

The bioactive compounds of sweet cherry fruits influenced by cultivar/rootstock combination

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Abstract: This paper investigates the effect of rootstock (Gisela 6, PiKu 1 and SL 64) on the total phenol content, total anthocyanin content, the content of the individual phenols and the antioxidant activity in fruits of two sweet cherry cultivars, 'Kordia' and 'Regina'. The total phenolic content determined by the spectrophotometric method using Folin-Ciocalteu reagent varied from 34.84 to 149.28 to mg GAE/100 g FW depending on the cultivar/rootstock combination. The concentration of total anthocyanins was determined by using the pH-differential method and it ranged from 0.46 to 11.54 mg CGE/100 g FW. Highest level of the total phenolic content and concentration of the total anthocyanins content was detected in the cultivar 'Regina' grafted onto the Gisela 6 rootstock. Neochlorogenic acid, catechin, chlorogenic acid and quercetine-3-O-glucoside were detected using HPLC method. Significant variation of detected individual polyphenols in sweet cherry fruits grafted on different rootstocks was observed. The lowest content of individual polyphenols was measured in 'Regina' cultivar grafted on the SL 64. Ferric reducing antioxidant power assay indicated that all investigated fruits possessed similar antioxidant activity. There was a statistically significant correlation observed between the total phenolic content and antioxidant activity (correlation coefficient 0.972, *P*-value below 0.01), as well as between the anthocyanins and antioxidant activity (correlation coefficient 0.855, *P*-value below 0.01).

Keywords: sweet cherry; antioxidants; polyphenols; anthocyanins; rootstock

Sweet cherry (*Prunus avium* L.) presents one of the most popular fruits worldwide based on its high content of many phytonutrients and bioactive compounds which may significantly contribute to a health-promoting activity (Gonçalves et al. 2018). Bioactive compounds in sweet cherries are mostly polyphenols, including phenolic acids and flavonoids, which are associated with their antioxidant activity (Gonçalves et al. 2004; Usenik et al. 2008; González-Gómez et al. 2010; Usenik et al. 2010; Pacifico et al. 2014). It is generally known that phenolic compounds have a protective

effect against oxidative stress (Szajdek, Borowska 2008), and numerous studies have identified various beneficial health effects associated with their intake (Antognoni et al. 2020; Domínguez-Perles et al. 2020). Several studies have shown a direct relation between the levels of phenolic compounds and antioxidant activity in cherry extracts (Tomás-Barberán et al. 2013).

The production of sweet cherry in the world, and in Bosnia and Herzegovina, has been constantly increasing for the last 20 years. In 2023, sweet cherry was grown on 5.479 ha and 9.715 t were produced

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(FAOSTAT 2023) in Bosnia and Herzegovina and were characterized by low yields. The main reason for such occurrence was found to be the use of old varieties and generative rootstock which are characterized by high vigour, difficult harvesting, and high costs of production (Drkenda et al. 2012). The goals set in the creation of new cherry cultivars were large fruits, less susceptibility to fruit cracking, self-fertilization, extension of the ripening season, light or dark red colour, firmness, sweetness, and taste (Sansavini, Lugli 2005). The red colour of the sweet cherry fruit is used as an indicator of ripeness and is directly related to the content and concentration of anthocyanins (Usenik et al. 2015).

The quality of the sweet cherry fruit mainly depends on the genotype of the cultivar (Usenik et al. 2008), environmental conditions of the area where it is grown (Tomás-Barberán et al. 2013; Skrzyński et al. 2016), maturity stages (Serradilla et al. 2012), as well as the genotype of the rootstock on which the cultivar is grafted (Scalzo et al. 2005).

Many studies have been conducted on the physical, chemical, pomological, and nutritional properties of sweet cherry in neighbouring countries (Usenik et al. 2008, 2010, 2015; Milinović et al. 2016; Skrzyński et al. 2016; Đorđević et al. 2021; Milatović et al. 2021). There has been no detailed research on the rootstock/cultivar effect on the chemical properties and synthesis of bioactive substances in sweet cherry fruit in Bosnia and Herzegovina. The aim of this research was to evaluate the effect of rootstock on bioactive compounds in 2 sweet cherry cultivars ('Kordia' and 'Regina') under the specific climatic conditions in Herzegovina region.

