Sugar, Acid and Phenols in Fruit of the Sharka-Tolerant Autochthonous Plum Genotype ‘Mrkosljiva’

Pakeza Drkenda1 · Osman Music1 · Amila Oras1 · Selma Haracic2 · Sanel Haseljic1 · Michael Blanke3 · Metka Hudina4

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Abstract
The self-rooted plum genotype ‘Mrkosljiva’ was first selected as it was devoid of Sharka leaf symptoms during the 5-year study (2009–2014), which was subsequently confirmed by negative ELISA test results. Hence, the aim of the study was to investigate the biochemical variability of the autochthonous ‘Mrkosljiva’ plum genotype, based on its sugar, acid and phenolic compounds content during 3 years. In 2010 and 2011, the plums tasted sweet with favourable sugar:acid ratios of 55:1 to 65:1. These plum fruits were collected at the full ripeness stage from an extensive orchard located in northeastern Bosnia and Herzegovina (45 °N). Analysis by HPLC identified the following polyphenolic compounds—chlorogenic acid, cyanidin-3-O-glucoside, cyanidin-3-O-rutinoside, neochlorogenic acid, quercetin-3-O-galactoside and quercetin-3-O-rutinoside—in all 3 years that were evaluated (2009–2011), but in different amounts, depending on the environmental conditions of each year. In 2009, the plums were healthier with a threefold greater phenol content than during the other 2 years of growth. In 2009, protocatechuic acid was detected, whereas catechin, procyanidin B1, procyanidin B2 and quercetin-3-O-rhamnoside were not detected. The combination of high phenol and sugar content in ‘Mrkosljiva’ provides healthy and tasty plums suitable primarily for processing, and to a lesser extent, due to their size, for fresh fruit marketing.

Keywords Plum (Prunus domestica L.) · Autochthonous · Phenols · Plum pox virus (PPV) · Sharka virus · Sugar processing fruit · Taste

Introduction
The world’s annual plum production exceeds 12 mil t (FAOSTAT 2018). In the Balkan countries, plum is an economically important crop, notably represented by a significant number of old autochthonous varieties such as ‘Pozegaca’, ‘Sisaca’, ‘Turgulja’, ‘Mrkosljiva’, ‘Havaca’, ‘Korajka’ and ‘Ljubinka’. In 2018, Bosnia and Herzegovina (B&H) produced ca. 190,000 t European plums (FAOSTAT 2018) and in 2019 ca. 115,000 t and in 2020 ca. 160,000 t and exported ca. 8100 t of fresh fruit.

Pakeza Drkenda
p.drkenda@ppf.unsa.ba
Osman Music
o.music@ppf.unsa.ba
Amila Oras
a.vranac@ppf.unsa.ba
Selma Haracic
selmaharacic@yahoo.com
Sanel Haseljic
s.haseljic@ppf.unsa.ba
Michael Blanke
mmblanke@uni-bonn.de
Metka Hudina
metka.hudina@bf.uni-lj.si

1 Faculty of Agriculture and Food Sciences, University of Sarajevo, Zmaja od Bosne 8, Sarajevo, Bosnia and Herzegovina
2 Faculty of Forestry, University of Sarajevo, Zagrebačka 20, Sarajevo, Bosnia and Herzegovina
3 INRES-Gartenbauwissenschaft, Universität Bonn, Bonn, Germany
4 Biotechnical Faculty, Department of Agronomy, University of Ljubljana, Jamnikarjeva 101, Ljubljana, Slovenia
The most famous plum production regions in B&H include Podrinje, Majevica, Potkazare, Gradacac, Gracanica, Brcko and central Bosnia (Drkenda et al. 2014). Plum fruits are sold on the domestic and international markets in various forms: fresh, as jam, compote, as well as dried, frozen and in the form of juice. Modern plum production in B&H is based on the cultivation of commercial plum varieties that are tolerant to plum pox virus (PPV). In addition to commercial genotypes, various autochthonous plum genotypes such as ‘Pozegaca’, ‘Turgulja’, ‘Sisaca’, and ‘Mrkosljiva’ among others can also be found (Drkenda et al. 2014, 2019; Halapija Kazija et al. 2014; Drkenda and Kurtovic 2012). Sharka (PPV) is one of the most dangerous viruses in plum cultivation. More and more fruit growers are dissatisfied with the resistance or tolerance of plum varieties cultivated today (Hartmann 2019). The most interesting research currently is to develop a new Sharka-resistant plum cultivar. The native plum ‘Pozegaca’ has mainly been used for processing. More and more fruit growers are dissatisfied with the resistance or tolerance of plum varieties cultivated today (Hartmann 2019). The most interesting research currently is to develop a new Sharka-resistant plum cultivar. The native plum ‘Pozegaca’ has mainly been used for processing. However, this plum is one of the most sensitive to Sharka virus. It will therefore be interesting to find an autochthonous plum genotype with similar fruit quality and tolerance to Sharka virus.

Traditional and autochthonous fruit genotypes are rich in various phenolic compounds (Begić-Akagić et al. 2011; Akagić et al. 2019; Drkenda et al. 2019; Đurić et al. 2015; Balik et al. 2019). Schmitz-Eiberger and Blanke (2012) reported that food producers and consumers are showing increasing interest in phenolic compounds as health-promoting substances. Plums are an important source of compounds influencing human health, such as phenolic acids, anthocyanins, flavanols, organic acids (mostly citric and malic acids), pectin, tannins, aromatic substances, enzymes and minerals, as well as vitamins A, B, C and K. The plum is classified as a health-promoting fruit (Lammerich et al. 2020). According to Birwal et al. (2017), the predominant phenolic compounds in plums are caffeic acid, 3-O-caffeicquinic (neochlorogenic acid), 5-O-caffeicquinic (chlorogenic acid) and 4-O-caffeicquinic (cryptochlorogenic acid). Milosevic et al. (2019) reported lower sugar, acid and phenol content in Sharka-tolerant varieties. By contrast, Usenik et al. (2014, 2017) reported that the composition of phenolics in plum fruit was significantly modified by PPV infection in that the plant responds with a synthesis of flavonoids and exhibits high natural resistance to pests and diseases (the p-coumaroylquinic acid content was higher in infested plum tissues than in healthy tissues). Cinnamic acid has been recorded as being highly toxic to V. inaequalis (Kirkham and Flood 1963).

