovalis* Med., *Amelanchier alnifolia* Nutt., Canadian serviceberry (*Amelanchier canadensis* L.) and juneberry (*Amelanchier lamarckii* F. G. Schroed.) (Ochmian, Kubus, & Dobrowolska, 2013). The species differ from each other in terms of the shape of the inflorescences and the colour of the leaves. Juneberry has thicker inflorescences, while the Canadian serviceberry has rarer inflorescences (Gough, 2008). The fruits are not berries but pomes, which grown in clusters of 5-12 together. In Europe they ripen at the end of June and beginning of July. Mature fruits are dark- red to blue-violet, sweet, and about the size of peas (diameter 1-1.5 cm) with a waxy coating on long stems. The size of the fruit differs depending on the type, variety, soil and weather conditions etc. The fruits have been used in the traditional diet of indigenous people in the United States and Canada (Jurikova et al., 2012). Nowadays, fruits can be consumed fresh or can be used for juices, jams, syrups, wines, teas, pies etc (Opalko, Andrienko, & Opalko, 2016).

Amelanchier fruits have numerous required components. Ripe fruits are rich in minerals: calcium, magnesium**,** phosphorus, potassium, vitamins C, E, B6, proteins, cellulose and fibre (Jurikova et al., 2012). In comparison with blueberries (*Vaccinium corymbosum* L.), saskatoon fruits contain a higher content of calcium, fibre, proteins, magnesium, iron, thiamin and riboflavin (Mazza, 2005). In particular, the fruits contain a high level of anthocyanins (cyanidin derivatives) (Ozga, Saeed, Wismer, & Reinecke, 2007), flavonol glycosides (especially different quercetin glycosides) (Jurikova et al., 2013), tannins (Donno, Cerutti, Mellano, Prgomet, & Beccaro, 2016) and hydroxycinnamic acids (caffeic and coumaric acid derivatives)

(Lachowicz, Oszmianski, & Pluta, 2017). The composition of various chemical substances in fruits is known to depend on various factors, such as genotype, growth site, climatic conditions, degree of maturity etc.

Interest in different *Amelanchier* species is increasing year by year, mainly due to the high content of phenolic compounds in mature fruits. Phenolic compounds are extremely important in human nutrition because they have antioxidant, antimicrobial, antimutagenic and anti-inflammatory activity and thus have a positive effect on human health (Rop, Mlcek, Jurikova, Sochor, & Kizek, 2013). In addition to the above, they also contribute to resistance to the emergence of cancerous diseases (Folmer et al., 2014; Zengin, Sarikurkcu, Aktumsek, Ceylan, & Ceylan, 2014) and cardiovascular diseases (Zhao et al., 2014). The leaves and bark of serviceberry also have health effects and can be used in treatments against diabetes (Zhang, Rimando, Fish, Mentreddy, & Mathews, 2012) and as a disinfectant (Lim, 2012). Compounds derived from plants have recently been getting much attention as an alternative method for the inhibition and control of chronic degenerative disorders. Due to the increasingly fast human lifestyle, juneberry has great potential for production, because of its high nutritional and antioxidants properties (Donno et al., 2016). This fruit species will surely spread in home gardens and also in orchards, because it is a fairly undemanding plant species, which also thrives on poor soils and under hard weather conditions (high frost resistance). In Europe, juneberry has only a few diseases and pests. Birds are usually the major problem, so the fruit needs to be picked in time (Lim, 2012).

Consumers are increasingly focusing on the fruit, which has a special taste, an appropriate chemical composition and is rich in antioxidants. In some parts of Europe, cultivation of saskatoon (*Amelanchier alnifolia*) has recently been expanded, research having shown that it

contains many flavonoids that have positive health effects. In a review of scientific literature, we found that studies on *Amelanchier lamarckii* are lacking, so our aim was to analyse the content of bioactive compounds in the mature fruit of juneberry (sugars, organic acid and phenolic compounds). Additionally, we planned to examine how the different processes of product preparation affect the content of phenolic substances. It is known that different processing methods may affect the effectiveness of extraction from fruits. Extraction efficiency is affected by many elements, such as the kind of solvents and their concentration, time, pH, temperature etc. We therefore decided to perform an experiment to determine how different fruit processing procedures affect the content of phenolic compounds in a single processed product. For this purpose, we analysed the content of phenolics in fresh fruits and compared it with various food products: tea, juice, jam and liqueur. Due to the high content of natural colours (anthocyanins) in *Amelanchier* spp. fruits, this species could be useful in the future for the food industry, since synthetic colouring could be replaced by juneberry fruit extracts.