MATERIAL AND METHODS

The research was conducted in a commercial orchard „Jaffa komerc” Blagaj (43°16'58.44"N 17°50'45.16"E; 45 m above mean sea level) in the Herzegovina region during 2022. The average annual temperature in the region is about 14.8 °C, and the total precipitation is 1439.3 mm. The orchard was planted in 2014 and planting distance was 4.5 × 3.6 m. The soil in the orchard is described as sandy slightly alkaline clay loam (pH H₂O 8.04; pH KCl 7.35) and with 3.78% of humus. The soil is water-permeable, well aerated with stable microaggregates. Drip irrigation was used in the experimental orchard and all the cherry trees were grown under the same stan-

dard agronomic techniques (fertilization, irrigation, and pest control). Fruits from two sweet cherry cultivars, 'Kordia' and 'Regina', grafted on three rootstocks, Gisela 6, PiKu 1 and SL 64; were investigated. The ripening period, corresponding to the commercial maturity of the fruit, was determined based on the maturity indicators such as firmness, colour, and soluble solids content.

All chemicals used in this research were of analytical grade and were obtained from Sigma-Aldrich (Germany).

Sample preparation. 100 g of frozen sweet cherry fruits from each cultivar was homogenized using a blender. 2 g of homogenized sample was extracted with 25 mL of 80% acidified methanol (1% HCl) for 15 minutes at 35 °C in ultrasonic bath. Samples were then centrifuged at a room temperature for 5 minutes at 2 000 rpm. For HPLC analysis samples were ultra-filtrated into vials. The extracts were stored at –20 °C until use.

HPLC analysis of polyphenolic compounds. High performance liquid chromatography (HPLC) analysis was performed on Shimadzu HPLC system equipped with a UV/Vis detector. The chromatographic separation was performed on an Eclipse plus C18 (3.5 µm, 4.6 × 150 mm) column. The flow rate was 0.8 mL/min, the sample injection was 10 µL. The temperature in the column oven was set to 35 °C. Total run time was 110 minutes per sample extract injection. For separation mobile phase composed of an aqueous solution of phosphoric acid concentration of 0.01 M (mobile phase A) and methanol (mobile phase B) was used, run as a gradient with conditions described in Table 1. Measurements were performed at 320 nm for neochlorogenic acid, catechin and chlorogenic acid, 280 nm for *p*-coumaric acid and 370 nm for quercetin-3-*O*-glucoside. Polyphenolic compounds were identified by comparing retention times with those of authentic standards. Calibration curve of the standards was made by diluting standard mix in acidified 80% methanol to 5–20 µg/mL for standards. Samples were spiked with standard solution for confirmation. All results were expressed as mg per 100 g of fresh weight (mg/g FW).

Determination of total phenolic content (TP). Total phenolic content was determined by Folin-Ciocalteu method as described by Singleton et al. (1999) and as previously described by Kazazic et al. (2016). Gallic acid was used to prepare the standard curve, and the results were expressed as mg

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Table 1. HPLC mobile phase gradient program for HPLC analysis of polyphenols

| Time (min) | Volume (%) mobile phase A | Volume (%) mobile phase B |
|------------|---------------------------|---------------------------|
| 0–30 | 82 | 18 |
| 30–70 | 70 | 30 |
| 70–75 | 55 | 45 |
| 75–80 | 0 | 100 |
| 80–110 | 82 | 18 |

Mobile phase A – phosphoric acid concentration of 0.01 M; mobile phase B – Methanol

of gallic acid equivalents per 100 g of fruits FW (mg GAE/100 g FW).

