Original article

Geographic and seasonal variation of biochemical parameters of the European native plum ‘Pozegaca’ (*Prunus domestica* L.)

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Summary

‘Pozegaca’ is the most important native plum genotype in southeast Europe. In this study, the phytochemical fruit composition of the ‘Pozegaca’ ecotype from four different growing regions was evaluated over three growing years. HPLC was used for identification and quantification of sugars, organic acids and phenolic compounds in the fruits. The total effects of growing years and regions on different phytochemical characteristics of ‘Pozegaca’ fruit were analysed using principal component analysis (PCA). Glucose, fructose, sucrose and sorbitol were detected as major sugars in ‘Pozegaca’ fruit. Malic, citric, fumaric and shikimic acids were identified in the studied plum ecotype. Glucose was the predominant sugar and malic acid was the predominant organic acid. A range of phenol compounds was identified, including hydroxycinnamic acids, flavonols, anthocyanins and flavan-3-ols. The main factor affecting the fruit quality and phenol composition of the ‘Pozegaca’ plum ecotype in skin and pulp was the growing year. The geographical origin of the ‘Pozegaca’ ecotype appears to have no major influence on the phenol profile of ‘Pozegaca’ fruit. The results showed that air temperature has a strong correlation with sugar compounds (particularly with fructose and sucrose) and with some organic acids (fumaric, shikimic and citric). Related to the accumulation of phenol compounds, air temperature strongly correlated with flavanols (particularly with quercetin 3-rutinoside) and anthocyanins in pulp. Precipitation and altitude were strongly correlated with total phenols in pulp, chlorogenic acid in skin and pulp, neochlorogenic acid in pulp, and all three identified flavanols in pulp. The results showed that specific seasonal and geographic environmental conditions could significantly affect the content of secondary metabolites in ‘Pozegaca’ fruit.

Keywords

acid, growing region, growing season, HPLC-MS, phenol compounds, sugar

Significance of this study

What is already known on this subject?

- The phenotypical characteristics of certain local plum ecotypes, as well as some basic characteristics of their chemical composition.

What are the new findings?

- The biochemical richness of a native plum genotype from the Balkans in correlation with geographic and seasonal variation.

What is the expected impact on horticulture?

- Plum accessions from Balkan countries are a valuable source of plant genetic material, worthy of conservation, sustainable use and breeding programs.

tance in this area (Milošević and Milošević, 2012). Total plum production in Bosnia and Herzegovina amounted 31,579 tons in 2016 (Agency for Statistics of Bosnia and Herzegovina, 2017). The ‘Pozegaca’ plum is a well-known regional cultivar (also called ‘Bistrica’, ‘Madjarica’, ‘Vânăt Românesc’, ‘Bistrițene’, ‘Kiustendilska’, or ‘Vengerka’ in Moldova, or ‘Quetsche’, ‘Hauszwetsche’, or ‘Domaca cesplja’). This cultivar is valued for the quality of its fruit for both fresh consumption and processing (baking, jam, brandy, dried fruit, juice and sweetener). Although this plum cultivar has been grown in the region for over 300 years, its origins are unknown. However, this native cultivar can only be found having a stable yield in a number of regions and orchards (‘Pozegaca’ is one of the most sensitive to Sharka virus). In Bosnia and Herzegovina, because of the unstable yield of ‘Pozegaca’, the average yield per plum tree is only 10.6 kg (Agency for Statistics of Bosnia and Herzegovina, 2017). There has been significant polymorphism noted in the population of plum genotypes in Bosnia and Herzegovina. Because of the number of plum species and/or cultivars, as well as the various types of propagation employed (both suckers and seeds), plum populations are highly heterogeneous (Ercisli, 2004) and show environmentally dependent morpho-anatomical traits. Some genotypes are described in the literature, but others are merely mentioned under local names (Kapetanović and Prica, 1976; Bubić, 1977; Jovancevic, 1977; Rankovic, 1986; Paunovic et al., 1988). The morphological characteristics of certain local plum genotypes and some characteristics of their chemical composition are more or less known, thanks to studies of primary metabolites (Kapetanovic et al., 1982; Rankovic, 1986; Paunovic et al., 1988; Drkenda and Kurtovic, 2012; Milošević and Milošević, 2012). Usenik et al. (2013) emphasized that plants synthesize a large range of organic compounds that

Introduction

There is a long tradition of plum production in Serbia, Romania, and Bosnia and Herzegovina, similar to other countries in southeast Europe (Croatia, Bulgaria and Montenegro). Plum is the most widespread fruit species, because of the good climatic conditions, the fruit value (energetic, nourishment, dietetic) and it is also considered a traditional fruit species. Plums have high economic, social and supply impor-

are traditionally classified as primary and secondary metabolites, although the precise boundary between the two groups can be unclear. The 'Pozegaca' ecotype has small fruit (up to 25 g), dark blue skin, with golden yellow fleshy pulp. Stone separability is good. It belongs to prune plums (for drying), given the high level of total soluble solids. Interest in secondary metabolites and in polyphenols has grown markedly, because of their antioxidant properties and ability to alleviate chronic diseases (Rice-Evans et al., 1997; Vinson et al., 2001; Gardner et al., 2000). Plant polyphenols have shown potential as natural anti-carcinogenic compounds, which, on the basis of their mechanisms of action, may have potential as cancer-blocking and/or cancer-suppressing agents (Noratto et al., 2009). Plums (*Prunus domestica* L.) contain a number of healthy bioactive substances, including vitamins, anthocyanins and other phenolics and carotenoids (Stacewicz-Sapuntzakis et al., 2001). Fruit polyphenols include a wide range of compounds, including hydroxycinnamates, flavan-3-ols (condensed tannins), gallic acid derivatives (hydrolysable tannins), flavonols and anthocyanins (Tomás-Barberán et al., 2001). The phenol composition of the fruit varies greatly among cultivars, and peel tissue contains larger amounts of phenols, anthocyanins, and flavonols than pulp tissue. Mukhtar et al. (2013) and Wisuthiphaet et al. (2014) observed that the wax bloom may protect phenols against high levels of radiation. A certain level of astringent substance is considered vital, contributing to the rich flavour of the fruit and its derived products. Certain phenolic compounds increase resistance to rot caused by pathogens (Herrmann, 1989).

The first objective of this study was to determine the phe-

nolic profile of the native plum ecotype 'Pozegaca', originating from four different growing regions in Bosnia and Herzegovina over three growing years, under extensive orchard conditions; the second objective was to determine the influence of three different growing regions on the fruit quality of the 'Pozegaca' ecotype.

Materials and methods

Plant material

The 'Pozegaca' trees for this study were chosen after morphological and agronomical observation over three years (2009–2011). Only selected ecotypes of 'Pozegaca' had a stable yield in all three observed years. The most important regions in Bosnia and Herzegovina in which 'Pozegaca' trees are grown were chosen for this experiment. All trees were planted in 'on-farm collections' and mainly grown without any special plant cultivation and protection (extensive cultivation). The trees were planted in their own roots (suckers) and the trees were trained as a pyramidal system. Calcocambisol was the soil type in Glavaticveo and Gorazde; in Persovici and Lukavica Rijeka it was Dystric Cambisol. In 2009, when we started the study, the inventoried trees were 17 years old.