According to the results of a large-scale survey for the presence of PPV (Fig. 1) in the leaves of autochthonous B&H plum cultivars (Fig. 2) over a period of 5 years, the genotype ‘Mrkosljiva’ was found to be uninfected (Drkenda et al. 2014). ‘Mrkosljiva’ was thus marked as an interesting target for future investigations. Since the presence or absence of PPV is associated with the amount of sugar and phenols, this study aimed to provide more details on the chemical content of the ‘Mrkosljiva’ autochthonous plum genotype through the identification and quantification of its sugars, organic acids and phenols in its fruit during three subsequent years.

**Material and Methods**

**Sample Collection, Trees and Location**

Specimen were collected in 2009, 2010 and 2011 at fruit maturation, when the fruit peel was completely dark blue,
Table 1  Average monthly and yearly air temperatures and precipitation at Lukavica Rijeka (44.8 °N)

| Year | I    | II   | II   | IV   | V    | VI   | VII  | VIII | IX   | X    | XI   | XII  | Year Mean | Year Mean | Vegetation |
|------|------|------|------|------|------|------|------|------|------|------|------|------|-----------|-----------|------------|
|      | Temperature (°C) | Rainfall (mm) |      |      |      |      |      |      |      |      |      |      | | Total | Total |
| 2009 | –1.1 | 2.8  | 7.4  | 14.6 | 18.6 | 19.4 | 23.3 | 23.1 | 19.8 | 12.2 | 10.2 | 4.0  | 12.9      | 16.5      |            |
| 2010 | –0.5 | 3.1  | 7.5  | 12.3 | 16.6 | 20.1 | 22.9 | 22.0 | 15.9 | 9.6  | 10.9 | 1.9  | 11.9      | 15.3      |            |
| 2011 | 2.0  | 1.4  | 7.5  | 13.8 | 16.5 | 21.1 | 22.6 | 24.0 | 21.6 | 11.8 | 3.1  | 4.7  | 12.5      | 15.8      |            |

at the locality Lukavica Rijeka in the northeastern part of B&H (44.8 °N 18.2 °E) at an elevation of 117 m. The soil is a dystric cambisol. The plum trees were planted in 1991, grown on their own roots (suckers) and trained in a pyramidal system.

Environmental Conditions

In 2010, significant precipitation was noted, while higher air temperatures were recorded in 2009 and 2011, and 2011 had less rainfall and a slightly lower average air temperature (Table 1). In 2009, the average air temperature was higher and the amount of precipitation was between the other two analysed years, thus the 2009 growing season was favourable in terms of the environmental conditions measured. By contrast, 2011 was characterized by a water deficit and higher temperatures (drought was the main weather characteristic of this growing season). The location of the extensive plum orchard (Figs. 3 and 4) has a temperate continental climate with an average temperature of 10.1 °C.

Fruit Collection

Plum fruits were harvested on the last 10 days of August (on each of the three experimental years), at fully matured stage. Fully matured fruit samples (considered as a pooled sample) were collected from all parts of five trees (10 fruits per tree) to represent the plum genotype fully. Immediately after harvesting, the weight of the fruits and stones was measured. The data obtained were used for later calculation of the fruit flesh ratio (the percentage [%] of fruit flesh in the total fruit weight). The following operation required placing the samples into containers and transporting them to the laboratory, where the fruits were frozen and stored at –20 °C until analysis.
Table 2  Results of the ELISA test based on the difference in absorption at 405 nm after 60 min

| Year | At 405 nm (‘Mrkosljiva’) | At 405 nm (positive control) |
|------|--------------------------|-----------------------------|
| 2009 | 0.144 | 2.010 |
| 2010 | 0.129 | 1.964 |
| 2011 | 0.224 | 2.027 |

Serological Test for PPV

The serological test for plum pox virus (PPV) was performed through the ELISA method (diagnostic protocols for regulated pests PM7/32 [1], Bulletin OEPP/EPPO 34, 247–256) with the reagents AGRITEST Srl., Italy, in 2011 and BIOREBA AG, Switzerland, in 2009 and 2010. The results of serological tests for PPV are shown in Table 2 based on the absorption values at 405 nm after 60 min of leaf extracts.

Plum pox virus was not detected in serological tests carried out every year by DAS-ELISA.

Extraction, Standards and HPLC Analysis

Identification and quantification of the organic acids (citric, fumaric and shikimic acids) and individual sugars (fructose, sucrose, glucose and sorbitol) were carried out using standards acquired from Fluka (Buchs, Switzerland). Malic acid was obtained from Merck (Darmstadt, Germany).

Chlorogenic acid, neochlorogenic acid and (+)-catechin were from Roth (Karlsruhe, Germany). Quercetin 3-O-glucoside, quercetin 3-O-galactoside, procyanidin B1, procyanidin B2, cyanidin 3-O-rutinoside standards were purchased from Fluka (Buchs, Switzerland), while quercetin 3-O-rutinoside and cyanidin 3-O-glucoside were obtained from Sigma (St. Louis, MO, USA). Methanol was purchased from Riedel-de Haën (Seelze, Germany) and acetone from Sigma-Aldrich (Steinheim, Germany). The Milli-Q system (Millipore, Burlington, VT, USA) was used for bi-distilled water production.