Determination of the total anthocyanin content (TA). The total anthocyanin content was determined by using the pH-differential method as described by Zhishen, Mengcheng and Jianming (1999) with some modifications described by Kazazic et al. (2022). The total anthocyanin content was expressed as mg of cyanidin-3-glucoside equivalent (CGE) per 100 g of fruits FW.

Ferric reducing antioxidant power (FRAP) assay. A modified FRAP method was used to determine antioxidant activity as described in work by Kazazic et al. (2016). The antioxidant capacity was determined using the calibration curve and represented as mmol FeSO₄ equivalents per 100 g of fruits FW.

Statistical analysis. All experiments were conducted in triplicates. The values are expressed as the mean ± standard deviation. Statistical analysis was carried out through Excel (Microsoft Corporation, USA) and SPSS software (V.18; Statistical software, SPPS. Inc., USA).

RESULTS AND DISCUSSION

There is a growing interest in food that not only tastes good and is safe to consume, but also offers health benefits. Fruits are known to contain high levels of antioxidant compounds which can protect against cancer and heart diseases. Cherries are particularly beneficial due to their high content of polyphenol groups, mainly anthocyanins. The content of biochemical compounds in sweet cherries is influenced by various factors such as the cultivar's genetic characteristics, rootstock, maturity, climatic and geographic conditions in which they are grown. Influence of rootstock on the quality of sweet cherries has been more extensively studied last decade. In our research two sweet cherry cultivars ('Kordia' and 'Re-

gina') grafted on 3 rootstocks (Gisela 6, PiKu 1 and SL 64) were examined. All fruits were harvested in full maturity and used for analysis. Cherry fruit samples were gathered when they reached commercial maturity, with a slight difference of only 3 days in the harvesting time for all the tested rootstock/variety combinations (from 31st May to 3rd June). Specifically, the combinations 'Kordia'/Gisela 6, 'Kordia'/PiKu 1, and 'Regina'/Gisela 6 were harvested on the 31st of May, while 'Kordia'/SL 64, 'Regina'/PiKu 1, and 'Regina'/SL 64 were collected on the 3rd of June. The close timing of ripening can be attributed to the relatively high daily temperatures during the ripening period, which led to an explosive ripening process for all the rootstock/variety combinations, resulting in a rapid change in skin color from green to red. Additionally, the presence of sandy soil had an impact, as it tends to accelerate fruit ripening compared to deep, heavy soils. Consequently, these factors caused significant fluctuations in the chemical composition of the fruits, as they were unable to accumulate their components gradually. The total phenolic content in the cultivar/rootstock combination varied from 34.84 to 149.28 mg GAE/100 g FW (Table 2).

The concentration of total anthocyanins in samples ranged from 0.46 to 11.54 mg CGE/100 g FW. The combination of the cultivar 'Regina' grafted onto Gisela 6 rootstocks showed the highest level of the total anthocyanin content (11.54 ± 0.36 mg CGE/100 g FW) and total phenol content (149.28 ± 2.81 mg GAE/100 g FW), while the combination of the cultivar 'Kordia' grafted onto Gisela 6 rootstocks showed the lowest content of total anthocyanins (0.46 ± 0.21 mg CGE/100 g FW) and low content of total phenols (36.40 ± 2.63 mg GAE/100 g FW). The lowest total phenol content was determined in 'Regina' cultivar grafted on SL 64 rootstock (34.84 ± 1.77 mg GAE/100 g FW). Total phenol content of 'Kordia' cultivar demonstrated less variation in combination with individual rootstocks, while this variation is higher in 'Regina' cultivar.