The fruit samples of 'Pozegaca' were taken from the four most important plum growing regions of Bosnia and Herzegovina. The Lukavica Rijeka growing region is located in the northeast part of the country at an altitude of 117 m. Gorazde is located in the southeast part at an altitude of 350 m. Glavaticveo and Persovici regions (Konjic locality) are located in the southern part of Bosnia and Herzegovina at altitudes

TABLE 1. Monthly and yearly mean daily air temperatures and precipitation by year and region.

Year	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Year	Vegetation
	Temperature (°C)												Year mean temp.	Vegetation mean temp.
	Rainfall (mm)												Year rainfall	Vegetation rainfall
Gorazde														
2009	-1.3	1.7	6.2	13.0	17.3	18.5	21.3	21.0	17.4	10.3	8.2	3.5	11.4	14.8
	62	44	99	32	56	160	22	87	11	136	72	126	906	674
2010	-0.3	1.8	6.3	11.2	15.1	19.0	21.1	20.7	14.9	8.7	9.6	1.3	10.8	14.1
	86	74	55	102	177	257	110	85	126	65	57	68	1261	1034
2011	0.9	0.9	6.1	12.1	14.8	19.2	21.2	22.0	19.4	9.6	2.8	3.0	11.0	14.1
	32	37	14	29	86	74	147	13	14	45	15	60	566	436
Lukavica Rijeka														
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
	59	29	77	13	83	87	62	90	4	94	59	150	805	568
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
	98	54	52	79	91	319	82	44	106	94	54	72	1145	921
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
	32	34	27	26	44	61	97	9	18	45	12	74	477	337
Konjic														
2009	6.3	5.9	9.5	15.6	21.3	22.0	26.3	26.8	22.3	14.8	10.4	7.5	15.7	18.8
	337	117	174	68	36	190	16	58	128	247	164	326	1860	1080
2010	5.4	6.3	9.3	14.8	17.8	22.8	26.8	26.7	20.2	13.9	11.9	6.0	15.2	18.2
	260	373	175	194	140	99	27	50	236	249	389	300	2491	1557
2011	5.8	7.8	10.0	15.8	19.6	24.7	24.9	27.2	24.9	15.2	10.3	7.8	16.2	19.2
	52	41	145	32	77	31	136	21	36	65	68	170	873	609

of 582 m (Glavaticevo) and 829 m (Persovici). Table 1 summarises the environmental and weather conditions for these plum regions during the three experimental years.

During the three experimental years, the orchards in northern Bosnia (Lukavica Rijeka and Gorazde) experienced similar weather conditions. The warmest season was 2009, followed by 2011. The lowest air temperature was measured in 2010. The difference in air temperature between the years was greater in August and September (time of plum ripening). The average air temperature in September 2010 was significantly lower than in 2011 and 2009. In these northernly situated regions the vegetation period in 2010 had more rainfall than in 2009 and 2011. The driest year was 2011, followed by 2009. Weather conditions in Glavaticevo and Persovici were warmer in all three years than in the aforementioned regions in the northern part of country. In these two regions, the warmest season was in 2011. There was also more precipitation in Glavaticevo and Persovici regions in 2010 than in the other two observed years. Compared to 2010, 2009 and 2011 were both drought years.

Three repetitions from the 'Pozegaca' trees were performed for each region. Each repetition included 10 randomly selected samples of fruit for analysis. The fruits were picked at the proper stage of maturity, following Kader (2002), when the skin colour was dark blue. Basic pomological analysis included fruit weight, fruit flesh ratio and total soluble solids (TSS) in the fruit. The fruit flesh ratio was calculated as the percentage of fruit flesh in the total fruit weight, and TSS was measured using a hand refractometer and expressed in °Brix. The identification and quantification of sugar, organic acids and phenolic compounds were performed using HPLC-MS. After harvesting, fruit samples were packed in plastic bags, frozen in liquid nitrogen and kept at -20°C until extraction.

Standards and chemicals

The following standards were used for quantification of individual polyphenolic compounds: chlorogenic acid, neochlorogenic acid and (+)-catechin from Roth (Karlsruhe, Germany), quercetin 3-glucoside, quercetin 3-galactoside, procyanidin B1, procyanidin B2, cyanidin 3-rutinoside from Fluka (Buchs, Switzerland) and quercetin 3-rutinoside, cyanidin 3-glucoside from Sigma (St. Louis, MO, USA). Methanol was purchased from Riedel-de Haën (Seelze, Germany) and acetonitrile from Sigma-Aldrich (Steinheim, Germany); both were of HPLC grade. Water was bi-distilled using the Milli-Q system (Millipore).

Extraction and determination of sugars and organic acids

Fruit samples were homogenized with a manual blender (Braun) for identification and quantification of the contents of individual sugars (glucose, fructose, sucrose and sorbitol) and organic acids (malic, citric, shikimic and fumaric). The procedure described by Usenik et al. (2008) was used for extraction and determination of sugars and organic acids. Ten g of homogenized fruit were extracted at room temperature with 50 mL of bi-distilled water for 30 min. Extracted samples were then centrifuged at 12,000 *g* for 7 min at 10°C (Eppendorf centrifuge 5810 R, Hamburg, Germany). The supernatant was filtered through a 0.45 µm cellulose ester filter (Macherey-Nagel, Düren, Germany), transferred into a vial and used for analysis. HPLC analysis of sugars was performed using a Thermo separation product (Riviera Beach, USA) HPLC and refractive index (RI) detector. Sugar separation

was carried out using a Rezex RCM-monosaccharide column (300 × 7.8 mm). The column temperature was maintained at 65°C. The samples were eluted according to the isocratic method described by Sturm et al. (2003). Organic acids were analyzed 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. Concentrations of sugars were expressed as g kg⁻¹ of fresh weight (FW), fumaric and shikimic acids as mg kg⁻¹ (FW). Sums of sugars and of organic acids were calculated using the data of individual sugars and individual organic acids.

Extraction and analysis of individual phenolic compounds

The extraction of individual phenolic compounds consisted of submerging 10 g of fruit, homogenized with a 10 mL extraction solution (methanol containing 3% formic acid and 1% 2,6-di-tert-butyl-4-methylphenol (BHT) to prevent degradation of the phenolic compounds), in an ultrasonic ice bath for one hour before centrifuging at 10,000 rpm for seven minutes at 5°C. The supernatant was filtered through a Chromafil AO-45/25 polyamide filter (Macherey-Nagel, Düren, Germany) into a vial. HPLC analysis was performed with the Surveyor system with a diode array detector (DAD) controlled by Chromo-Quest 4.0 chromatography workstation software systems (Thermo Scientific, San Jose, CA). The column used for the separation was a Gemini C18 (150 × 4.6 mm; Phenomenex, Torrance, CA) with particle size of 3 µm maintained at 25°C. Phenolic compounds (catechin, chlorogenic and neochlorogenic acid, procyanidin B1, procyanidin B2) were analysed at 280 nm, flavonols (quercetin 3-galactoside, quercetin 3-rutinoside and quercetin 3-glucoside) were analysed at 350 nm, and anthocyanins (cyanidin 3-glucoside and cyanidin 3-rutinoside) were analysed at 530 nm and identified by retention time and the use of external 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 by HPLC-Finnegan MS detector and an LCQ Deca XP MAX (Thermo Finnegan, San Jose, CA) instrument with electro spray interface (ESI) operating in negative ion mode. Analyses were performed using full-scan, data dependent MS2 scanning from *m/z* 115 to 2,000. Column and chromatographic conditions were identical to those used for the HPLC-DAD analyses. Quantification of individual phenol compounds was achieved according to the concentrations of the corresponding external standard and was expressed in mg kg⁻¹ of FW. Total phenols were calculated from the data of individual phenols.