Extraction and HPLC analysis were done at the Biotechnical Faculty, University of Ljubljana, Slovenia. The analysed samples were prepared according to the method described by Usenik et al. (2008). For identification and quantification of the contents of individual sugars (glucose, fructose, sucrose and sorbitol) and organic acids (malic, citric, shikimic and fumaric), 10 g of fresh plum samples was mixed with 50 mL of bi-distilled water. The mixtures were homogenized with a T-25 Ultra-Turrax (IKA—Labortecnik, Staufen, Germany) and left for extraction at room temperature for 30 min. The extracted samples were centrifuged at 12,000 g for 7 min at 4°C (Eppendorf centrifuge 5810 R, Hamburg Germany). Prior to HPLC analysis, the supernatants obtained were filtered through a 0.45-μm cellulose ester filter (Macherey-Nagel, Düren, Germany) directly into a vial.

Sugars were analysed using Thermo Separation Products (Riviera Beach, USA) HPLC instrument and refractive index (RI) detector. Sugars were separated using a Rezex RCM-monosaccharide column (300×7.8 mm), whereby the column temperature was maintained at 65°C. The samples were eluted according to the isocratic method described by Hudina et al. (2007). Organic acids were analysed by HPLC, using an Aminex HPX-87H column (300×7.8 mm; Bio-Rad, USA) associated with a UV detector set at 210 nm. Sugars and organic acids in plum extracts were identified by their retention time characteristics, as well as by the internal standard method. Contents of sugars and organic acids are expressed as g kg⁻¹ of fresh weight (FW). Total sugars and organic acids were calculated as a sum of individual sugars and individual organic acids.

For analysis of individual phenols, 10 g of plum sample was homogenized with 10 mL of extraction solution (methanol containing 3% formic acid and 1% 2,6-di-tert-butyl-4-methylphenol [BHT] to prevent degradation of phenolic compounds). The mixture was placed in an ultrasonic ice bath for 1 h before centrifuging at 10,000 rpm for 7 min at 4°C. The supernatant was filtered through a Chromafil AO-45/25 polyamide filter (Macherey-Nagel, Düren, Germany) and transferred into a vial.

The HPLC analysis was performed with the Surveyor system and a diode array detector (DAD) controlled by the Chromquest 4.0 chromatography workstation software system (Thermo Scientific, San Jose, CA, USA). The column used for the separation was a Gemini C18 (150×4.6 mm; Phenomenex, Torrance, CA, USA) with a particle size of 3 μm, maintained at 25°C.

Phenolic acids and flavonols (protocatechuic acid, catechin, chlorogenic and neochlorogenic acid, procyanidin B1, procyanidin B2) were analysed at 280 nm, flavonol glycosides (quercetin 3-O-galactoside, quercetin 3-O-rutinoside and quercetin 3-O-glucoside) were analysed at 350 nm, and anthocyanins (cyanidin 3-O-glucoside and cyanidin 3-O-rutinoside) were analysed at 530 nm. Phenolic compounds were identified according to peak retention time and UV/VIS spectra by comparing them with those of analytical standards. The elution solvents were 1% aqueous formic acid (A) and 100% acetonitrile (B) with the flow rate maintained at 1 mL min⁻¹.

All phenolic compounds presented in our results were identified using an HPLC-Finnegan MS detector and an LCQ Deca XP MAX (Thermo Finnegan, San Jose, CA, USA) instrument with an electro-spray interface (ESI) operating in negative ion mode. The analyses were performed using full-scan, data-dependent MS2 scanning from m/z 115 to 2000. Column and chromatographic conditions were identical to those used for the HPLC-DAD analyses. Quan-
tification of individual phenol compounds was achieved according to the concentrations of the corresponding external standards and is expressed in mg kg⁻¹ of FW. Total phenols were calculated from the data of individual phenols.

**Data Analysis**

All data analyses were carried out in three repetitions and in triplicate. One-way analysis of variance (ANOVA) was used for data analysis in SPSS 20. Pairwise comparisons between different parameters were made using the Tukey test \((p < 0.05)\). Differences between years, as well as between fruit skin and pulp, were also identified by PCA analysis (using STATGRAFICS Centurion XVI, version 16.1.11). Pearson correlation coefficients between fruit content of sugar, organic acids and total and individual phenolic compounds (phenolic acids, anthocyanins, flavonols, flavanols) were determined.

**Results and Discussion**

Table 3 shows the average fruit and stone weights, as well as fruit flesh percent of the autochthonous ‘Mrkosljiva’ plum fruit genotype.

The fruit weights of this plum genotype ranged from ca. 17 g (2011) to 25.8 g (2010), while stone weights ranged from 0.74 g (2009) to 1.18 g (2010). The wide range in fruit weight can be explained by the non-intensive orchard management and different weather from year to year. The fruit to flesh percent varied between 94% and 96.3%. Fruits from 2010 were notably larger than in other years, in terms of both fruit and stone weight. However, fruits from 2009 and 2010 had a statistically higher fruit flesh percentage. Similar results relating to fruit weight and fruit flesh percentage were obtained for the native plum ‘Pozegaca’ (Drkenda et al. 2019).

Sugar content in ‘Mrkosljiva’ plum fruits ranged from 137 to 196 g kg⁻¹ FW. These results are slightly higher than results obtained by Usenik et al. (2008, 2013) and Dugalic et al. (2014). Generally, individual sugar content in ‘Mrkosljiva’ plum varied within the following ranges: glucose 45.6–57.4 g kg⁻¹ FW, sorbitol 41.6–80.7 g kg⁻¹ FW, sucrose 33.9–37.6 g kg⁻¹ FW and fructose 12.3–21.7 g kg⁻¹ FW. The sorbitol level obtained is higher than previously found in the native plum ‘Pozegaca’ (Drkenda et al. 2019).

Plum fruits from 2010 and 2011 displayed a significantly higher content of total sugars compared to fruits from 2009. Among total sugars, glucose was the most abundant in fruits from 2009, followed by sorbitol, sucrose and fructose. On the other hand, fruits from 2010 and 2011 contained sorbitol as the dominant sugar, followed by glucose, sucrose and fructose. Such a high share of sorbitol in the total sugar content may be due to stress impact during those years (high precipitation in 2010 and prolonged dry period in 2011). Many authors have already reported a significant influence of stress conditions on higher accumulation of sorbitol during fruit growth and maturation (Dietrich et al. 2007; Usenik et al. 2007, 2008; Wilford et al. 1997).