Table 2. The content of total phenols (TP), total anthocyanins (TA) and antioxidant activity (AA) by FRAP method in sweet cherries.

| Variety/root-stock | TP (mg GAE/100 g FW) | TA (mg CGE/100 g FW) | AA (mmol Fe ²⁺ /100 g FW) |
|--------------------|----------------------|----------------------|--------------------------------------|
| 'Regina'/SL 64 | 34.84 ± 1.77 | 1.04 ± 0.44 | 0.28 ± 0.01 |
| 'Regina'/PiKu 1 | 110.82 ± 6.97 | 9.52 ± 1.38 | 0.65 ± 0.01 |
| 'Regina'/Gisela 6 | 149.28 ± 2.81 | 11.54 ± 0.36 | 0.61 ± 0.01 |
| 'Kordia'/SL 64 | 57.43 ± 4.38 | 3.10 ± 1.02 | 0.40 ± 0.01 |
| 'Kordia'/PiKu 1 | 81.77 ± 6.10 | 7.15 ± 0.36 | 0.75 ± 0.03 |
| 'Kordia'/Gisela 6 | 36.40 ± 2.63 | 0.46 ± 0.21 | 0.26 ± 0.00 |

According to Kim et al. (2005), the total phenolic content of sweet cherry cultivars ranged from 92.1 to 146.8 mg GAE/100 g FW, which aligns well with the findings of this study. 'Regina' cultivar had total phenolic content of 104.3 ± 6.6 mg/100 g FW (Kim et al. 2005). Vangdal and Slimestad (2006) reported that sweet cherry cultivars had a total phenolic content ranging from 23 to 168 mg/100 g FW in a study conducted in Norway. However, Milinovic et al. (2016) found a higher total phenolic content in 'Regina' and 'Kordia' cultivars grown on PiKu 1 and Gisela 6. These differences could be explained by the influence of geographical location, temperature, and rain amount. Different UV index can cause differences in the concentration of flavonoids, while the average temperature in different locations can cause differences in the concentration of anthocyanins.

Out of the three different rootstocks investigated in this research, two are of heterogenic origin (PiKu 1 and Gisela 6) (Milinovic et al. 2016). Observed difference in the total phenol content depends not only on genetic origin of rootstock but on cultivars as well. Metabolism adjustments of genetically different rootstock and cultivar can cause high stress levels which can be used to explain higher content of total phenols in sweet cherries grafted on heterogenic rootstock (Usenik et al. 2005). Jakobek et al. (2009) previously reported that cultivar Lapins had higher total phenol content grafted on heterogenic rootstocks and highest content of total phenols was detected when this cultivar was grafted on PiKu 1.

Reductive capacity of methanol sweet cherry extracts determined by the FRAP method is in the range of 0.26 ± 0.00 mmol Fe²⁺/100 g FW detected for 'Kordia'/Gisela 6 to 0.75 ± 0.03 mmol Fe²⁺/100 g FW in 'Kordia'/PiKu 1. Antioxidant activity of the investigated sweet cherry cultivars were lower than previously reported (Halvorsen et al. 2002; Vangdal, Slimestad 2006; Djapo et al. 2020).

The correlations between total phenolic content and antioxidant activity (0.972, $P < 0.01$) and anthocyanins and antioxidant activity (0.855, $P < 0.01$) were statistically significant.

The content of individual polyphenolic compounds was determined by HPLC using the mobile phase composition and gradient conditions as described in Table 1.

It was previously reported that major phenolic compounds in sweet cherries are hydroxycinnamic acids, namely neochlorogenic acid and *p*-coumaroylquinic acid. The flavonol glycosides in cherries included quercetin 3-rutinoside, quercetin 3-glucoside, kaempferol 3-rutinoside, and isorhamnetin 3-rutinoside. Jakobek et al. (2009) reported that phenolic acids such as chlorogenic acid, neochlorogenic acid, *p*-coumaric acid derivative and quercetin-3-rutinoside were found in sweet cherry cultivar Lapins grown on PiKu 1 rootstock. Variation in the content of individual polyphenols is shown in Table 3. Neochlorogenic acid, catechin, chlorogenic acid and quercetin-3-*O*-glucoside were detected.

Out of the researched polyphenolic compounds, most abundant was catechin followed by neochlorogenic and chlorogenic acid. We were not able to detect presence of *p*-coumaric acid. The lowest content of individual polyphenols was measured in 'Regina' grown on the SL 64.