Materials and methods

Description of the experimental field and plant material

The fruits of juneberries were collected at edible maturity (8.6.2016) in the experimental plantation of the Agricultural Institute of Slovenia at Brdo pri Lukovici (latitude, 46° 10′ N; longitude, 14°41′ E). The soil texture is silty loam, rich in potassium and nitrogen and poor in phosphorus. Organic matter is high. The climate of the plantation is continental, with an excess of precipitation in spring and autumn. The annual sum of precipitation is about 1300 mm and average annual temperature 12.5 °C. We picked approximately 5 kilograms of mature fruits from different trees.

Measurements of fruit colour

The colour of the fruit was measured with a colorimeter (CR-10 Chroma, Minolta, Osaka, Japan) in the middle of the berry, by which we read C* (colour intensity or chroma), h° (colour shade - 0-360 °: 0 ° - red , 90 ° - yellow, 180 ° - green, 270 ° - blue) and L* (brightness: 0 - black, 100 - white). Measurements were made on fifty fruits.

Analysis of sugars and organic acids

Sugars and organic acids were extracted from the fresh juneberry. Ten repetitions were carried out (n = 10); each repetition included several fruits. Four grams of chopped fruit material were extracted in 16 ml of bidistilled water for half-an-hour at room temperature with frequent shaking, as reported by Mikulic-Petkovsek et al. (2019). The homogenate was centrifuged (Eppendorf Centrifuge 5810 R) at 10,000 rpm for 8 min at 10 °C. The supernatant was filtered through a 0.20 µm cellulose ester filter (Macherey-Nagel; Düren, Germany), transferred into a vial, and 20 µL of the sample was used for the analysis. The analysis of sugars and acids was done using a high-performance liquid chromatograph (HPLC) from Thermo Separation Products. For the sugars, we used a Rezex RCM-monosaccharide Ca+ (2%) column (300 mm x 7.8 mm) and for organic acids a Rezex ROA – organic acid H+ (8%) (300 mm x 7.8 mm) (Phenomenex). The mobile phase was bidestilled water for sugars and 4 mM sulfuric acid for the analysis of acids. We used the same parameters for both primary metabolites: flow rate 0.6 mL min^{-1}, column temperature 65 °C and total run 30 min. For identification and measurement of sugars, a refractive index (RI) detector was used and for acids a UV detector set at 210 nm. The concentration of an individual metabolite was calculated according to a calibration curve of corresponding standards and expressed as g kg^{-1} FW.

Preparation and extraction of juneberry products

For the extraction of phenolic compounds with the control treatment, we weighed 4 g of ground fruit and poured over 10 ml of 70% methanol containing 3% formic acid. Samples were extracted in an ice-cooled ultrasonic bath for 60 minutes. To prepare tea, we weighed 2.5 g of dried berries, ground them to a powder with liquid nitrogen and poured over 100 ml of boiling water. The extraction from powder of dry berries was done on a shaker for 15 minutes. Alcoholic liqueur was prepared by squeezing 12 grams of fresh fruit and adding 12 ml of bidistilled water. The sample was heated for 5 minutes at a temperature of 80 °C. After cooking, 20 ml of 100% ethanol was added and the bottles were closed. Liqueurs were left for 7 days at room temperature. Juice was prepared from fresh fruits; they were pressed with a juicer and cooked for 7 minutes at 80 °C. To prepare jam, we used 60 g of ground fruits, added 10 ml of bi-distilled water and mixed. The fruit mixture was boiled for 12 minutes at 80 °C. For the extraction of polyphenolic compounds from jam, we weighed 4 g of jam, added 10 ml of 70% methanol containing 3% formic acid and transferred it to an ice-cooled ultrasonic bath for 1 hour. All the prepared extracts were centrifuged for 12 minutes at 9000 rpm. The supernatant was filtered through polyamide filters (Chromafil® A-20/25, Macherey-Nagel) into vials, which were appropriately labelled. Fresh and dehydrated fruit samples were oven-dried at 105 °C for 48 hours to determine DW (AOAC Method 934.06).

For the extraction of phenolic substances during the control treatment, we weighed 4 g of ground fruit and added 10 ml of 70% methanol containing 1% formic acid. Samples were extracted in an ultrasonic bath for 60 minutes. Alcoholic liqueur was prepared be squeezing 12 grams of fresh fruit and adding 12 ml of bidistilled water. The sample was boiled for 5 minutes at a temperature of 80 °C and a power of 400 W. After cooking, 20 ml of 100% ethanol was added and the bottles closed. The flasks were left for 7 days at room temperature. Juice from fresh fruits was pressed with a juicer and cooked for 7 minutes at induction at 400 W and 80

°C. To prepare the jam, we used 60 g of ground fruit, covered with 10 ml of bidistilled water and mixed. The mixture was boiled for 12 minutes at 80 °C. For the extraction of polyphenolic substances from the jam, we weighed 4 g of chilled jam, added 10 ml of 70% methanol containing 1% formic acid and transferred it to an ultrasonic bath for 1 hour. To prepare tea, we weighed 2.5 g of dried berries, ground them into powder with liquid nitrogen and added 100 ml of boiling water. The extraction took place on a shaker for 15 minutes. All the prepared extracts were centrifuged for 12 minutes at 9000 rpm. The supernatant was filtered through polyamide filters into vials, which were appropriately labelled.