Data analysis

Analysis of variance (ANOVA) and multiple comparisons (Tukey post hoc test) were used to evaluate the significant difference of the data at *p* < 0.05. Correlations between traits to reveal possible relationships were calculated from raw data of the three years, using the Pearson correlation coefficient at *p* ≤ 0.05. Principal component analysis was applied to standardized variables. Principal component analysis (PCA) was also performed to establish the relationship between experimental factors (growing years and growing regions) and phenol compounds. The principal components scores were plotted for individual observations in relation to the

most significant components whose eigenvalues were greater than 1.0. All statistical analyses, except PCA analysis, were performed using the statistical system "StatBox" (Version 6.7, Grimmersoft, France). The statistical system Statgraphics Centurion XVI, Version 16.1.11. was used for PCA analysis.

Results and discussion

Basic physical and chemical properties

Basic physical and chemical properties, fruit weight, fruit flesh ratio and total soluble solids content in fruit are presented in Table 2.

There were statistically significant differences between growing regions and years for fruit weight and total soluble solids (TSS), as shown in Table 2. There was no significant influence of growing region and season on the fruit flesh ratio of 'Pozegaca' plum. Statistically, the Persovici (21.10 g) and Lukavica Rijeka (19.05 g) ecotypes had greater fruit weight than the other two 'Pozegaca' ecotypes. In 2010, a higher fruit weight was measured than in the other two observed years.

Total soluble solids content (TSS) ranged from 10.2 to 17.9 °Brix. The lowest value of TSS was measured in 2010. Higher air temperatures and dry weather conditions during ripening characterized this year. The obtained results of TSS content are comparable to the results of Usenik et al. (2008), Tomás-Barberán et al. (2001) and Robertson et al. (1992), but Dugalic et al. (2014), and Milošević and Milošević (2012) reported higher values of TSS. The highest value of TSS was observed in plums from Glavaticevo (except in 2011 when it was the ecotype from Lukavica Rijeka). Weather conditions in Lukavica Rijeka in 2011 were marked by a lack of precipitation and higher temperatures during the ripening period. Ionica et al. (2013) reported soluble solids contents between 12.4 to 20.46% in some commercial plum varieties.

Sugar content

Table 3 presents data of sugar composition in 'Pozegaca' plums. The studied 'Pozegaca' ecotypes had considerable variation in sugar contents among years and regions (Table 3). The content of sugars and organic acids depends

on the genotype (Begic-Akagic et al., 2013; Drkenda et al., 2014), but it is also influenced by environmental factors and cultivation measures in an orchard (Hudina et al., 2006).

The total sugar content varied from 91.9 to 169.2 g kg⁻¹ FW. Usenik et al. (2008) and Dugalic et al. (2014) obtained similar results. Sucrose, glucose, fructose and sorbitol were detected in 'Pozegaca' fruit as the major sugars. Glucose was the major sugar, with an average content of 46.99 g kg⁻¹ FW, followed by sorbitol (31.24 g kg⁻¹ FW), sucrose (28.04 g kg⁻¹ FW) and fructose (18.42 g kg⁻¹ FW). The observed plums had a slightly higher glucose content than native plum selections from Slovenia (Usenik et al., 2008).

There were only differences in total sugar, glucose, fructose and sucrose content between growing regions in 2009. In 2009, the total sugar content was notably lower in fruits from Persovici than in fruits from other regions. Difference in sugars content might be explained by differences in environmental conditions between years. Sucrose is one of the main transport sugars (Dugalic et al., 2014). Abidi et al. (2011) indicated that harvesting time could cause variability in sucrose content among years.

Plums from Glavaticevo had a higher content of glucose than plums from Lukavica Rijeka. Over the studied seasons, fructose varied from 12.4 to 29.4 g kg⁻¹ FW. Data analysis showed that in the 2010 season, the lowest fructose content was found in 'Pozegaca' plums. Fructose content was significantly lower in fruits from Glavaticevo and Gorazde than in fruits from Lukavica Rijeka and Persovici. Data analysis showed significant differences in sucrose content between regions only in the 2009 season, when Glavaticevo had notably higher content than other regions.

The sorbitol content varied significantly among the years, ranging from 20.53 to 39.47 g kg⁻¹ FW. The lowest content was also found in 2010. This year had higher rainfall than the 2009 and 2011 seasons. Li and Li (2005) reported that sorbitol has an important role in osmotic adjustment during water deficit. In 2011, growing region had no influence on sorbitol content in 'Pozegaca' plums. In 2009, lower sorbitol content was measured in plums from Persovici and Gorazde than in plums from Glavaticevo and Lukavica Rijeka.

TABLE 2. Some basic physical and chemical properties of native plum 'Pozegaca' ecotypes (mean ± standard deviation).

Ecotype Glavaticevo				Ecotype Gorazde				Ecotype Lukavica Rijeka				Ecotype Persovici			
2009 ^x	2010	2011	Mean	2009	2010	2011	Mean	2009	2010	2011	Mean	2009	2010	2011	Mean
Fruit weight															
16.3 ^b	18.1 ^b	16.6 ^b	17.0 ^b	16.3 ^b	18.4 ^b	13.0 ^b	15.9 ^b	20.5 ^a	21.9 ^a	15.3 ^b	19.1 ^a	17.2 ^b	23.7 ^a	22.8 ^a	21.1 ^a
±2.80	±3.70	±2.60	±3.03	±2.10	±2.01	±1.40	±1.83	±1.87	±2.12	±3.35	±2.44	±1.80	±2.50	±2.90	±2.45
2009 ^y				2010				2011							
17.57 ^e ± 2.14				20.52 ^d ± 2.59				16.92 ^e ± 2.57							
Fruit flesh ratio															
95.5 ^a	95.4 ^a	95.0 ^a	95.3 ^a	95.7 ^a	95.7 ^a	95.0 ^a	95.5 ^a	95.3 ^a	96.2 ^a	94.1 ^a	95.3 ^a	95.6 ^a	96.0 ^a	96.1 ^a	95.9 ^a
±0.60	±0.51	±0.61	±2.37	±0.51	±0.41	±0.42	±0.43	±0.42	±0.38	±1.70	±1.29	±0.42	±0.52	±1.71	±1.02
2009				2010				2011							
95.82 ^d ± 0.45				95.03 ^d ± 0.46				95.03 ^d ± 0.70							
TSS (°Brix)															
17.9 ^a	10.9 ^a	16.0 ^b	14.9 ^a	14.8 ^b	10.5 ^a	14.5 ^b	13.0 ^b	14.5 ^b	11.0 ^a	17.5 ^a	13.4 ^b	12.6 ^b	10.2 ^a	14.8 ^b	13.2 ^b
±1.87	±2.36	±0.70	±1.65	±3.06	±0.72	±0.72	±1.43	±3.63	±0.70	±0.70	±1.62	±1.87	±2.75	±0.29	±1.31
2009				2010				2011							
14.97 ^d ± 3.29				10.72 ^e ± 0.68				15.35 ^d ± 2.32							

^x Different letters a to c for physical and chemical properties indicate significantly different values between ecotypes in the three growing seasons at $p \leq 0.05$.

^y Different letters d to e for physical and chemical properties indicate significantly different values between growing seasons at $p \leq 0.05$.