Our results indicated lower content of neochlorogenic acid and higher content of chlorogenic acid for 'Kordia' and 'Regina' cultivar grafted on Gisela 6 and PiKu 1 compared to results by Milinovic et al. (2016). However, the exception was the content of neochlorogenic acid in combination 'Regina'/PiKu 1 where we detected higher values.

The content of neochlorogenic acid and catechin of 'Kordia' cultivar in combination with rootstocks was decreasing in following order: PiKu 1 > SL 64 > Gisela 6, whereas for chlorogenic acid PiKu 1 > Gisela 6 > SL 64. Higher content

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Table 3. The content of individual polyphenols expressed in mg/100 g FW

| Cultivar/root-stock | Neochlorogenic acid | Catechin | Chlorogenic acid | <i>p</i> -coumaric Acid | Quercetin-3-O-glucoside |
|---------------------|-----------------------------|------------------------------------|-----------------------------------|-------------------------|--------------------------------|
| ‘Regina’/SL 64 | B.D.L. | 1.86 ± 0.01 ^a | 0.18 ± 0.01 ^a | N.D. | 0.89 ± 0.01 ^a |
| ‘Regina’/PiKu 1 | 9.53 ± 0.09 ^e | 6.57 ± 0.19 ^{b, a} | 7.66 ± 0.34 ^f | N.D. | 1.60 ± 0.10 ^{c, a} |
| ‘Regina’/Gisela 6 | 3.74 ± 0.02 ^{b, e} | 17.98 ± 0.33 ^d | 3.10 ± 0.01 ^d | N.D. | 1.89 ± 0.12 ^e |
| ‘Kordia’/SL 64 | 4.59 ± 0.01 ^c | 19.86 ± 0.57 ^{e, a, b} | 1.29 ± 0.01 ^{b, a, d} | N.D. | 1.76 ± 0.04 ^{d, a} |
| ‘Kordia’/PiKu 1 | 7.76 ± 0.21 ^{d, c} | 34.82 ± 1.10 ^{f, d} | 6.87 ± 0.50 ^{e, f} | N.D. | N.D. |
| ‘Kordia’/Gisela 6 | 3.20 ± 0.02 ^a | 17.68 ± 0.39 ^{c, a, b, e} | 2.06 ± 0.08 ^{c, a, d, b} | N.D. | 1.06 ± 0.06 ^{b, e, d} |

Means in the same columns followed by the same letter (a–f) are not significantly different at the 5% level of probability ($P < 0.05$)

B.D.L. – below detection level; N.D. – not detected

of quercetine-3-*O*-glucoside was detected in ‘Kordia’/SL 64 combination compared to ‘Kordia’/ Gisela 6, while no quercetine-3-*O*-glucoside was detected in ‘Kordia’/ PiKu 1.

The content of neochlorogenic acid and chlorogenic acid of ‘Regina’ cultivar in combination with rootstocks was decreasing in following order: PiKu 1 > Gisela 6 > SL 64. Catechin and quercetin-3-*O*-glucoside content was decreasing in following order: Gisela 6 > PiKu 1 > SL 64.

The results revealed that the content of the individual polyphenols in sweet cherries differed significantly depending on the rootstock.

CONCLUSION

The obtained results indicate that the content of bioactive components in the fruits of the investigated cultivars ‘Kordia’ and ‘Regina’ is significantly influenced by the researched rootstocks (PiKu 1, Gisela 6 and SL 64) and the cultivar/rootstock interaction. The highest content of investigated individual polyphenols was detected in fruits harvested from PiKu 1 rootstock in both cultivars. The process of graft incompatibility involves complex physiological factors, and despite the ongoing research efforts, its mechanism remains largely unclear. Particularly in *Prunus* plants, there is a lack of understanding and limited approaches to studying the metabolic processes that take place during the union of scion and rootstock. Developing methods to identify potentially incompatible graft combinations early on can help prevent financial losses and delays.

The obtained results will serve as a basis for recommendations to agricultural producers when

choosing grafting components when raising new cherry plantations, as well as to consumers when choosing better quality fruits in terms of their health value, primarily as a significant source of bioactive compounds.

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