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INFLUENCE OF ROOTSTOCK/CULTIVAR COMBINATIONS ON BIOACTIVE COMPOUNDS IN SWEET CHERRY FRUITS

SUMMARY

The present study aimed to investigate the influence of different rootstocks (Gisela 6, Pi-Ku 1, and SL 64) on various parameters, including total phenol content, total anthocyanin content, individual phenol content, and antioxidant activity in the fruits of two sweet cherry cultivars, namely Early Lory and Prime Giant. The determination of total phenolic content was carried out using the Folin-Ciocalteu reagent and spectrophotometric method, resulting in a range of 34.77±1.76 to 88.58±8.83 mg GAE/100 g FW, depending on the specific combination of cultivar and rootstock. The concentration of total anthocyanins, determined through the pH-differential method, varied from 1.08±0.07 to 18.62±0.66 mg CGE/100 g FW. Among the different combinations, the highest levels of total phenolic content and total anthocyanin concentration were found in Early Lory cultivar grafted onto Pi-Ku 1 rootstock. Using HPLC analysis, neochlorogenic acid, catechin, chlorogenic acid, *p*-coumaric acid and quercetin-3-*O*-glucoside were detected as individual phenols, exhibiting significant variation among sweet cherry fruits grafted on different rootstocks. The lowest content of the investigated individual polyphenols was observed in Early Lory grafted onto Pi-Ku 1 rootstock. Furthermore, the ferric reducing antioxidant power assay indicated higher antioxidant activity in Early Lory cultivar compared to Prime Giant. A statistically significant correlation was observed between total phenolic content and antioxidant activity (0.978 $p<0.01$), as well as between anthocyanins and antioxidant activity (0.956 $p<0.01$).

Keywords: anthocyanins, antioxidant activity, cultivar, rootstock, total phenol content

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INTRODUCTION

Sweet cherry (*Prunus avium* L.) is a widely popular fruit globally due to its abundant phytonutrients and bioactive compounds, which contribute significantly to its health-promoting properties (Gonçalves *et al.,* 2018). The bioactive compounds in sweet cherries primarily consist of polyphenols, including phenolic acids and flavonoids, which are known for their antioxidant activity (Gonçalves *et al*., 2004; Usenik *et al*., 2008; González-Gómez *et al.,* 2010; Usenik *et al.,* 2010; Pacifico *et al.,* 2014; Llupa *et al.,* 2022). Phenolic compounds are recognized for their protective effect against oxidative stress (Szajdek and Borowska, 2008), and numerous studies have associated their consumption with various beneficial health effects (Malaguti *et al.,* 2013; Antognoni *et al.,* 2020; Domínguez-Perles *et al.,* 2020). Furthermore, the levels of phenolic compounds in cherry extracts have been directly linked to their antioxidant activity (Tomás-Barberán *et al.,* 2013).

The production of sweet cherries has been steadily increasing worldwide, including in Bosnia and Herzegovina. In 2023, 22 sweet cherry cultivars covered 5,479 hectares, yielding a total of 9,715 tons (FAOSTAT, 2023) in Bosnia and Herzegovina, although the yields remained relatively low. This is mainly attributed to the use of old varieties and generative rootstocks, which possess high vigor, making harvesting difficult and increasing production costs (Drkenda *et al*., 2012). The quality of sweet cherry fruit primarily relies on the variety genotype (Usenik *et al*., 2008; González-Gómez *et al.,* 2010), environmental conditions in the growing area (Tomás-Barberán *et al*., 2013; Skrzyński *et al.,* 2016), maturity stages (Serradilla *et al.,* 2012), and the rootstock genotype onto which the variety is grafted (Scalzo *et al.,* 2005; Tavarini *et al.,* 2011).