Analysis of phenolic compounds on PDA - HPLC MS^n system

Analysis of individual phenolic compounds in different extracts was performed on an HPLC (Thermo Scientific) system with a PDA detector at three wavelengths (280, 350 and 530 nm) using a mass spectrometer (MS). For mobile phase A, we used bidistilled water/acetonitrile/formic acid (96.9/3/0.1, v/v/v) and for mobile phase B: acetonitrile/bidistilled water/formic acid (96.9/3/0.1, v/v/v). Samples were eluted according to a linear gradient from 5% to 20% B in the first 15 min, followed by a linear gradient from 20% to 30% B for 5 min, then an isocratic mixture for 5 min followed by a linear gradient from 30% to 90% B for 5 min, and then an isocratic mixture for 15 min before returning to the initial conditions. Measurements were made with a Gemini C18 (Phenomenex) column at 25 °C. Determination of phenolic compounds was carried out in positive (anthocyanins) and negative ionization (all other phenolic compounds). The analyses were carried out using full scan data-dependent MS^n scanning from *m/z* 115 to 1900. The capillary temperature was 250 °C, the sheath gas and auxiliary gas were 60 and 15 units, the source voltage was 3 kV and normalized collision energy was between 20-35%. Spectral data were elaborated using the Excalibur software (Thermo Scientific). Identification of the phenolic compounds was established based on their retention

176 times and their PDA spectra in comparison with standard phenolics, and based on fragmentation
177 patterns in different MS^n modes compared with literature data. The content of individual
178 phenolic compounds was calculated using standard curves of different phenolics and expressed
179 in mg kg^{-1} DW.

180

181 Statistical analysis

182 The statistical analysis was done with the Statgraphics Plus 4.0 program (Manugistics, Inc.).
183 The analysis was performed with a one-way variance analysis (ANOVA). Statistically
184 significant differences of sugars, acids and phenolic compounds between the juneberry products
185 were compared with multiple comparison tests (Duncan test) at a 95% confidence interval and
186 between juneberry beverages with LSD test. Means and standard errors are presented (mean ±
187 SE) and statistical differences among treatments are denoted by different letters.

188

189 **Results and discussion**

190

191 Fruit quality depends on external and internal quality parameters. Colour plays a very important
192 role in external fruit quality since it is an indicator of maturity and a first characteristic in the
193 consumer's decision to buy. The results of the colour parameters for juneberry were the
194 following: C* 35.32, h° 350.86 and L* 14.16, which means that the fruits were red. In terms of
195 internal fruit quality, the content of various chemical substances, such as sugars, acids and
196 aromatic compounds, plays an important role. The average measured pH of juneberry juice
197 prepared from mature fruits was 4.00, which means that is fairly acidic.

198

199 The content of sugars and acids in berries, and their relationship, contribute to the taste of fruit.
200 The main sugars in juneberry are glucose (61 g/kg FW), fructose (64 g/kg FW) and sorbitol (51

g/kg FW). Rogiers and Knowles (1997) also reported that the mentioned three sugars were major in saskatoon berry, accounting for approximately 99% of total sugars. Sucrose gave only about 3% of all analysed sugars content. Our results of sugar content in juneberry are a little higher than with saskatoon (Rogiers & Knowles, 1997). This difference is probably explained by the different maturity stages of the fruits or the different extraction or analytical protocols used. Organic acids from the fruits can also serve as natural preservatives for various food products and they have an impact on the colour and flavour of the product. Of organic acids in the fresh fruits of juneberry, we analysed malic, tartaric, oxalic, citric, succinic and fumaric acid. Approximately half of the total acid content was malic acid (5.85 g/kg FW), followed by citric acid (2.61 g/kg FW), oxalic (1.52 g/kg FW) and tartaric acid (1.28 g/kg FW). Succinic and fumaric acid were present only in traces. As previously reported by Rogiers and Knowles (1997), malic acid was also the major organic acid in *Amelanchier alnifolia* fruit and its level was similar to our results. Malic and citric acids have not been identified in the fruits of *Amelanchier canadensis* and the amount of tartaric acid (2.9 g/kg FW) was in the same range as in our samples of juneberry fruits (Donno et al., 2016). Comparing the content of total sugars with some other berry fruit species, it can be concluded that the total sugars in juneberry (183 g/kg FW) are much higher than in red and black currants (46-118 g/kg FW) and gooseberries (78-96 g/kg FW) (Mikulic-Petkovsek et al., 2015) and in the same range as the sugar content in chokeberry (114 g/kg), rowanberry (218 g/kg FW) and eastern shadbush (159 g/kg FW) (Mikulic-Petkovsek, Schmitzer, Slatnar, Stampar, & Veberic, 2012). The high content of total sugars in juneberry is certainly due to the high content of sorbitol, which some berry species do not have. High sugar content and low acids are responsible for the high sugar:acids ratio, which gives the fruit a sweetish taste.