TABLE 3. Content of sucrose, glucose, fructose, sorbitol, and the sum of sugars (g kg⁻¹ fresh weight) in 'Pozegaca' fruit in relation to ecotype and year (mean ± standard deviation).

Ecotype Glavaticvevo				Ecotype Gorazde				Ecotype Lukavica Rijeka				Ecotype Persovici			
2009 ^x	2010	2011	Mean	2009	2010	2011	Mean	2009	2010	2011	Mean	2009	2010	2011	Mean
Sucrose															
27.1 ^b	21.1 ^a	31.2 ^a	25.5 ^a	31.4 ^{ab}	20.4 ^a	34.2 ^a	28.2 ^a	40.8 ^a	20.01 ^a	32.0 ^a	30.6 ^a	38.4 ^{ab}	20.4 ^a	29.0 ^a	29.6 ^a
±3.51	±2.73	±2.79	±5.32	±9.67	±1.15	±1.33	±9.91	±11.9	±1.21	±10.3	±8.56	±4.16	±0.88	±5.07	±6.7
2009 ^y				2010				2011							
34.85 ^d ± 4.315				20.67 ^f ± 2.03				30.93 ^e ± 4.16							
Glucose															
58.9 ^a	41.8 ^a	54.5 ^a	49.3 ^a	49.3 ^{ab}	42.7 ^a	47.9 ^a	46.2 ^{ab}	40.4 ^b	41.5 ^a	58.0 ^a	43.4 ^b	41.0 ^a	43.3 ^a	51.3 ^a	46.7 ^{ab}
±7.58	±7.39	±7.39	±4.52	±4.52	±4.38	±3.41	±4.72	±17.2	±3.29	±0.14	±7.49	±6.91	±1.21	±6.90	±7.52
2009				2010				2011							
47.15 ^e ± 6.95				42.16 ^f ± 3.36				52.24 ^d ± 6.04							
Fructose															
29.4 ^a	14.8 ^a	23.7 ^a	20.6 ^a	28.4 ^a	12.4 ^a	20.8 ^a	19.3 ^a	16.9 ^b	13.9 ^a	23.4 ^a	16.5 ^b	15.5 ^a	12.6 ^b	18.21 ^a	16.2 ^b
±2.63	±2.63	±6.76	±4.22	±3.37	±0.70	±2.16	±6.68	±5.40	3.34	±2.31	±4.14	±3.43	±0.55	±6.77	±4.29
2009				2010				2011							
21.72 ^d ± 7.48				13.85 ^e ± 2.04				20.91 ^d ± 6.24							
Sorbitol															
53.8 ^a	20.8 ^b	40.5 ^a	33.8 ^a	28.5 ^b	19.7 ^b	32.2 ^a	26.5 ^b	36.7 ^a	24.6 ^a	51.2 ^a	33.6 ^a	21.1 ^b	15.7 ^c	39.8 ^a	29.2 ^{ab}
±7.57	±2.64	±10.6	±5.18	±16.6	±1.66	±1.48	±8.64	±17.9	±13.2	±3.68	±4.69	±6.15	±1.73	±13.5	±4.71
2009				2010				2011							
35.97 ^d ± 7.68				20.53 ^e ± 3.42				39.47 ^d ± 6.52							
Total sugar															
169.2 ^a	98.6 ^a	149.9 ^a	129.1 ^a	137.7 ^{ab}	95.4 ^a	135.1 ^a	120.4 ^b	134.8 ^{ab}	100.0 ^a	164.6 ^a	124 ^{ab}	116.0 ^a	91.9 ^c	138.4 ^a	121.6 ^b
±18.8	±6.99	±23.6	±5.58	±37.8	±2.25	±7.26	±2.28	±36.3	±7.40	±6.19	±4.19	±18.7	±2.88	±27.5	±8.46
2009				2010				2011							
139.69 ^d ± 1.96				97.22 ^e ± 6.83				143.55 ^d ± 1.25							

^x Different letters a to c for chemical properties indicate significantly different values between ecotypes in the three growing seasons at $p \leq 0.05$.

^y Different letters d to f for chemical properties indicate significantly different values between growing seasons at $p \leq 0.05$.

TABLE 4. Content of citric, malic, shikimic and fumaric acids (g kg⁻¹ fresh weight) in 'Pozegaca' fruit in relation to growing region and year (mean ± standard deviation).

Ecotype Glavaticvevo				Ecotype Gorazde				Ecotype Lukavica Rijeka				Ecotype Persovici			
2009 ^x	2010	2011	Mean	2009	2010	2011	Mean	2009	2010	2011	Mean	2009	2010	2011	Mean
Citric acid															
7.76 ^a	3.25 ^a	2.74 ^a	4.03 ^a	6.12 ^a	2.55 ^a	2.97 ^a	3.53 ^{ab}	6.20 ^a	2.38 ^a	3.48 ^a	4.17 ^a	4.47 ^b	3.05 ^a	2.18 ^a	3.00 ^b
±1.22	±0.81	±1.00	±2.16	±1.27	±0.27	±0.29	±1.58	±1.31	±1.19	±0.10	±2.16	±0.97	±0.30	±1.13	±1.34
2009 ^y				2010				2011							
6.14 ^a ± 1.679				2.93 ^e ± 0.838				2.61 ^e ± 0.992							
Malic acid															
15.99 ^a	27.38 ^a	28.85 ^a	25.48 ^a	14.10 ^{ab}	28.19 ^a	27.74 ^a	24.76 ^a	16.39 ^a	22.91 ^b	28.67 ^a	20.90 ^b	12.51 ^b	21.30 ^b	27.72 ^a	22.10 ^b
±1.81	±2.21	±3.67	±5.61	±1.82	±4.14	±2.35	±6.73	±0.62	±2.83	±0.06	±5.02	±1.31	±1.40	±2.80	±6.89
2009				2010				2011							
14.84 ^f ± 2.340				25.60 ^e ± 3.695				28.17 ^d ± 2.883							
Shikimic acid															
0.059 ^a	0.908 ^a	0.073 ^a	0.240 ^b	0.048 ^a	0.653 ^{ab}	0.065 ^a	0.190 ^b	0.057 ^a	0.837 ^a	0.058 ^b	0.390 ^a	0.053 ^a	0.531 ^b	0.052 ^b	0.180 ^b
±0.014	±0.174	±0.009	±0.035	±0.002	±0.048	±0.004	±0.026	±0.005	±0.234	±0.011	±0.42	±0.013	±0.026	±0.007	±0.02
2009				2010				2011							
0.74 ^d ± 0.217				0.07 ^e ± 0.011				0.05 ^e ± 0.012							
Fumaric acid															
0.063 ^a	0.025 ^a	0.026 ^a	0.030 ^b	0.045 ^b	0.021 ^a	0.022 ^a	0.027 ^b	0.069 ^a	0.023 ^a	0.025 ^a	0.040 ^a	0.048 ^b	0.021 ^a	0.022 ^a	0.030 ^b
±0.011	±0.005	±0.005	±0.01	±0.005	±0.003	±0.002	±0.01	±0.017	±0.002	±0.002	±0.02	±0.005	±0.004	±0.003	±0.01
2009				2010				2011							
0.060 ^d ± 0.014				0.020 ^e ± 0.005				0.023 ^e ± 0.004							

^x Different letters a to b for chemical properties indicate significantly different values between ecotypes in the three growing seasons at $p \leq 0.05$.

^y Different letters d to f for chemical properties indicate significantly different values between growing seasons at $p \leq 0.05$.