The breeding objectives for new cherry varieties include larger fruit size, reduced susceptibility to fruit cracking, self-fertilization, extended ripening season, desirable red color (either light or dark), firmness, sweetness, and taste (Sansavini and Lugli, 2005). At the same time, the quality of fruits and their health effects need to be considered. There has been no detailed research on the effect of rootstock on the content of polyphenols in sweet cherry grown in Herzegovina region. This research was undertaken to evaluate the composition of bioactive compounds of 2 sweet cherry cultivars (Early Lory and Prime Giant) on 3 rootstocks (Gisela 6, Pi Ku-1 and Santa Lucia 64) under climatic conditions in the Herzegovina region.

MATERIAL AND METHODS

The study took place in 2022 at a commercial orchard in Blagaj, located in the Herzegovina region (Figure 1). The average annual temperature in this region is approximately 14.8 °C and the total precipitation is 1,439.3 mm. The orchard was established in 2014, with a planting distance of 4.5×3.6 m. The soil in the orchard is characterized as slightly alkaline clay loam with a sandy texture (pH H2O 8.04; pH KCl 7.35), and it contains 3.78% humus. The soil has good water permeability, adequate aeration, and stable microaggregates. Drip irrigation was employed and all cherry trees were cultivated using standardized agronomic practices for fertilization, irrigation, and pest control.

To determine the optimal harvest time corresponding to the fruit's commercial maturity, the ripening period was assessed based on indicators such as firmness, color, and soluble solids content.

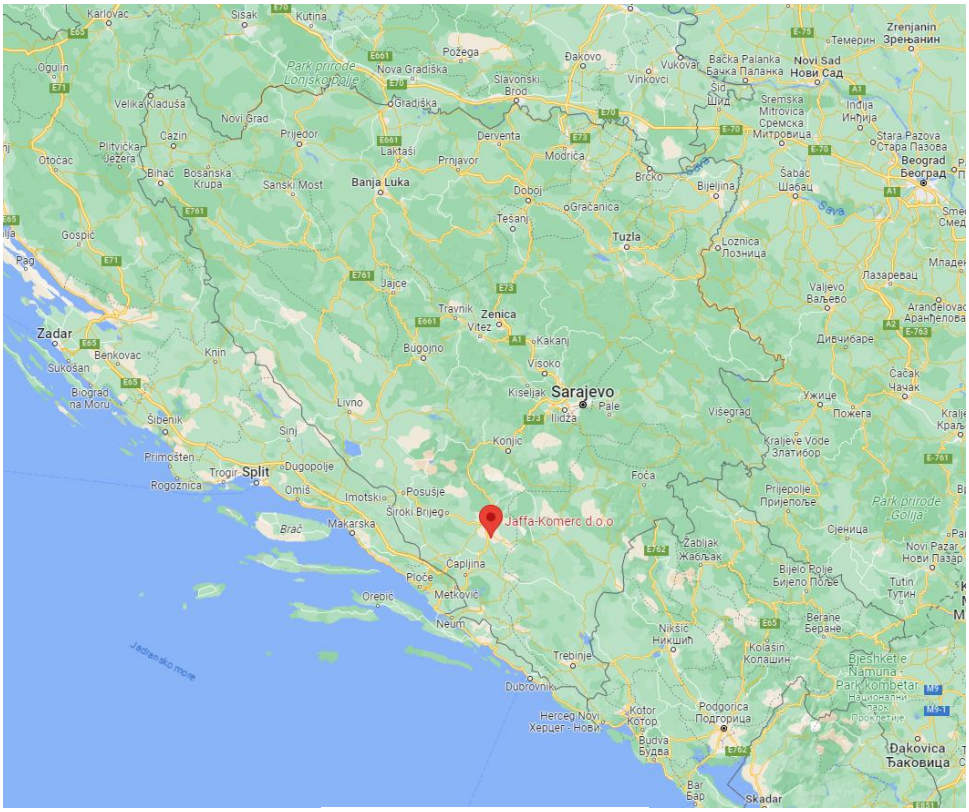

Figure 1. Sampling location

Sample preparation

All fruits were harvested at their full maturity stage and were subsequently utilized for analysis. A blender was used to homogenize 100 g of frozen sweet cherry fruits from each cultivar. From the homogenized sample, 2 g were extracted using 25 mL of 80% acidified methanol (1% HCl) in an ultrasonic bath for 15 minutes at a temperature of 35 °C. The samples were then centrifuged at room temperature for 5 minutes at a speed of 2000 rpm. Subsequently, the samples were ultra-filtrated and stored at -20 °C until analysis.