Among the phenolics in the fruits, 8 hydroxycinnamic acid derivatives, 4 anthocyanins, one hydroxybenzoic acid, 6 flavanols, 2 flavones and 24 flavonol glycosides were identified (Table 1). Derivatives of hydroxycinnamic acid are the major phenolic group, being approximately half of all analysed phenolic compounds in fresh juneberry fruits (control 3837 mg/kg DW). The second big group is flavonol glycosides, representing one quarter of total analysed phenolic content. Their content reached 1973 mg/kg DW berries. From the group of flavanols, catechin, epicatechin and four procyanidins were detected in the fruits. The content of all flavanols was 15% of all phenolics. Only two substances were found in the group of flavones: apigenin dirhamnoside and apigenin hydroxyhexoside. Their analysed content was 246 mg/kg DW fruits.

In terms of content, hydroxycinnamic acids were the most important phenolic group, representing 47% of total analysed phenolics (Table 2). Similarly, Lavola et al. (2012) reported that hydroxycinammic acids were the main phenolic acids in saskatoon fruit. Hydroxybenzoic acids were present only in small amounts (Lavola et al., 2012). The main phenolic acid was *trans* 5-*O*-caffeoylquinic acid (chlorogenic acid) (3594 mg/kg DW), which represented 93% of total hydroxycinnamic acids (Table 2). In other *Amelanchier* species, too, it has been reported that chlorogenic acid is the main component in their fruits (Jurikova et al., 2012; Lavola et al., 2012). It has been found that chlorogenic acid is the most valuable compound in various berries and it was confirmed that it has antioxidant properties (Agunloye et al., 2019). Two isomers of 5-caffeoylquinic acid were identified in our samples. On the chromatogram, first 5-caffeoylquinic acid was confirmed with a commercial standard of *trans* 5-*O*-caffeoylquinic acid (λ_{max} 328 nm). We suggest that the other form is *cis* 5-*O*-caffeoylquinic acid; its content was 88.5 mg/kg DW (Table 2). This isomer had a similar MS spectrum but a different UV spectrum (λ_{max} 319 nm) to chlorogenic acid. According to the finding of Jaiswal et al. (2010), chlorogenic acid exposed to natural UV light freely converts from the *trans* to the *cis* isomer in plants.

Various hexosides of *p*-coumaric, ferulic and caffeic acid, and two forms of 5-*p*-coumaroylquinic acids, were also identified in the juneberry (Table 1). The liqueur had a 17% higher content of total phenolic acids than the control and the jam had a 14% higher content than the control, calculated on the dry weight of the fruit (Table 2). In the tea, the content of hydroxycinnamic acids was only 314 mg/kg of DW fruits (Table 2). The results show that the procedure of fruit processing significantly affects the content of hydroxycinnamic acids in the final product, which was also reported by Makila et al. (2017). The consumer is certainly most interested in how many ingredients he can get by eating a particular product. Consequently, the contents were expressed per 100 ml and it was found that 205 mg of phenolic acids per 100 ml could be consumed with juice, only 42 mg/100 ml with liqueur and only 4.2 mg of phenolic acids/100 ml with aqueous extract (tea) (Figure 1A).

Another abundant group, in terms of content as well as the numbers of individual phenolics, was the group of flavonols. This group included one isorhamnetin derivative, 13 kaempferol and 10 quercetin derivatives (Table 1). In fresh fruits, the content of quercetin glycosides was 1838 mg/kg DW, kaempferol glycosides 1133 mg/kg DW and isorhamnetin-3-rutinoside 11 mg/kg DW. The major quercetin derivatives in terms of their content were quercetin dirhamnoside, two quercetin dirhamnosides, quercetin-3-galactoside, quercetin-3-rhamnoside, quercetin rhamnosyl pentoside, quercetin-3-arabinopyranoside and quercetin-3-rutinoside (Table 2). The total flavonol content in juneberry is comparable to that reported for saskatoon berry (Lachowicz et al., 2017). The highest content of quercetin (1432 mg/kg DW) and kaempferol derivatives (1852 mg/kg DW) was found in liqueur. In second position was jam, with 1521 mg/kg DW of kaempferol derivatives and 1134 mg/kg DW of quercetin derivatives. The lowest content of flavonol glycosides was found in juice and tea (Table 2). If the results of the prepared products are expressed per volume unit, it can be seen that the highest content of