Organic acid content

The organic acid composition of 'Pozegaca' fruit is presented in Table 4. As can be seen from the table, malic, citric, shikimic and fumaric acids were detected in 'Pozegaca' fruit. This does not agree with Usenik et al. (2008, 2013), who detected only malic, shikimic and fumaric acids in European plum cultivars. Ionica et al. (2013) reported that the organic acids present in fruit, especially malic, tartaric and citric acids, give the acidity of plum fruit. Malic acid was the predominant acid, followed by citric, shikimic and fumaric acids.

The results showed statistically significant differences in all acid components among years and growing regions. The total acids in 'Pozegaca' plums ranged from 17.56 to 32.23 g kg⁻¹ FW. This is slightly more than reported by Usenik et al. (2008, 2013). In the observed 'Pozegaca' fruits, the content of malic acid ranged from 12.51 to 28.85 g kg⁻¹ FW. Analysis of data showed significant differences in the content of malic acid among years and regions. The 2011 season, which had the lowest rainfall, was a favourable year for malic acid content.

There was no significant difference in malic acid content between fruits from Glavaticevo and Gorazde regions and these two regions had greater malic acid content than the other two regions. Over the studied years, citric acid varied from 2.61 to 6.14 g kg⁻¹ FW, with 2009 being a favourable year for citric acid. In 2010 and 2011, there was no significant difference in citric acid content among the observed 'Pozegaca' regions. In 2009, fruits from the Persovici region had significantly less citric acid than fruits from the other three regions.

The content of shikimic acid in 'Pozegaca' plums ranged from 0.048 to 0.908 g kg⁻¹ FW. In 2009, the shikimic acid content was notably higher than in other years. Lukavica Rijeka was a favourable region for accumulation of shikimic acid. The 2009 season was favourable for accumulation of fumaric acid. The fumaric acid content in 'Pozegaca' plums ranged

from 0.021 to 0.069 g kg⁻¹ FW. There was no significant difference in fumaric acid content in the fruit during 2010 and 2011. A significant difference in fumaric acid content was registered among the regions in 2009. Fruit from Lukavica Rijeka showed a significantly higher content of fumaric acid than fruit from the other three regions.

Phenolic content

The level of total and individual phenol compounds detected in the skin and pulp of 'Pozegaca' plum is shown in Tables 5–8. Different phenol compounds were identified, including hydroxycinnamic acids (HCA) (neochlorogenic, chlorogenic), flavonols (quercetin 3-rutinoside, quercetin 3-glucoside, quercetin 3-galactoside and quercetin 3-ramnoside), as well as anthocyanins (cyanidin 3-rutinoside and cyanidin 3-glucoside). Plums have been known to contain various phenolics, containing HCA, flavonols, and anthocyanins (Macheix et al., 1990; Tomás-Barberán et al., 2001; Mattila et al., 2006; Usenik et al., 2008, 2009). The distribution and composition of phenol phytochemicals are affected by maturity, cultivar, horticultural practices, geographic origin, growing season, post-harvest storage conditions and processing procedures (Kim et al., 2003; Usenik et al., 2013).

The results presented in Table 5 show that HCA were the major phenol compounds. Among the identified HCA, neochlorogenic acid was the predominant phenol compound. The neochlorogenic acid content in plum skin varied among the years from 114.67 to 445.58 mg kg⁻¹ FW. It has been reported that neochlorogenic acid is the predominant phenol, with 73% of total phenolics, following by chlorogenic acid (Usenik et al., 2008). The results of the present study are slightly lower, but agree with previous studies that found hydroxycinnamates to be the dominant phenolic compounds (Tomás-Barberán et al., 2001; Usenik et al., 2008).

TABLE 5. Content of dominant hydroxycinnamic acids (HCA) in fruit skin and pulp (mg kg⁻¹ FW) for 'Pozegaca' plum ecotypes (mean ± standard deviation).

Ecotype Glavaticevo				Ecotype Gorazde				Ecotype Lukavica Rijeka				Ecotype Persovici			
2009 ^x	2010	2011	Mean	2009	2010	2011	Mean	2009	2010	2011	Mean	2009	2010	2011	Mean
Neochlorogenic skin															
610 ^a	181 ^b	265 ^a	297 ^a	290 ^b	110 ^b	18 ^a	117 ^b	450 ^b	159 ^b	52 ^a	268 ^{ab}	350 ^b	275 ^a	46 ^a	181 ^{ab}
±127	±65	±527	±159	±139	±27	±4	±124	±121	±67	±20	±188	±84	±79	±27	±151
2009 ^y				2010				2011							
445.58 ^d ± 162.254				180.72 ^e ± 78.45				114.67 ^e ± 71.26							
Neochlorogenic pulp															
140 ^a	206 ^a	48 ^a	143 ^a	13 ^a	121 ^{ab}	23 ^b	86 ^b	110 ^a	87 ^b	41 ^a	92 ^b	120 ^a	189 ^{ab}	41 ^a	96 ^b
±28	±8	±17	±92	±5	±65	±7	±6	±20	±29	±4	±33	±27	±38	±12	±65
2009				2010				2011							
117.41 ^e ± 27.40				188.93 ^d ± 38.22				41.27 ^f ± 11.73							
Chlorogenic skin															
160 ^a	117 ^b	282 ^a	178 ^a	90 ^a	179 ^{ab}	562 ^a	109 ^a	150 ^a	115 ^{ab}	23 ^a	116 ^a	120 ^a	242 ^a	49 ^a	112 ^a
±41	±97	±56.2	±83	±44	±27	±25	±66	±72	±38	±15	±66	±23	±59	±16	±84
2009 ^y				2010				2011							
134.46 ^d ± 53.44				147.96 ^d ± 0.092				125.50 ^d ± 33.19							
Chlorogenic pulp															
7 ^a	19 ^b	1 ^a	11 ^{bc}	5 ^a	37 ^a	0.8 ^{ab}	16 ^a	8 ^a	11 ^b	1 ^a	9 ^{bc}	6 ^a	10 ^b	0.6 ^b	4
±1	±5	±0.2	±9	±1	±17	±0.2	±2	±5	±5	±0.3	±5	±1	±3	±1	±2
2009				2010				2011							
445.58 ^d ± 162.25				180.72 ^e ± 78.45				114.67 ^e ± 71.26							

^x Different letters a to c for chemical properties indicate significantly different values between ecotypes in the three growing seasons at $p \leq 0.05$.

^y Different letters d to f for chemical properties indicate significantly different values between growing seasons at $p \leq 0.05$.

This study found flavonol quercetin 3-rutinoside to be the second major phenol compound. In addition to quercetin 3-rutinoside, quercetin 3-galactoside and quercetin 3-glucoside flavonols were also found. The anthocyanins, cyanidin 3-rutinoside and cyanidin 3-glucoside, were minor phenol compounds (the content of cyanidin 3-rutinoside was higher than that of cyanidin 3-glucoside).