The year 2009 was favourable for sucrose accumulation in fruits. A significant difference in the content of sucrose may be explained by the differences in environmental conditions between the years (higher temperature and lower precipitation during the vegetation period in 2009). Soluble solid and sugar contents usually increase in response to reduced irrigation (Li and Li 2005; Génard et al. 2003; Crisosto et al. 1997; Killili et al. 1996), but irrigation results in both dilution and metabolic effects on fruit sugar contents (Mills et al. 1996). Sucrose is one of the main transporting sugars, which are products of photosynthesis in leaves and not produced in the fruits. Sorbitol and sucrose are thought to be biosynthesized in leaves and then translocated to the developing fruit (Bielecki 1977; Ishida et al. 1985). A part of sorbitol, translocated into fruit during maturation (Ishida et al. 1971), is continuously converted into fructose, sucrose and glucose, instead of accumulating, and its actual concentration in the fruit would seem to remain constant. The accumulation of sugars during fruit development and maturation takes place in various ways, depending on the fruit species (Hubbard et al. 2006). Sugars and organic acids in Japanese plums (Prunus salicina Lindell) were influenced by maturation, harvest date and storage temperature (Singh et al. 2009).

Dugalic et al. (2014) cited the sugar model established by Wu et al. (2012). Using this model, it is possible to predict the partitioning of carbon into sucrose, glucose, fructose and sorbitol in the fruit mesocarp of peach cultivars with normal and high glucose:fructose ratio (G:F ratio). The extended sugar model (Wu et al. 2012) presupposes a high

| Year | Fruit weight (g) | Stone weight (g) | Fruit flesh percent (%) |
|------|-----------------|-----------------|------------------------|
| 2009 | 18.2 b ± 2.09   | 0.74 b ± 0.06   | 95.9 a ± 0.29          |
| 2010 | 25.8 a ± 3.75   | 1.18 a ± 0.22   | 95.4 a ± 1.04          |
| 2011 | 17.0 b ± 1.67   | 0.96 c ± 0.09   | 94.4 b ± 0.41          |

Different letters (“a–c”) in columns indicate significant differences between growing seasons at \(p \leq 0.05\)
ratio of G: F for preferential transformation of sorbitol to glucose and fructose, or the use of preferential conversion of fructose to glucose. The relative speed of the transformation of sucrose to glucose and fructose depends on the cultivar, but not on the G:F status.

Total organic acid content in ‘Mrkosljiva’ plum fruits during the 3 years was in the range 24.5–28.55 g kg\(^{-1}\) FW and it was not of statistical significance. Among organic acids, malic acid was the most abundant, followed by citric acid, while the lowest amount was found for fumaric acid. This does not agree with the results reported by Drkenda et al. (2019) or by Usenik et al. (2007, 2013), who did not detect citric acid in European plum cultivars. Individual acid contents in ‘Mrkosljiva’ plum varied in the following ranges: malic acid 16.8–25.5 g kg\(^{-1}\) FW; citric acid 2.94–6.90 g kg\(^{-1}\) FW; shikimic acid 0.07–0.76 g kg\(^{-1}\) FW; and fumaric acid 0.03–0.07 g kg\(^{-1}\) FW. These results are similar to the results of Drkenda et al. (2019). In 2009, the content of citric and fumaric acid was statistically greater than in the other 2 years, while 2010 was generally the year with the highest total organic acids content. The plums in the years 2010 and 2011 tasted pleasantly sweet with favourable sugar:acid ratios of 55:1 to 65:1 (Tables 4 and 5).

Shiratake and Martinoia (2007) reported that in the early stages of fruit development, fruits accumulate organic acids, and thus have an acidic taste. During the process of fruit maturation and ripening, sugars stored in vacuoles (Yamaki and Ishikawa 1986) generally increase in concentration, together with a simultaneous decrease in organic acids, except in highly acidic fruits such as citrus (Echeverria and Burns 1989). Yu (1999) stated that sugars are synthesized throughout the process of photosynthesis, and used for respiratory substrates and in the cell wall structure. Total acidity and total soluble solids therefore increase as the fruits ripen.

### Polyphenols

Phenolic profiles together with enzyme patterns of the bark of P. domestica trees could be useful for distinguishing between cultivars (Groh et al. 1994) and identifying the geographical origin of plums (Nunes et al. 2008). The following phenolic compounds (ranked by their average amount in the whole fruit) were identified and quantified in fruits of ‘Mrkosljiva’: neochlorogenic acid, cyanidin 3-O-rutinoside, quercetin 3-O-rutinoside, cyanidin 3-O-glucoside, chlorogenic acid, procyanidin B1, catechin, protocatechuic acid, procyanidin B2, quercetin 3-O-glucoside, quercetin 3-O-galactoside and quercetin 3-O-rhamnoside. Protocatechuic acid was detected and quantified in fruit skin collected in 2009.

Among the hydroxycinnamic acids analysed in ‘Mrkosljiva’ plum fruit, neochlorogenic acid was the most abundant phenolic compound in all 3 years (Table 6). In 2009, statistically higher contents of neochlorogenic and chlorogenic acid were measured in the fruit skin of ‘Mrkosljiva’ than in 2010 and 2011. Moreover, in 2011, neochlorogenic acid was measured in the highest amount in the fruit pulp, while the lowest amount was determined in 2009. There were no significant differences between seasons in the content of chlorogenic acids in fruit pulp.

Neochlorogenic acid is the predominant hydroxycinnamic acid in nearly all plum varieties, with the exception of the genotype ‘Spate Myrobalane’, in which this compound could not be detected (Jaiswal et al. 2012). It has been suggested that plum fruits are rich in phenolic content and are important for human health (Donovan et al. 1998). The results of this study are mostly in agreement with those of other researchers. The content of neochlorogenic acid ranged from 6.55 mg kg\(^{-1}\) FW (in the pulp in 2009) to 325.84 mg kg\(^{-1}\) FW (in the skin in 2009). The neochlorogenic acid level obtained is lower than previously found in European plums (Usenik et al. 2008; Drkenda et al. 2019).