total flavonol derivatives was found in juice (56.3 mg/100 ml), followed by liqueur with 30.8 mg/100 ml and tea only 4.2 mg/100 ml (Figure 1B). The results show that the process of juneberry pressing in a juicer contributed to the highest yield of flavonols. In the preparation of liqueur, the success of flavonol extraction was about half that with the juicer (Figure 1B). This is probably because the juicer crushes the berry skin more effectively, since it is known that the main source of flavonols is the skin. Various publications have reported that the skin has a higher concentration of flavonol glycosides than does berry pulp (Inglett & Chen, 2011; Ribera, Reyes-Diaz, Alberdi, Zuniga, & Mora, 2010). Previous results have also shown that a fruit maceration process achieved the best effect of phenolic extraction (Cujic et al., 2016).

The main anthocyanin in juneberry was cyanidin-3-galactoside, which represented 90% of the content of all anthocyanins analysed (Table 2). The other three anthocyanins (cyanidin-3-glucoside, cyanidin-3-xyloside and peonidin-3-glucoside) were present in a lower amount, 80 mg/kg of DW fruits. In *Amelanchier alnifolia* fruits, too, the same anthocyanin glycosides were confirmed except peonidin-3-glucoside, which was absent (Mazza, 1986; Ozga et al., 2007). The procedures of fruit processing had a significant impact on the changed levels of anthocyanins (Lafarga et al., 2019; Zorenc, Veberic, & Mikulic-Petkovsek, 2018). Our results showed that the lowest content of anthocyanins was found in tea and juice (Table 2). Only cyanidin-3-glucoside was identified in tea, the other three anthocyanins were missing. In liqueur and jam, the total anthocyanins content was statistically the highest, since they had a 1.37- to 1.43-fold higher content than the control. Previous studies have reported that the effect of anthocyanin extraction from fruits was poorest when 96% ethanol or water was used as the solvent. The maximum effect of anthocyanins extraction was achieved using 50% ethanol (Cujic et al., 2016). In our case, we used 62.5% ethanol for liqueur and the content of anthocyanins was highest in this treatment. The size of the particles of plant material also plays

an important role in the success of anthocyanins extraction. Cujic et al. (2016) found that the best effect of anthocyanins extraction was achieved when *Aronia melanocarpa* fruits were chopped into smaller particles (0.75 mm); with bigger particles the effect of extraction was worse. The reason is that smaller particles have a higher surface, which enables better mass transport.

Two monomeric forms, catechin and epicatechin, and three procyanidin dimers and one procyanidin trimer were identified among the group of flavonols in juneberry (Table 1). Total procyanidins in fresh fruits represented almost 60% of all flavanols, epicatechin accounted for 30% and catechin was present in a minor share (Table 2). It has been reported that procyanidins and epicatechin have antioxidant properties, and also anticancer activity (Santos-Buelga & Scalbert, 2000). Ursini et al. (2001) found that the level of their polymerization has an impact on their antioxidant activity. Flavanols were not found in tea, in juice their content was very low, 58.4 mg/kg DW, while liqueur had significantly the highest flavanol content (2020 mg/kg DW) (Table 2). They have a very low content per unit volume (Figure 1C). The liqueur contained only 18.9 mg of flavanols/100 ml and juice 18.4 mg/100 ml. It can be concluded from this that the extraction of flavanols from the fruits and seeds of juneberry with different product preparation processes was fairly poor. This is actually very good, since a high content of flavanols in food products is not desired by consumers, because they contribute to a bitter and unpleasant astringent taste when consumed (Aron & Kennedy, 2008). It is known that high procyanidin content is characteristic of young and unripe fruits (Lesschaeve & Noble, 2005).

Two flavones, apigenin dirhamnoside and apigenin hydroxyhexoside, and gallic acid from the group of hydroxybenzoic acids provide a minor share of the total phenolic content. All three phenolics together represent only 3% of total phenolics in fresh juneberry. We did not identify

any of the listed phenolics in tea, only apigenin dirhamnoside was found in juice, while liqueur had the highest content of all of these phenolics. Fresh juice contained per 100 ml 6.72 mg of apigenin derivatives and liqueur 5.05 mg apigenin derivatives/100 ml (data not shown). The effectiveness of polyphenolic extraction is influenced by various factors, such as the method of plant grinding or maceration, the type of solvent, the ratio between the organic and the aqueous phases of the solvent, the temperature and the time of extraction. Cujic et al (2016) reported that the effect of total phenolics extraction from chokeberry fruits was greatest with 50% ethanol, while it was reduced by increasing the concentration of ethanol in the extraction solvent. The results of some studies suggest that the use of an aqueous-organic solvent mixture is a better choice for good and efficient extraction of phenolic substances than using only organic solvent or only water (Felix et al., 2018; Yang, Ou, Zhang, Zhou, & Ma, 2017). Different procedures for the preparation of food products certainly affect the content of chemical substances in the final product (Kim et al., 2018; Shinwari & Rao, 2018). Michalczyk and Macura (2010) found that pasteurization drastically decreases the total phenolic content of the final product in comparison with freezing.