The levels of neochlorogenic and chlorogenic acids in 'Pozegaca' plum are given in Table 5. The main factor affecting the level of neochlorogenic acid (in skin and pulp) was the growing season. Ecotype also significantly affected the neochlorogenic acid content. Neochlorogenic acid in the skin was in a range from 18 to 610 mg kg⁻¹ FW. This neochlorogenic acid level is lower than found previously in European plums (Usenik et al., 2009) or in Californian prunes (Donovan et al., 1998), but higher than in some sweet cherries (Usenik et al., 2010) or peaches (Orazem et al., 2011). Tomás-Barberán et al. (2001) identified neochlorogenic acid in plum cultivars in a range from 16.3 to 342.6 mg kg⁻¹ FW. Although 2009 was a favourable year for neochlorogenic accumulation in plum skin, 2010 was a favourable year for neochlorogenic accumulation in plum pulp. The differences may indicate the

different ripening stages of the fruit. Tomás-Barberán et al. (2001) found that there is no clear trend in phenol content in terms of the ripening of peach, nectarine and plum cultivars. Differences in neochlorogenic acids might be explained by differences in precipitation during the growing seasons. The highest content of neochlorogenic acid was found in the Glavicevo region.

Table 6 presents the average content of dominant flavonols in 'Pozegaca' plum. A slight variation in quercetin 3-rutinoside measured in fruit skin was only noted between years (from 216.8–357.8 mg kg⁻¹ FW). In 2010, no notable content of any flavonols was found in the fruit pulp. However, variation in quercetin 3-rutinoside measured in fruit pulp was influenced by growing season as well as growing region. A slight variation in quercetin 3-rutinoside in pulp was noted in two years (from 0.53–0.96 mg kg⁻¹ FW). Gorazde had a lower amount of quercetin 3-rutinoside in pulp than Glavicevo.

The main factor affecting levels of quercetin 3-galactoside (in skin and pulp) was the growing season. Growing region also had an influence on the content of this flavonol in fruit pulp. There was higher quercetin 3-galactoside accumu-

TABLE 6. Dominant flavonols in fruit skin and pulp (mg kg⁻¹ FW) for 'Pozegaca' plum ecotypes (mean ± standard deviation).

Glavicevo				Gorazde				Lukavica Rijeka				Persovici			
2009 ^x	2010	2011	Mean	2009	2010	2011	Mean	2009	2010	2011	Mean	2009	2010	2011	Mean
Q-Rut skin															
326.3 ^a	320.5 ^a	411.7 ^a	350.0 ^a	186.1 ^b	472.8 ^a	94.6 ^b	261.2 ^a	195.3 ^a	211.9 ^a	85.9 ^b	186.8 ^a	214.0 ^b	522.5 ^a	136.7 ^b	245.5 ^a
±120.2	±89.1	±75.8	±321.9	±116.3	±70.7	±57.1	±191.4	±64.5	±94.2	±20.8	±83.3	±59.6	±41.9	±61.8	±166.5
2009 ^y				2010				2011							
236.8 ^{de} ± 121.66				357.8 ^d ± 134.38				216.8 ^e ± 144.58							
Q-Rut pulp															
1.51 ^a	–	0.52 ^a	0.92 ^a	0.87 ^a	–	0.33 ^b	0.53 ^b	0.73 ^b	–	0.94 ^a	0.78 ^{ab}	0.68 ^b	–	0.56 ^a	0.60 ^{ab}
±0.62		±0.15	±0.63	±0.09		±0.08	±0.29	±0.4		±0.6	±0.48	±0.2		±0.2	±0.16
2009				2010				2011							
0.96 ^d ± 0.52				–				0.53 ^e ± 0.259							
Q-Gal skin															
20.1 ^a	17.9 ^a	5.02 ^a	14.4 ^a	9.23 ^{ab}	26.1 ^a	0.74 ^b	12.5 ^{ab}	8.09 ^{ab}	9.02 ^b	0.89 ^b	7.46 ^b	6.30 ^b	25.3 ^a	1.05 ^b	8.00 ^{ab}
±12.6	±8.11	±9.07	±11.1	±8.54	±11.5	±0.43	±13.9	±5.22	±6.37	0.26	±5.84	±2.88	±5.22	±0.59	±10.2
2009				2010				2011							
11.17 ^e ± 7.56				18.76 ^d ± 6.75				2.36 ^f ± 0.54							
Q-Gal pulp															
0.41 ^a	–	0.03 ^a	0.18 ^{ab}	0.29 ^a	–	0.03 ^a	0.13 ^{ab}	0.34 ^a	–	0.05 ^a	0.25 ^a	0.32 ^a	–	0.01 ^a	0.12 ^b
±0.08		±0.03	±0.16	±0.18		±0.04	±0.11	±0.23		±0.004	±0.21	±0.13		±0.013	±0.10
2009				2010				2011							
0.35 ^d ± 0.16				–				0.02 ^e ± 0.0025							
Q-Glu skin															
23.3 ^a	26.8 ^b	3.44	18.8 ^a	11.6 ^a	48.9 ^a	0.65	21.7 ^a	17.9 ^a	15.9 ^b	0.85	14.6 ^a	9.3 ^a	49.7 ^a	0.83	14.2 ^a
±14.4	±8.79	±6.11	±13.9	±9.22	±19.1	±0.39	±25.5	±13.7	±11.3	±0.14	±12.5	±2.97	±8.87	±0.44	±20.4
2009				2010				2011							
16.07 ^e ± 9.16				32.10 ^d ± 15.75				1.67 ^f ± 0.036							
Q-Glu pulp															
0.09 ^a	–	0.008 ^a	0.04 ^b	0.10 ^a	–	0.01 ^a	0.04 ^b	0.14 ^a	–	0.009 ^a	±0.11 ^a	0.12 ^a	–	0.009 ^a	0.05 ^a
±0.03		±0.004	±0.04	±0.01		±0.01	±0.05	±0.01		±0.01	0.09	±0.04		±0.006	±0.06
2009				2010				2011							
0.12 ^d ± 0.051				–				0.008 ^e ± 0.0025							

^x Different letters a to c for chemical properties indicate significantly different values between ecotypes in the three growing seasons at $p \leq 0.05$.

^y Different letters d to f for chemical properties indicate significantly different values between growing seasons at $p \leq 0.05$.

lation in fruit skin in 2010 (18.76 mg kg⁻¹ FW). Glavaticevo had a higher amount of this flavonol in skin than did Lukavica Rijeka and Persovici. A significant variation of quercetin 3-galactoside in pulp was noted in two years (from 0.02 in 2011 to 0.35 mg kg⁻¹ FW in 2009).

A variation in quercetin 3-glucoside measured in fruit skin and pulp was only noted between growing seasons. The year 2010 was also a favourable year for quercetin 3-glucoside accumulation in fruit skin. Significant variation in quercetin 3-galactoside in pulp was noted between the 2009 and 2011 seasons (from 0.008 in 2011 to 0.12 mg kg⁻¹ FW in 2009).

The average contents of the predominant anthocyanins in the fruit of the 'Pozegaca' ecotype in different growing seasons and regions are presented in Table 7.

The presence of cyanidin 3-glucoside and cyanidin 3-rutinoside was noted in this study. The main factor affecting the content of anthocyanins (in skin and pulp) was the growing season. Growing region had no significant influence on the content of anthocyanins in fruit skin.

The content of cyanidin 3-rutinoside in fruit skin in the three growing seasons ranged from 125.21 to 345.34 mg kg⁻¹ FW. Statistically, the lowest amount of cyanidin 3-rutinoside in fruit skin was in 2011. In the 2010 season, no notable content of any anthocyanin was found in the fruit pulp. Between two seasons (2009 and 2011), there was more cyanidin 3-rutinoside and cyanidin 3-glucoside in fruit pulp in 2009 (1.17 and 0.43 mg kg⁻¹ FW) than in 2011 (0.42 and 0.15 mg kg⁻¹ FW). A significantly higher content of cyanidin 3-rutinoside was found in fruit pulp from the Glavaticevo region than from Gorazde and Persovici. The most favourable year for cyanidin 3-glucoside accumulation in fruit skin was 2009 (215.49 mg kg⁻¹ FW) and the lowest content of this anthocyanin was

in 2011. Higher contents of cyanidin 3-glucoside in fruit pulp were found in Glavaticevo and Gorazde than in the other two regions.