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**Table 4** Contents of sucrose, glucose, fructose, sorbitol and total sugars (g kg\(^{-1}\) FW) in ‘Mrkosljiva’ plum fruits in relation to growing year (mean±SD)

| Year | Sucrose | Glucose | Fructose | Sorbitol | Total sugars content |
|------|---------|---------|----------|----------|---------------------|
| 2009 | 37.6±2.72 | 45.6±3.88 | 12.3±1.66 | 41.6±4.47 | 137±10.39 |
| 2010 | 33.9±1.78 | 57.3±6.13 | 19.3±2.15 | 78.0±5.89 | 188±14.24 |
| 2011 | 36.2±4.83 | 57.4±4.49 | 21.7±2.69 | 80.7±14.02 | 196±21.15 |

Different letters (“a, b”) in columns indicate significant differences between growing seasons at \(p \leq 0.05\)

**Table 5** Contents of malic, citric, shikimic and fumaric acids (g kg\(^{-1}\) FW) in ‘Mrkosljiva’ fruits in relation to growing year (mean±SD)

| Year | Malic acid | Citric acid | Shikimic acid | Fumaric acid | Total organic acids content |
|------|------------|-------------|--------------|-------------|---------------------------|
| 2009 | 16.8±0.75  | 6.90±0.78   | 0.76±0.04    | 0.07±0.001  | 24.5±1.54                |
| 2010 | 25.5±1.46  | 2.94±0.17   | 0.09±0.01    | 0.03±0.002  | 28.5±1.39                |
| 2011 | 23.9±6.59  | 3.11±0.41   | 0.07±0.004   | 0.03±0.003  | 27.1±1.21                |

Different letters (“a, b”) in columns indicate significant differences between growing seasons at \(p \leq 0.05\)
some wild types of *Prunus domestica* L. (Jaiswal et al. 2012) or in Californian prunes (Donovan et al. 1998), but higher than in some sweet cherries (Usenik et al. 2010), peaches (Orazem et al. 2011), *Prunus salicina* L. or *Prunus cerasifera* L. (Jaiswal et al. 2012; Celik et al. 2017).

Environmental conditions of the growing years significantly affected the neochlorogenic and chlorogenic acids contents. The fruit skins analysed in 2009 showed significantly higher contents of neochlorogenic and chlorogenic acids (Table 6). The most favourable year for neochlorogenic acid accumulation in pulp was 2011.

Protocatechuic acid was identified in the skin of fruits collected in 2009. The presence of protocatechuic acid agrees with Fang et al. (2002), Macheix et al. (1990), Usenik et al. (2017), Miletić et al. (2019) and Slimestad et al. (2009). Drkenda et al. (2019) and Usenik et al. (2013) did not detect protocatechuic acid in European plum varieties. Protocatechuic acid has been reported for its potential activities, such as antioxidant, antibacterial, anticancer, antiulcer, antidiabetic, antiangiogenic, antifibrotic, antiviral, anti-inflammatory, analgesic, antiatherosclerotic, cardiac, hepatoprotective, neurological and nephro-protective (Kakkar and Bais 2014). Protocatechuic acid (PCA) is an antiviral agent against avian influenza virus (AIV) and infectious bursal disease (IBD) virus, but its antiviral mechanism is unknown (Birwal et al. 2017). Protocatechuic acid demonstrated efficacy against *Botrytis cinerea* on strawberry fruits and it could be a promising biofungicide to prevent this disease (Nguyen et al. 2015).

Among the analysed flavonols in ‘Mrkosljiva’ fruits, quercetin-3-*O*-rutinoside was the most abundant flavonol glycoside in all 3 years analysed (Table 6), which is in agreement with Kim et al. (2003).

Significantly higher contents of quercetin-3-*O*-rutinoside, quercetin-3-*O*-galactoside and quercetin-3-*O*-glucoside were registered in fruit skins in 2009 than in the other 2 years. Quercetin-3-*O*-ramnoside was registered in fruit samples from 2010 and 2011. However, there was no significant difference in the content of quercetin-3-*O*-ramnoside between these 2 years.

Quercetin-3-*O*-rutinoside content ranged from 0.2 (in pulp) to 250.7 mg kg⁻¹ FW in skin, quercetin-3-*O*-glucoside from 0.01 (in pulp) to 13.8 mg kg⁻¹ FW in skin, quercetin-3-*O*-galactoside from 0.02 (in pulp) to 9.4 mg kg⁻¹ FW in skin and quercetin-3-*O*-ramnoside from 0.02 (in pulp) to 0.16 mg kg⁻¹ FW in skin. Quercetin-3-*O*-ramnoside was not detected in 2009. Treutter et al. (2012) did not detect quercetin-3-*O*-ramnoside, quercetin 3-*O*-rutinoside and quercetin 3-*O*-glucoside levels in peel.

Higher amounts of phenolics, anthocyanins and flavonols were located in the fruit skin (Table 6). This is in agreement with Tomás-Barberán et al. (2001), Usenik et al. (2008, 2013), Cosmulescu et al. (2015). However, Drkenda et al. (2019) cited a slightly higher content of all flavanols in fruit skin of the native plum ‘Pozegaca’. Statistically, the most favourable growing year for accumulation of all flavonol glycosides in both fruit skin and pulp (except for quercetin-3-*O*-ramnoside) was 2009 (Table 7).

Rutin contents in samples analysed by Celik et al. (2017) ranged from 0.091 to 0.467 mg kg⁻¹ FW, which is lower than our results. Jaiswal et al. (2012) measured the content of rutin as 66.1 mg per 100 g. In the study by Treutter et al.