Conclusion

Juneberry fresh fruits are rich in sugar content and bioactive compounds, especially in the content of chlorogenic acid, cyanidin-3-galactoside and in different kaempferol- and quercetin glycosides. The fruits of juneberry (*Amelanchier lamarckii*) can therefore be used as a source of important bioactive components in various food products, such as tea, juice, food supplements, alcoholic beverages etc. The results of the study showed that the different methods of fruit processing of various products significantly affect the changes in the content of certain bioactive substances. The results showed that alcoholic liqueur had the highest content of phenolic substances per unit of the used fruit weight, followed by jam. The lowest phenolic

content was found in the aqueous extract (tea). The results of our research on the content of chemical components in the fruits of juneberry open new possibilities of introducing this fruit species into orchard production and its use in the food, nutraceutical and pharmaceutical industries.

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Authors contributions

D. Koron collected plant material. M. Mikulic-Petkovsek and D. Rusjan performed the experiment, interpreted the results and prepared the manuscript.

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505 TABLES

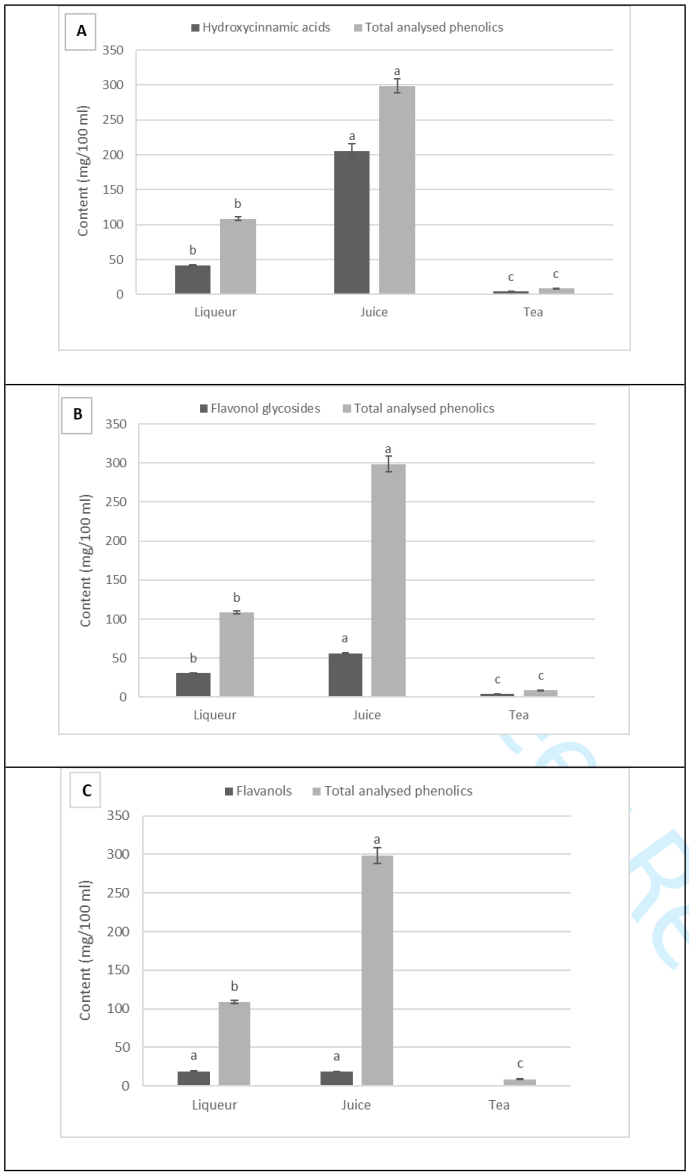
506 Table 1: Identification of phenolic compounds and their presence in different juneberry products in negative and positive ions with HPLC-MS and MS^2.