HPLC analysis

HPLC analysis was performed on Shimadzu HPLC system equipped with a UV/Vis detector as described by Escarpa and González (1999) with some modifications. The chromatographic separation was performed on an Eclipse plus C18 (3.5 µm, 4.6x150 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 min per sample. 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 with a gradient elution as follows: 18% B, 0-30 min; 30% B, 30-70 min; 45% B, 70-75 min; 100% B, 75-80 min; 18% B, 80-110 min.

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

Determination of total anthocyanin content (TA)

Total anthocyanin content was determined using the pH-differential method as described by Zhishen *et al.* (1999) with some modifications described by Kazazic *et al*. (2022). The absorbance was measured simultaneously at 510 nm and 700 nm with spectrophotometer after 20 min. The total anthocyanin content was expressed as mg of cyanidin-3-glucoside equivalent (CGE) per 100 g of fruits FW (mg CGE/100 g 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). This method is based on the reduction of the colorless iron (III)-tripyridyltriazine (Fe^{3+}-TPTZ) complex to the ferrous form (Fe^{2+}) of intense blue color. The antioxidant activity was determined using the calibration curve and represented as mmol $FeSO_4$ equivalents per 100 g of fruits FW (mmol Fe^{2+}/100 g FW).

Statistical analysis

The results obtained in each analysis were analyzed with Excel (Microsoft Corporation, USA) and IBM SPSS Statistics 25 software (USA). All experiments were conducted in triplicates. The values were expressed as the mean ± standard deviation.

RESULTS AND DISCUSSION

The sweet cherry is an excellent source of many nutrients and secondary metabolites and can contribute to a healthy diet. Polyphenols are secondary metabolites that are involved in antioxidative defense of plants against biotic and abiotic stresses. Polyphenols are bioactive compounds that play a key role in sweet cherry quality attributes since they contribute to color, taste, aroma and flavor but also have beneficial effects on human health. Influence of rootstock on the content of bioactive compounds in sweet cherry cultivars has been more extensively studied last decade.

Total phenolic content in examined cultivar/rootstock combination varied from 34.77±1.76 to 88.58±8.83 mg GAE/100 g FW (Table 1).

Table 1. The content of total phenols (TP), total anthocyanins (TA) and antioxidant activity (AA) by FRAP method in sweet cherries

Cultivar/rootstock	TP (mg GAE/100 g FW)	TA (mg CGE/100g FW)	AA (mmol Fe^{2+} /100g FW)
E.Lory/Gisela 6	83.73±1.85	17.53±0.88	0.79±0.01
E.Lory/Pi-Ku 1	88.58±8.83	18.62±0.66	0.81±0.00
E.Lory/SL 64	71. 80±7.01	10.35±0.29	0.63±0.02
P.Giant/Gisela 6	41.17±4.36	1.08±0.07	0.30±0.00
P.Giant/Pi-Ku 1	34.77±1.76	2.86±0.07	0.30±0.01
P.Giant/SL 64	42.11±0.01	4.79±0.07	0.35±0.02

The cultivar Early Lory grafted onto Pi-Ku 1 showed the highest level of total phenolic content (88.58±8.83 mg GAE/100 g FW) and highest total anthocyanin content (18.62±0.66 mg CGE/100 g FW).