The analysed 'Pozegaca' fruit (whole) contained many phenolic compounds, ranging from 0.23 to 1.89 g kg⁻¹ FW (Table 8). However, no notable differences were determined in the total phenolic content among the examined regions. Differences in total phenolic amounts were a consequence of year. The most favourable years for total phenols were 2009 and 2010. In 2009, the total phenolic content did not differ significantly among the plum regions. Plums from Glavaticevo had the highest amount of total phenolic content in 2011 and plums from Persovici in 2010.

The maximum amount of phenolics was detected in fruit skin, ranging from 0.19 to 1.89 g kg⁻¹ FW. Differences in the amount of polyphenols in skin were associated with year. Data analysis and a post hoc test showed a significant difference in phenolic content in fruit skin in two of the investigated years (2010 and 2011). The highest phenolic content in fruit skin in 2011 was measured in fruit from Glavaticevo. In 2010, fruit from Gorazde and Persovici had higher phenolic content in skin than fruit from the other two regions. Fruits from Glavaticevo showed significantly higher polyphenols content in pulp than the plums from Gorazde in 2011 and from Lukavica Rijeka in 2010.

Principal component analysis

In order to overview the variability and homogeneity of data for growing years and growing regions, PCA, using a correlation matrix, was applied. Of the PCA that assisted in classification of the experimental factors, the first two principal components, with eigenvalue over 1 (Keiser criteria), accounted for 71.98% of the variability (Table 9).

TABLE 7. Dominant anthocyanins in fruit skin and pulp (mg kg⁻¹ FW) for 'Pozegaca' plum genotype (mean ± standard deviation).

Glavaticevo				Gorazde				Lukavica Rijeka				Persovici			
2009 ^x	2010	2011	Mean	2009	2010	2011	Mean	2009	2010	2011	Mean	2009	2010	2011	Mean
CY-rutinoside skin															
408.23 ^a	251.0 ^b	284.43 ^a	293.88 ^a	290.47 ^b	448.48 ^a	50.82 ^b	259.07 ^a	338.48 ^{ab}	172.9 ^b	21.19 ^b	222.17 ^a	316.74 ^{ab}	530.57 ^a	47.68 ^b	230.87 ^a
±82.02	±77.57	±155.6	±139.88	±183.10	±117.67	±24.92	±128.98	±227.76	±61.71	±19.23	±187.11	±132.31	±112.90	±17.18	±218.65
2009 ^y				2010				2011							
345.34 ^d ± 155.81				314.86 ^d ± 155.78				125.21 ^e ± 93.96							
CY-rutinoside pulp															
2.08 ^a	–	0.48 ^a	0.97 ^a	0.72 ^b	–	0.26 ^b	0.34 ^b	1.37 ^a	–	0.27 ^b	0.63 ^{ab}	0.32 ^b	–	0.47 ^a	0.43 ^b
±1.4		±0.27	±0.68	±0.36		±0.16	±0.30	±0.8		±0.09	±0.33	±0.28		±0.42	±0.37
2009				2010				2011							
1.17 ^d ± 1.01				–				0.42 ^e ± 0.33							
CY-glucoside skin															
196.09 ^a	105.6 ^b	64.53 ^a	111.59 ^{ab}	144.35 ^a	206.64 ^a	10.30 ^b	116.75 ^{ab}	331.36 ^a	73.07 ^b	4.96 ^b	174.18 ^{ab}	154.26	242.11 ^a	7.94 ^b	101.07 ^b
±82.61	±39.66	±12.79	±88.64	±95.33	±58.88	±5.22	±94.68	±123.94	±39.50	±2.13	±64.30	±61.30	±66.73	±2.39	±89.82
2009				2010				2011							
215.49 ^d ± 165.46				138.70 ^e ± 78.27				27.02 ^f ± 17.60							
CY-glucoside pulp															
0.66 ^a	–	0.17 ^a	0.37 ^a	0.19 ^b	–	0.12 ^a	0.14 ^b	0.60 ^a	–	0.06 ^b	0.47 ^a	0.16 ^b	–	0.16 ^a	0.16 ^b
±0.038		±0.08	±0.03	±0.003		±0.006	±0.007	±0.30		±0.03	±0.27	±0.12		±0.14	±0.12
2009				2010				2011							
0.43 ^d ± 0.33				–				0.15 ^e ± 0.11							

^x Different letters a to c for chemical properties indicate significantly different values between ecotypes in the three growing seasons at $p \leq 0.05$.

^y Different letters d to f for chemical properties indicate significantly different values between growing seasons at $p \leq 0.05$.

TABLE 8. Content of total phenols in fruit skin and pulp (g kg⁻¹ FW) for 'Pozegaca' plum ecotypes (mean ± standard deviation).

Ecotype Glavaticevo				Ecotype Gorazde				Ecotype Lukavica Rijeka				Ecotype Persovici			
2009 ^x	2010	2011	Mean	2009	2010	2011	Mean	2009	2010	2011	Mean	2009	2010	2011	Mean
Skin															
1.75 ^a	1.02 ^b	1.32 ^a	1.26 ^a	1.02 ^a	1.49 ^a	0.23 ^b	0.90 ^a	1.49 ^a	0.76 ^b	0.19 ^b	0.99 ^a	1.16 ^a	1.89 ^a	0.29 ^b	0.89 ^a
±0.22	±0.25	±0.55	±0.94	±0.59	±0.317	±0.114	±0.66	±0.79	±0.267	±0.072	±0.71	±0.26	±0.347	±0.09	±0.70
2009 ^y				2010				2011							
1.79 ^d ± 0.80				0.76 ^{de} ± 0.27				0.19 ^e ± 0.07							
Pulp															
0.15 ^a	0.23 ^a	0.05 ^a	0.15 ^a	0.14 ^a	0.16 ^{ab}	0.02 ^b	0.10 ^a	0.13 ^a	0.10 ^b	0.04 ^{ab}	0.10 ^a	0.13 ^a	0.20 ^{ab}	0.04 ^{ab}	0.10 ^a
±0.03	±0.08	±0.02	±0.08	±0.004	±0.049	±0.008	±0.07	±0.025	±0.034	±0.002	±0.04	±0.03	±0.041	±0.01	±0.068
2009				2010				2011							
0.13 ^f ± 0.02				0.18 ^e ± 0.08				0.42 ^d ± 0.02							
Whole fruit															
1.89 ^a	1.25 ^{ab}	1.36 ^a	1.41 ^a	1.16 ^a	1.65 ^{ab}	0.25 ^b	1.00 ^a	1.61 ^a	0.86 ^b	0.23 ^b	1.09 ^a	1.29 ^a	2.09 ^a	0.33 ^b	0.99 ^a
±0.23	±0.29	±0.75	±1.12	±0.79	±0.769	±0.11	±0.70	±0.81	±0.990	±0.07	±0.75	±0.66	±1.364	±0.096	±0.66
2009				2010				2011							
1.54 ^d ± 0.56				1.37 ^d ± 0.50				0.66 ^e ± 0.44							

^x Different letters a to c for chemical properties indicate significantly different values between ecotypes in the three growing seasons at $p \leq 0.05$.