Phenolic compounds	$[M]^+$ or $[M-H]^-$ (*m/z*)	MS^2 (*m/z*)	Control	Juice	Jam	Liqueur	Tea
Hydroxycinamic acid derivatives							
cis-5-Caffeoylquinic acid	353	191, 179	+	+	+	+	+
Caffeic acid hexoside	341	179, 161	+	+	+	+	+
p-Coumaric acid hexoside	325	163	+	+	+	+	+
Ferulic acid hexoside 1	355	193	+	+	+	+	+
Ferulic acid hexoside 2	355	193	+	nd	+	+	nd
Chlorogenic acid	353	191, 179, 173, 135	+	+	+	+	+
5-*p*-Coumaroylquinic acid 1	337	191, 163	+	+	+	+	+
5-*p*-Coumaroylquinic acid 2	337	191, 163	+	+	+	+	+
Hydroxycinnamic acid derivatives							
Gallic acid	169	125	+	nd	nd	+	nd
Flavones							
Apigenin dirhamnoside	561	269	+	+	+	+	nd
Apigenin hydroxyhexoside	449	269	+	nd	+	+	nd
Flavanols							
Epicatechin	289	245	+	+	+	+	nd
Catechin	289	245	+	nd	+	+	nd
Procyanidin dimer 1	577	451, 425, 407, 289	+	nd	nd	+	nd
Procyanidin dimer 2	577	451, 425, 407, 289	+	nd	+	+	nd
Procyanidin dimer 3	577	451, 425, 407, 289	+	nd	+	+	nd
Procyanidin trimer	865	577, 425, 407, 289	+	+	+	+	nd
Flavonol derivatives							
Isorhamnetin-3-rutinoside	623	315	+	nd	nd	+	nd
Kaempferol-3-galactoside	447	285	+	+	+	+	nd
Kaempferol-3-glucoside	447	285	+	+	+	+	+
Kaempferol-3-rutinoside	593	285	+	nd	nd	+	nd
Kaempferol pentoside 1	417	285	+	+	+	+	+

508 **Table 2.** The content of phenolic compounds analysed in different juneberry products (mg/kg DW).