Total phenolic content of 91.3 mg GAE/100 g in Early Lory cultivar was previously reported by Eroğul (2016) which is in accordance with our results. Legua *et al.* (2017) reported that Prime Giant cultivar had total phenolic content 30 mg GAE/100 g, which is slightly lower than the results reported in this study. Prime Giant grafted onto SL 64 rootstock had total phenolic content of 42.11±0.01 mg GAE/100 g which is lower than 74.84±4.23 mg GAE/100 g reported by Carrión-Antolí *et al.* (2022). Schmitz-Eiberger *et al.* (2012) determined total phenol content of Prime Giant cultivar grafted onto Gisela 5 (35.6±0.8 mg GAE /100 g FW). These differences can be due to the complexity of phenols and various environmental conditions during the growing period of

cultivars. Weather conditions during cherry growth may have a profound influence on the total phenolics level. Composition of phenolics and antioxidant activity of the cherry fruit is strongly influenced during ripening stages (Mahmood *et al.,* 2013). The synchronization of harvesting time within a commercial orchard would benefit to growers. The distribution of the s-alleles linked with the flowering and fruit ripening time for cherries cultivars has provided valuable insights into the compatible cultivars with overlapping flowering and fruit ripening time (Dervishi *et al.,* 2022).

Total anthocyanin content varied from 1.08±0.07 to 18.62±0.66 mg CGE/100 g FW in investigated samples. Total anthocyanins of Prime Giant cultivar were in accordance with the results reported by Serrano *et al.* (2009). Prime Giant cultivar showed lower concentration of total anthocyanin content compared to Early Lory cultivar. This can be explained due to the fact that Prime Giant is considered as light-colored cultivar. Díaz-Mula *et al.* (2009) reported direct relationship between color parameters and anthocyanin concentration. The combination of Prime Giant grafted on Gisela 6 showed the lowest concentration of total anthocyanin content (1.08±0.07 mg CGE/100 g FW). Carrión-Antolí *et al*. (2022) analyzed total anthocyanins in Prime Giant grated onto SL 64 rootstock and reported higher concentration compared to the findings presented in this study.

According to the FRAP assay antioxidant activity was higher in Early Lory cultivar compared to Prime Giant with the highest antioxidant activity detected in Early Lory cultivar grafted onto Pi-Ku 1 rootstock (0.81±0.00 mmol Fe^{2+}/100 g FW). Early Lory cultivar also showed the highest antioxidant activity determined by FRAP method in the study by Eroğul (2016), which aligns with the results of this study.

Previous reports found that higher polyphenol content led to higher antioxidant activity.

The correlations between total phenolic content and antioxidant activity (0.978 $p<0.01$) and total anthocyanins and antioxidant activity (0.956 $p< 0.01$) were statistically significant.

A strong genotype, genotype of rootstock and interaction between the cultivar/rootstock influences the phenolic profile of sweet cherry fruits. Usenik *et al.* (2010) reported that there were significant differences between rootstocks in terms of the content of individual polyphenols in sweet cherry cultivars.

Previous studies show that sweet cherries are a good source of polyphenols such as phenolic acids (Kim *et al*., 2005), anthocyanins, flavanols and flavan–3-ols (Gonçalves *et al*., 2004). In the studied sweet cherry fruits the following individual polyphenols were found: neochlorogenic acid, catechin, chlorogenic acid, *p*-coumaric acid and quercetin-3-*O*-glucoside (Table 2).