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### Table 6

| Year | Neochlorogenic acid Skin | Chlorogenic acid Skin | Protocatechuic acid Skin | Neochlorogenic acid Pulp | Chlorogenic acid Pulp | Protocatechuic acid Pulp |
|------|--------------------------|-----------------------|--------------------------|--------------------------|-----------------------|--------------------------|
| 2009 | 325.8a ± 28.96           | 129.1a ± 27.59        | 41.1 ± 3.44              | 6.55c ± 0.33             | 0.38b ± 0.15          | 0.01b ± 0.01              |
| 2010 | 30.1b ± 7.05             | 61.1b ± 31.11         | n. d.                    | 35.0b ± 6.69             | 2.27b ± 0.34          | 0.16a ± 0.05              |
| 2011 | 80.1b ± 11.99            | 79.0b ± 15.31         | n. d.                    | 61.1b ± 31.11            | 0.16a ± 0.05          | 0.02a ± 0.002             |

Different letters in columns ("a–c") indicate significantly different values between growing seasons at *p* ≤ 0.05

### Table 7

| Year | Quercetin-3-*O*-Rutinoside Skin | Quercetin-3-*O*-Rutinoside Pulp | Quercetin-3-*O*-Galactoside Skin | Quercetin-3-*O*-Galactoside Pulp | Quercetin-3-*O*-Glucoside Skin | Quercetin-3-*O*-Glucoside Pulp | Quercetin-3-*O*-Ramnoside Skin | Quercetin-3-*O*-Ramnoside Pulp |
|------|--------------------------------|---------------------------------|---------------------------------|---------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| 2009 | 250.7a ± 44.7                  | 9.40a ± 2.45                   | 0.13a ± 0.06                   | 13.8a ± 2.47                   | 1.14a ± 0.19                  | n. d.                         | n. d.                         |
| 2010 | 133.6b ± 25.1                  | 2.27b ± 0.34                   | 0.05a ± 0.02                   | 1.12b ± 0.16                   | 0.03b ± 0.02                  | 0.16a ± 0.05                  | 0.02a ± 0.002                 |
| 2011 | 113.3b ± 24.7                  | 0.77b ± 0.11                   | 0.02b ± 0.01                   | 0.88b ± 0.04                   | 0.01b ± 0.01                  | 0.13a ± 0.05                  | 0.02a ± 0.002                 |

Different letters in columns ("a–c") indicate significantly different values between genotypes and growing seasons at *p* ≤ 0.05

*n. d.* not detected
to 383 mg kg \(^{-1}\) FW in skin, followed by cyanidin 3-
O-glucoside (Table 8). Statistically higher contents of cyanidin 3-
O-glucoside obtained here was 2009 (Table 8). Among the anthocyanins analysed
in ‘Mrkosljiva’ plum fruits, cyanidin 3-
O-glucoside was the most abundant anthocyanin in all 3 years (Table 9). Statisti-
cally higher contents of cyanidin 3-O-rutinoside and cyanidin 3-O-glucoside in fruit skin and pulp were measured in
2009. Five anthocyanins were identified in European plum
varieties, and the predominant anthocyanin in most juices
was cyanidin 3-O-rutinoside (followed by peonidin 3-ruti-
oside) according to Usenik et al. (2008), Treutter et al.
(2012) and Goldner et al. (2015). Anthocyanins contribute
significantly to the attractive pigmentation of red and blue
plums (Goldner et al. 2019). The content of cyanidin 3-O-
rutinoside and cyanidin 3-O-glucoside obtained here was
slightly higher than that reported by Usenik et al. (2008)
and Drkenda et al. (2019). Treutter et al. (2012) measured
a higher content of cyanidin 3-O-rutinoside in some plum
cultivars (fruits of ‘President’ plum achieved even 793 mg
per 100 g FW).

Notable differences in total phenols existed between
years (Table 10) and varied in whole fruit from 434 to
1526 mg kg \(^{-1}\) FW, while the value of total phenols of fruit
skin varied from 322 to 1383 mg kg \(^{-1}\) FW. The pulp had
total phenols from 47.2 to 143 mg kg \(^{-1}\) FW and 2009 was
identified as a favourable year for the accumulation of
phenols.

Drkenda et al. (2019) reported that fruit of the native
plum ‘Pozegaca’ contained a greater content of total phe-
nomlors than ‘Mrkosljiva’. According Treutter et al. (2012),
the total phenolic contents in the skin of plum varieties showed
large differences (0.4–29.9 mg g \(^{-1}\) FW).

Principal component analysis (PCA) was performed in
order to establish the relation between the growing year
and the pomological and biochemical characteristics of the
fruits. Two-factor components (groups of correlated vari-
ables) allow 83% of total variability to be explained. The
basic component (1) explains ca. 73% of total variabil-
ity, while the second component (2) explains the remain-

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### Table 8

| Year | Procyanidin B1 Skin | Procyanidin B1 Pulp | Procyanidin B2 Skin | Procyanidin B2 Pulp | Catechin Skin | Catechin Pulp |
|------|---------------------|---------------------|---------------------|---------------------|--------------|--------------|
| 2009 | n. d.               | n. d.               | n. d.               | n. d.               | n. d.        | n. d.        |
| 2010 | 21.5a±4.33          | 3.21a±0.66          | 12.6a±2.19          | 1.3b±0.24           | 15.0b±5.22   | 3.1b±0.68    |
| 2011 | 31.2a±10.1          | 4.31a±1.12          | 13.4a±4.30          | 2.2a±0.76           | 20.4a±7.41   | 6.1a±1.38    |

Different letters in columns (“a–c”) indicate significantly different values between genotypes and growing seasons at \(p \leq 0.05\). n. d. not detected.

### Table 9

| Year | Cy-3-O-rutinoside Skin | Cy-3-O-rutinoside Pulp | Cy-3-O-glucoside Skin | Cy-3-O-glucoside Pulp |
|------|------------------------|------------------------|-----------------------|------------------------|
| 2009 | 383.55a±73.97          | 2.33a±0.31             | 229.80a±57.41         | 2.33a±0.31             |
| 2010 | 75.80b±26.75           | 0.76b±0.41             | 33.73b±12.07          | 0.63b±0.81             |
| 2011 | 73.61b±14.02           | 0.85b±0.39             | 27.29b±5.87           | 0.57b±0.80             |

Different letters in columns (“a–b”) indicate significantly different values between growing seasons at \(p \leq 0.05\).