Phenolic compounds	Control	Juice	Jam	Liqueur	Tea
Hydroxycinamic acid derivatives					
cis-5-Caffeoylquinic acid	88.5 ± 3.0 c	11.0 ± 0.4 d	127 ± 5.0 b	194 ± 6.0 a	9.59 ± 0.56 e
Caffeic acid hexoside	41.7 ± 2.4 c	6.94 ± 0.64 e	64.9 ± 3.3 b	85.1 ± 5.1 a	10.6 ± 1.0 d
p-Coumaric acid hexoside	9.25 ± 0.17 c	1.71 ± 0.04 e	12.4 ± 0.6 b	15.9 ± 0.4 a	4.33 ± 0.23 d
Ferulic acid hexoside 1	45.8 ± 2.3 c	6.27 ± 0.11 d	57.2 ± 2.1 b	64.5 ± 2.1 a	4.64 ± 0.59 d
Ferulic acid hexoside 2	6.62 ± 0.27 c	-	10.3 ± 0.4 b	15.0 ± 0.5 a	-
Chlorogenic acid	3594 ± 219 a	559 ± 34 b	4060 ± 234 a	4024 ± 134 a	279 ± 10 c
5-*p*-Coumaroylquinic acid 1	23.1 ± 1.4 c	2.85 ± 0.09 d	32.5 ± 1.2 b	49.3 ± 2.3 a	2.85 ± 0.44 d
5-*p*-Coumaroylquinic acid 2	28.1 ± 2.0 c	3.47 ± 0.11 d	38.4 ± 1.5 b	50.3 ± 2.5 a	3.75 ± 0.40 d
Hydroxycinnamic acid derivatives					
Gallic acid	8.11 ± 0.56 b	-	-	16.4 ± 2.5 a	-
Flavones					
Apigenin dirhamnoside	223 ± 9.0 c	21.4 ± 1.3 d	348 ± 14 b	507 ± 16,0 a	-
Apigenin hydroxyhexoside	23.5 ± 0.8 c	-	29.1 ± 1.5 b	34.0 ± 0.9 a	-
Flavanols					
Epicatechin	353 ± 26.0 b	30.8 ± 0.8 c	502 ±14.0 a	530 ± 50 a	-
Catechin	154 ± 8.0 b	-	183 ± 8.0 a	107 ± 5.0 c	-
Procyanidin dimer 1	157 ± 10 b	-	-	312 ± 14 a	-
Procyanidin dimer 2	204 ± 7.0 b	-	243 ± 14 b	380 ± 24 a	-
Procyanidin dimer 3	126 ± 7.0 c	-	157 ± 6.0 b	337 ± 11 a	-
Procyanidin trimer	215 ± 12 c	27.6 ± 1.0 d	268 ± 11 b	355 ± 13 a	-
Flavonol derivatives					
Isorhamnetin-3-rutinoside	11.1 ± 1.1 c	-	-	16.8 ± 1.0 a	-
Kaempferol-3-galactoside	40.5 ± 1.9 c	2.58 ± 0.06 d	56.9 ± 2.3 b	76.4 ± 2.8 a	1.57 ± 0.15 e
Kaempferol-3-glucoside	81.8 ± 3.5 c	5.46 ± 0.14 d	113 ± 5.0 b	139 ± 2.0 a	-
Kaempferol-3-rutinoside	1.05 ± 0.02 b	-	-	1.48 ± 0.02 a	-
Kaempferol pentoside 1	87.6 ± 4.1 c	5.14 ± 0.13 d	120 ± 5.0 b	158 ± 5.0 a	3.46 ± 0.39 e
Kaempferol pentoside 2	27.6 ± 0.8 c	1.86 ± 0.04 d	35.6 ± 1.5 b	44.4 ± 1.1 a	-
Kaempferol dirhamnoside	288 ± 15 c	26.3 ± 0.4 e	393 ± 16 b	499 ± 16 a	40.4 ± 4.0 d
Kaempferol coumaroyl acetylrhamnoside	80.4 ± 4.7 c	6.40 ± 0.10 d	99.1 ± 4.7 b	130 ± 12 a	-
Kaempferol rhamnoside	48.3 ± 2.2 b	3.38 ± 0.10 d	73.4 ± 3.7 a	74.9 ± 6.6 a	3.95 ± 0.17 c
Kaempferol rhamnosyl pentoside 1	112 ± 5.0 c	12.2 ± 0.2 e	149 ± 6.0 b	172 ± 2.0 a	24.2 ± 2.0 d
Kaempferol rhamnosyl pentoside 2	49.0 ± 1.3 c	-	58.3 ± 2.4 b	69.3 ± 1.0 a	11.7 ± 1.0 d
Kaempferol rhamnosyl hexoside 1	106 ± 4.0 c	10.9 ± 0.1 e	139 ± 5.0 b	154 ± 3.0 a	27.8 ± 2.6 d
Kaempferol rhamnosyl hexoside 2	175 ± 8.0 c	19.6 ± 0.3 e	235 ± 10 b	277 ± 6.0 a	39.9 ± 3.7 d
Kaempferol rhamnosyl hexoside 3	35.6 ± 1.1 c	3.15 ± 0.05 e	49.6 ± 2.1 b	57.2 ± 1.7 a	8.49 ± 0.55 d
Quercetin-3-galactoside	74.7 ± 3.9 c	6.81 ± 0.11 d	102 ± 4.0 b	129 ± 4.0 a	-
Quercetin-3-glucoside	32.0 ± 0.7 c	2.91 ± 0.04 d	37.9 ± 1.5 b	45.2 ± 0.7 a	-
Quercetin-3-rutinoside	69.2 ± 2.2 c	6.17 ± 0.12 e	86.4 ± 3.7 b	95.2 ± 1.8 a	25.3 ± 1.3 d
Quercetin-3-rhamnoside	66.4 ± 4.8 b	6.71 ± 0.67 c	87.6 ± 2.4 b	195 ± 17 a	8.71 ± 1.28 c
Quercetin-3-arabinopyranoside	72.6 ± 3.4 c	4.63 ± 0.11 d	102 ± 4.0 b	137 ± 5.0 a	-
Quercetin dirhamnoside	103 ± 5.0 c	11.1 ± 0.2 e	136 ± 5.0 b	157 ± 2.0 a	22.1 ± 1.9 d
Quercetin glycoside 1	110 ± 4.0 c	11.0 ± 0.2 e	143 ± 6.0 b	179 ± 4.0 a	26.3 ± 2.9 d
Quercetin glycoside 2	127 ± 6.0 c	14.2 ± 0.4 d	173 ± 7.0 b	189 ± 3.0 a	26.6 ± 3.2 d
Quercetin rhamnosyl pentoside	160 ± 7.0 c	17.2 ± 0.3 e	236 ± 10 b	262 ± 7.0 a	36.9 ± 2.1 d
Quercetin coumaroyl acetylrhamnoside	14.3 ± 1.0 b	1.45 ± 0.15 c	19.0 ± 0.5 b	42.2 ± 3.8 a	1.89 ± 0.28 c
Anthocyanins					
Cyanidin-3-galactoside	787 ± 75 a	21.8 ± 1.3 b	921 ± 64 a	870 ± 35 a	23.4 ± 1.2 b
Cyanidin-3-glucoside	24.7 ± 1.9 c	7.24 ± 0.49 d	105 ± 5.0 b	176 ± 7.0 a	-
Cyanidin-3-xyloside	41.4 ± 3.2 b	5.86 ± 0.40 c	127 ± 2.0 a	152 ± 3.0 a	-
Peonidin-3-glucoside	13.4 ± 1.0 b	1.90 ± 0.13 c	41.0 ± 0.8 a	49.2 ± 1.1 a	-

510 FIGURES



511

512 **Figure 1.** The content of some phenolic groups and total analysed phenolics analysed in different juneberry
513 beverages (mg/100 ml). Different letters (a-c) indicate significant differences in each phenolic group or in total
514 analysed phenolics among different products by LSD test.