Table 2. The content of individual polyphenols expressed in mg/100 g FW

Cultivar/rootstock	Neochlorogenic Acid	Catechin	Chlorogenic Acid	*p*-coumaric Acid	Quercetin-3-*O*-glucoside
E.Lory/Gisela 6	B.D.L.	3.55± 0.01^{a}	B.D.L.	0.92± 0.01^{a}	3.30 ± 0.03^{e,d}
E.Lory/Pi-Ku 1	B.D.L.	N.D.	0.83 ± 0.01^{a}	N.D.	3.45 ± 0.01^{f,a,b}
E.Lory/SL 64	B.D.L.	3.11± 0.02^{a}	B.D.L.	18.54± 0.07^{d,a}	2.58 ± 0.02^{d}
P.Giant/Gisela 6	7.12 ± 0.03^{c,a}	31.66± 0.13^{d,b,c}	N.D.	N.D.	2.27 ± 0.03^{c}
P.Giant/Pi-Ku 1	3.51 ± 0.02^{a}	13.97± 0.03^{b,c}	B.D.L.	9.63± 0.03^{a,c}	1.93 ± 0.01^{b,c}
P.Giant/SL 64	4.52 ± 0.02^{b}	18.33± 0.07^{c}	B.D.L.	9.88± 0.02^{a,d}	1.46 ± 0.02^{a,b}

Note: B.D.L. - below detection level; N.D. - not detected. Means in the same columns followed by the same letter (a–f) are not significantly different at the 5% level of probability ($p<0.05$)

The lowest content of the investigated individual polyphenols was measured in Early Lory grafted onto Pi-Ku 1 rootstock. Content of quercetin-3-*O*-glucoside of Early Lory cultivar in combination with rootstocks was decreasing in following order Pi-Ku 1>Gisela 6>SL 64. Content of *p*–coumaric acid was decreasing in Early Lory cultivar in combination with rootstocks in following order SL 64>Gisela 6 but was not detected in Early Lory/Pi-Ku 1. Catechin was not detected in Early Lory/Pi-Ku 1, while higher content was detected in Early Lory/Gisela 6 compared to Early Lory/SL 64. Neochlorogenic acid was below detection level in Early Lory cultivar. Chlorogenic acid was only detected in Early Lory cultivar grafted onto Pi-Ku 1 rootstock.

Content of neochlorogenic acid and catechin of Prime Giant cultivar in combination with rootstock was increasing as following order Pi-Ku 1<SL 64<Gisela 6, whereas for quercetin-3-*O*-glucoside SL 64<Pi-Ku 1<Gisela 6. *p*-coumaric acid was not detected in Prime Giant/Gisela 6 cultivar/rootstock combination but higher concentration was detected in Prime Giant/SL 64 compared to Prime Giant/Pi-Ku 1 combination. Chlorogenic acid was below detection level in Prime Giant cultivar grafted onto Pi-Ku 1 and SL 64 and not detected in Prime Giant/Gisela 6.

Prime Giant cultivar showed higher concentration of catechin and *p*-coumaric acid than reported by Gonçalves *et al.* (2021), while the chlorogenic acid was below detection level.

There are no previous reports available to our knowledge about the concentration of individual polyphenols in Early Lory and Prime Giant cherry cultivars grafted onto Pi-Ku 1, Gisela 6 and SL 64 rootstocks. From this research we can conclude that individual phenols investigated in this study are not predominant polyphenols in these cultivars.

Results showed that the content of individual polyphenols in sweet cherries differed significantly depending on rootstock.

CONCLUSIONS

The results obtained demonstrate that the content of bioactive compounds in the fruits of the examined Early Lory and Prime Giant cultivars is significantly influenced by the rootstocks under investigation (Pi-Ku 1, Gisela 6, and Santa Lucia 64), as well as the interaction between the cultivar and rootstock. Based on the results we can conclude that total phenolic content, total anthocyanins, and antioxidant activity were highest when Early Lory cultivar was grafted onto Pi-Ku 1. On the contrary, when the Prime Giant cultivar was grown on the SL 64 rootstock, it displayed the greatest concentration of the studied bioactive compounds and demonstrated the highest antioxidant activity. Metabolic processes that occur during the joining of scion and rootstock are still not completely understood since it involves complex physiological factors. Therefore, it is important to detect incompatible graft combinations early to prevent financial losses and delays for agricultural producers. At the same time results can be used as recommendation to consumers in terms of their health value and quality of fruits.

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