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(2012), the measured content of rutin ranged from 55 to
1190 mg kg \(^{-1}\) FW.

During two growing seasons (2010 and 2011), catechin,
procyanidin B1 and procyanidin B2 were determined in ‘M-
rokosljiva’ plum fruits, while their content was not detected
in 2009. Procyanidin B1 was the most abundant flavanol
(Table 7). There was no significant difference in the con-
tent of procyanidin B1 or procyanidin B2 between growing
seasons (except for the content of procyanidin B2 in fruit
pulp). Statistically higher contents of catechin were mea-
sured in skin and pulp in 2010 than in 2011.

In terms of flavanols, previous studies have reported the occurrence of catechin, epicatechin and procyanidin dimers
(B1, B2, B4 and A-type dimers) and trimers (in smaller
amounts) in plums (Macheix et al. 1990; Tomás-Barberán
et al. 2001). Treutter et al. (2012) identified flavan-3-ols
such as catechin and epicatechin in Prunus domestica plum
cultivars.

In the 2010 growing season, both plum skins and pulp
contained catechin, procyanidin B1 and procyanidin B2.
The pulp of fruits harvested in 2011 had a significantly
higher content of catechin and procyanidin B2 of the fla-
vanols than pulp in 2010.

The dominant anthocyanin in ‘Mrkosljiva’ plum fruits
was cyanidin 3-O-rutinoside, ranging from 0.76 (in pulp)
to 383 mg kg \(^{-1}\) FW in skin, followed by cyanidin 3-O-
glucoside (from 0.57 in pulp to 229 mg kg \(^{-1}\) FW in skin).
There were statistically significant differences \((p < 0.05)\) be-
tween the examined growing years and anthocyanin content
in the evaluated autochthonous plum genotype. The most
favourable year for anthocyanin biosynthesis and accumula-
tion was 2009 (Table 8). Among the anthocyanins analysed
in ‘Mrkosljiva’ plum fruits, cyanidin 3-O-rutinoside was the
most abundant anthocyanin in all 3 years (Table 9). Statisti-
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Table 10  Content of total phenols in fruit skin and pulp (mg kg⁻¹ FW) of ‘Mrkoslijiva’ plum genotype in relation to growing year (mean ± standard deviation)

| Year | Total phenols Skin | Total phenols Pulp | Total phenols Whole fruit |
|------|--------------------|--------------------|--------------------------|
| 2009 | 1383.31a ± 191.72  | 143.30a ± 3.74     | 1526.61a ± 188.05        |
| 2010 | 322.45b ± 184.86   | 47.22b ± 8.08      | 434.17b ± 102.04         |
| 2011 | 440.04b ± 119.21   | 77.52b ± 14.76     | 517.56b ± 108.72         |

Different letters in columns ("a, b") indicate significantly different values between growing seasons at \( p \leq 0.05 \).

ing ca. 26%. This result (Fig. 5) implies that the data are well compressed and well presented. Figure 4 shows the distribution of variables (biplot component 1 vs. component 2). Component 1 of variability is significantly determined \( (r \geq 0.50) \) in the positive part of the biplot by the levels of the following components: malic acid, glucose, sorbitol, fructose, total sugar content, shikimic acid, quercetin 3-O-ramnoside in skin and peel, procyanidin B1 and B2 in skin and pulp, catechin in skin and pulp, quercetin 3-O-rutinoside and neochlorogenic acid in pulp.

The negative part of the component 1 plot is highly determined \( (r \geq 0.50) \) by the levels of the following components: cyanidin 3-O-rutinoside and cyanidin 3-O-glucoside in skin and pulp, protocatechuic acid in skin, quercetin 3-O-glucoside in skin and pulp, quercetin 3-O-galactoside in skin and pulp, quercetin 3-O-rutinoside, neochlorogenic and chlorogenic acids in skin, citric acid, total phenols (in whole fruit, skin and pulp), sucrose, fruit-to-flesh ratio, average of annual and vegetation air temperature.

The second component in the positive part was significantly described by the levels of fumaric acid, chlorogenic and neochlorogenic acids in pulp, total phenols in whole fruit and pulp, sucrose, catechin in pulp, procyanidin B1 in skin, procyanidin B2 in pulp, average of annual and vegetation air temperature. The negative part of component 2 is significantly determined by fruit weight, fruit-to-flesh ratio, shikimic acid, quercetin 3-O-rutinoside in pulp, total acids, and precipitation (in the whole year as well as in the vegetation period).

Plum fruits from 2010 and 2011 were grouped in the positive part of the first component. The fruits from 2009 were located in the negative part of component 1. The fruits from 2010 and 2011 were rich in quercetin 3-O-ramnoside and quercetin 3-O-rutinoside in pulp, procyanidin B1