^y Different letters d to f for chemical properties indicate significantly different values between growing seasons at $p \leq 0.05$.

The results presented in Figure 1 indicate that the first principal component (F1) strongly positively correlated with the amount of chlorogenic acid in pulp, all three identified flavonols in skin, total phenols in pulp, as well as precipitation. Altitude, neochlorogenic acid in pulp, malic acid, chlorogenic acid in skin, total phenols in skin and total phenols in whole fruit were also positively correlated with F1 but slightly less than the first group of fruit attributes. However, first principal component strongly negatively correlated with the amount of sorbitol, glucose, fructose, sucrose, total soluble solids, total sugars, quercetin 3-rutinoside in pulp, citric acid, fumaric acid, shikimic acid, cyanidin 3-glucoside in pulp and cyanidin 3-rutinoside in pulp. Air temperature and neochlorogenic acid were slightly negatively correlated with F1.

Precipitation (rainfall) was strongly positively correlated with F1 and air temperature was negatively correlated with F1. The content of fructose and sucrose significantly positively correlated with air temperature. Air temperature strongly positively correlated with some phenols in pulp (flavonols and anthocyanins). On the other hand, sugar compounds negatively correlated with precipitation (rainfall is situated on the opposite side of sugars compounds, particularly glucose and sorbitol, in F1 components of Figure 1). This indicates that a lack of precipitation induced greater glucose and sorbitol content. Dietrich et al. (2007) reported that juice produced from non-irrigated pear fruit had increased sorbitol content. Glucose, fructose, sucrose and sorbitol contents highly and positively correlated with each other. This agrees with Cantin et al. (2009), who found that individual sugar contents in peach and nectarine correlated with each other.

TABLE 9. Eigenvalues and proportion of total variability of the first two principal components (PC), and component scores for four ecotypes of 'Pozegaca' plum and three growing seasons.

Component	Eigenvalue	Percent of variance	Cumulative percentage
F1	11.43	36.88	36.88
F2	10.88	35.11	71.98

The second principal component (F2) strongly positively correlated with the amount of malic acid and total acid content. A slight positive correlation F2 component was noted with glucose and sorbitol. However, F2 strongly negatively correlated with content of neochlorogenic acid in skin, both identified anthocyanins in skin and pulp, shikimic and fumaric acids, quercetin 3-galactoside and quercetin 3-glucoside in skin and pulp, total phenols in whole fruit, skin and pulp, as well as with precipitation. Altitude, chlorogenic acid in skin and pulp, and quercetin 3-rutinoside in skin and pulp were slightly correlated with the negative part of the F2 component. Wang and Camp (2000), Gautier et al. (2005) and Burdon et al. (2007) reported that environmental factors influenced fruit acidity and malic accumulation.

The response of years to climatic conditions varies, which may be explained by differences in air temperature and precipitation (Figure 1). An effect of precipitation and altitude was noted for 2010 (positive part of F1 component in Figure 1). The highest effect of air temperature was noted in 2009 and 2011 (negative part of F1 component in Figure 1). However, there was difference between 2009 and 2011 related to component F2. Growing seasons 2009 and 2011 are situated on opposite sides of F2 components.

In terms of the accumulation of phenol compounds, air temperature strongly correlated with quercetin 3-rutinoside, quercetin 3-galactoside in pulp and cyanidin 3-rutinoside in pulp.

Precipitation had a strong correlation with total phenols in pulp, chlorogenic acid in skin and pulp, neochlorogenic acid in pulp, all three identified quercetins in pulp.

The results presented in Figure 1 indicate that 'Pozegaca' ecotypes from the southern part of Bosnia and Herzegovina (Persovici and Glavaticevo) are located in the negative part of F2 in 2009 and 2010. In 2009, ecotypes from Glavaticevo primarily stand out from the other ecotypes.

The year 2010 had the highest rainfall and ecotypes from Glavaticevo and Gorazde had mostly similar fruit attributes in this year. This could be attributed to the same type of soil in these two regions (Calcocambisol). The ecotype from Lukavac Rijeka in this year mostly had a high content of malic acid.

FIGURE 1. Principal component biplot of the phytochemical fruit attributes depend on growing seasons and regions. PGL: ecotype Glavicevo; PGO: ecotype Gorazde; PLR: ecotype Lukavac Rijeka; PP: ecotype Persovici; 9: year 2009; 10: year 2010; 11: year 2011; acids: total acids; alt: altitude; chlop: chlorogenic acid pulp; chlosk: chlorogenic acid skin; CY-Glp: cyanidin 3-glucoside pulp; CY-GlSk: cyanidin 3-glucoside skin; CY-R: cyanidin 3-rutinoside pulp; CY-Rsk: cyanidin 3-rutinoside skin; cit: citric acid; fruc: fructose; fum: fumaric acid; gluc: glucose; mal: malic acid; neop: neochlorogenic acid pulp; neosk: neochlorogenic acid skin; Q-GALp: quercetin 3-galactoside pulp; Q-GALsk: quercetin 3-galactoside skin; Q-Glp: quercetin 3-glucoside pulp; Q-GlSk: quercetin 3-glucoside skin; Q-Rp: quercetin 3-rutinoside pulp; Q-Rsk: quercetin 3-rutinoside skin; rain: precipitation; shic: shikimic acid; sorb: sorbitol; suc: sucrose; temp: air temperature; TPH: total phenols; TPp: total phenols pulp; TPsk: total phenols skin; TSS: total soluble solids; Ts: total sugars.

In 2011, the ecotype Glavicevo stood out from the other three ecotypes in content of glucose and sorbitol.

Conclusions

This study emphasizes that environmental conditions such as air temperature, precipitation and altitude can have a significant effect on plum fruit quality attributes. The main factor affecting fruit quality and the phenol composition of 'Pozegaca' plums (in skin and pulp) was growing season. Geographical region appears to have no major influence on the phenol profile of 'Pozegaca' fruit. In the tested 'Pozegaca' fruit, glucose, fructose, sucrose and sorbitol as major sugars were detected. Malic, citric, fumaric and shikimic acids were also identified in the studied plum fruit. Glucose was the predominant sugar and malic acid was the predominant organic acid. A range of phenolic compounds was identified, including hydroxycinnamic acids (HCA) (neochlorogenic and chlorogenic acids); flavonols (quercetin 3-rutinoside, quercetin 3-glucoside and quercetin 3-galactoside), as well as anthocyanins (cyanidin 3-rutinoside and cyanidin 3-glucoside), together with a small amount of flavan-3-ols (catechin and the dimmers procyanidins B1 and B2). The results of PCA indicated that air temperature had a strong correlation with sugar compounds (particularly with fructose and sucrose) and with some organic acids (fumaric, shikimic and citric). In terms of the accumulation of phenol compounds, air temperature strongly correlated with some flavonols (particularly quercetin 3-rutinoside) and anthocyanins in pulp.

Precipitation was strongly correlated with total phenols in pulp, chlorogenic acid in skin and pulp, neochlorogenic acid in pulp and cyanidin 3-rutinoside in skin. Precipitation was strongly correlated with total phenols in pulp, chlorogenic acid in skin and pulp, neochlorogenic acid in pulp and cyanidin 3-rutinoside in skin. The results indicate that altitude positively correlated with precipitation and had a similar effect on phenolic profiles of different 'Pozegaca' ecotypes. We suggest that in future research, differences among 'Pozegaca' ecotypes from different localities could be investigated using molecular markers.

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