Fig. 5 Principal component biplot based on the content of sugars, organic acids and phenolic compounds of the ‘Mrkoslijiva’ plum genotype. Cat P catechin in pulp, CatS catechin in skin, ChlP chlorogenic acid in pulp, ChlS chlorogenic acid in skin, CitrA citric acid, CyglucP cyanidin 3-O-glucoside in pulp, Cyglucs cyanidin 3-O-glucoside in skin, CyrutP cyanidin 3-O-rutinoside in pulp, CyrutS cyanidin 3-O-rutinoside in skin, FFR fruit to flash ratio, FumA fumaric acid, Fruc fructose, Fweight fruit weight, Gluc glucose, MalicA malic acid, NeoP neochlorogenic acid in pulp, NeoS neochlorogenic acid in skin, PB1P procyanidin B1 in pulp, PB1S procyanidin B1 in skin, PB2P procyanidin B2 in pulp, PB2S procyanidin B2 in skin, Prec annual precipitation, PrecV vegetation precipitation, ProtocS protocatechuic acid in skin, QugalP quercetin 3-O-galactoside in pulp, QugalS quercetin 3-O-galactoside in skin, QugluP quercetin 3-O-glucoside in pulp, Qugls quercetin 3-O-glucoside in skin, QrutP quercetin 3-O-rutinoside in pulp, QrutS quercetin 3-O-rutinoside in skin, Sah sucrose, ShicA shikimic acid, Temp annual air temperature, TempV vegetation air temperature, TPH total phenols in whole fruit, TPHP total phenols in pulp, TPHS total phenols in skin
Table 11  Pearson’s correlations between environmental parameters and content of sugars, acids and total phenols (in the skin and pulp of plum fruits)

| Environmental parameters | Sucrose | Glucose | Fructose | Sorbitol | Total soluble solids | Citric acid | Malic acid | Shikimic acid | Fumaric acid | Total acids | Total phenols in skin | Total phenols in pulp | Total phenols in whole fruit |
|---------------------------|---------|---------|----------|----------|---------------------|------------|------------|-------------|-------------|-------------|----------------------|------------------------|-----------------------------|
| Annual air temperature    | 1.00*   | -0.79  | -0.63    | -0.77    | -0.78              | 0.81       | -0.84     | -0.94       | 0.60         | -0.60       | 0.83                 | 0.92*                  | 0.92*                      |
| Vegetation air temperature| 0.97    | -0.91  | -0.78    | -0.88    | -0.89              | 0.91       | -0.93     | -0.85       | 0.42         | -0.42       | 0.93                 | 0.81*                  | 0.81*                      |
| Annual sum of precipitation| -0.63  | 0      | -0.24    | -0.05    | -0.03              | -0.02      | 0.07       | 0.84        | -1.00**      | 1.00**      | -0.06               | -0.87                  | -0.87                      |
| Vegetation sum of precipitation| -0.71 | 0.11  | -0.13    | 0.06     | 0.09               | -0.131     | 0.18       | 0.89        | -0.99        | 0.99*       | -0.17               | -0.92                  | -0.92                      |

*Correlation is significant at the 0.05 level (2-tailed); **correlation is significant at the 0.01 level (2-tailed)

and B2 and catechin and neochlorogenic acid in pulp, total content of sugars, content of glucose, sorbitol, fructose and malic acid. However, the fruits from 2010 had higher fruit weight, fruit-to-flesh ratio, content of shikimic acid and total content of acids than fruits from 2011. On the other hand, fruits from 2011 had a higher content of sucrose, fumaric acid, chlorogenic and neochlorogenic acids in pulp than the fruits from 2010. This difference between these 2 years could be explained by higher precipitation during 2010 and higher air temperatures in 2011. The fruits from 2009 were rich in anthocyanins, quercetin 3-O-glucoside, quercetin 3-0-galactoside, quercetin 3-O-rutinoside in skin, neochlorogenic acid and chlorogenic acids in skin, protocatechuic acid, total phenols (in whole fruit, skin and pulp), high fruit-to-flesh ratio, content of sucrose and citric acid. This could be explained by the higher air temperature in 2009 (average of the year and vegetation) than in 2010 and 2011 (Macheix et al. 1990; Saint-Cricq de Gaulejac et al. 1997; Hudina et al. 2006; Usenik et al. 2008). These results are of particular interest, since the phenolic content of fruit is related to their antioxidant activity and their health-promoting properties. Furthermore, the contribution of phenolic compounds and anthocyanins to the antioxidant activity is important (Kim et al. 2003; Gil et al. 2002).

Correlations

Table 11 shows Pearson’s correlations between environmental parameters and the content of sugars, organic acids and total phenols (in the skin and pulp of plum fruits).

Annual air temperature was significantly positively correlated with the sucrose content, total phenols in pulp and the total phenols in the whole fruit. According to the results in Table 10, the air temperature was negatively correlated with glucose, fructose and sorbitol. Dugalic et al. (2014) reported a positive correlation between air temperature and glucose and sorbitol content.

Vegetation air temperature was significantly positively correlated with the total phenols in both pulp and whole fruit. The annual sum of precipitation was significantly positively correlated with the total organic acid content. However, there was a negative correlation between the annual sum of precipitation and fumaric acid content. Dugalic et al. (2014) reported a positive corelation between sucrose and precipitation. Total organic acid content was also positively correlated with the vegetation sum of precipitation. Negative correlations were found between precipitation and total phenol content, but these correlations were not significant. According to Rieger and Duemmel (1992), drought stress severely limits successful cultivation of Prunus species fruits in arid climates and in areas with shallow soils. In large-fruited species such as peach, both the yield and the quality are negatively affected by drought stress, particularly during the 4–6-week period before harvest, when the fruits increase rapidly in weight and diameter.

Conclusion

Analyses of ‘Mrkosljiva’ plum fruit over 3 years showed that the levels of individual and total phenolic compounds as well as sugar and organic acid compounds depend on the environmental conditions in the growing years such as air temperature and precipitation and are not necessarily reduced in Sharka-tolerant varieties as suggested by Milosevic et al. (2019). Overall, high sugar and phenolic concentrations were obtained; phenol concentrations were greater in the fruit skin than in the flesh. This study confirms that the autochthonous Sharka-tolerant plum genotype ‘Mrkosljiva’ is a good source of natural phenolic compounds.
Further research will be conducted to determine the fruit content and phenolic compounds under different ecological conditions, as well as for genetic characterization of this plum genotype to differentiate it from other European plum varieties, which will be included in future research.

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Declarations

Conflict of interest P. Drkenda, O. Music, A. Oras, S. Haracic, S. Haseljic, M. Blanke, and M. Hudina declare that they have no competing interests.

Ethical standards For this article no studies with human participants or animals were performed by any of the authors. All studies mentioned were in accordance with the ethical standards indicated in each case.

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