Polyphenolic profile of selected varieties of Serbian berries

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ABSTRACT

Selected varieties of strawberries, blackberries, and blueberries grown in Serbia have been analyzed, and their polyphenolic profile was determined by HPLC analysis. The occurrence of phenolic acids, flavonols, and anthocyanins has been examined. Caffeic, *p*-coumaric, ferulic, and ellagic acid were identified and quantified. Six flavonols have been identified in berry samples: quercetin, quercetin-glucoside, quercetin-galactoside, kaempferol, kaempferol-glucoside, and rutin. The following anthocyanins were found in strawberries: pelargonidin-glucoside, pelargonidin-rutinoside, cyanidin-glucoside, and cyanidin-malonylglucoside. Cyanidin-glucoside, cyanidin-rutinoside, and cyanidin-malonylglucoside were found in blackberry samples. The following anthocyanins were found in blueberries: delphinidin-galactoside, delphinidin-glucoside, delphinidin-arabinoside, cyanidin-galactoside, cyanidin-glucoside, cyanidin-arabinoside, petunidin-arabinoside, petunidin-galactoside, peonidin-galactoside, and malvidin-galactoside. Statistical multivariate method - principal component analysis (PCA) was used to classify phenolic acids, flavonols, and anthocyanins according to their contents in berry samples. Cluster analysis (CA) was used to classify samples based on the individual polyphenolics content.

Keywords: anthocyanins, flavonols, phenolic acids, HPLC, berry fruits, PCA analysis

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Introduction

Specific aroma, attractive color, and juiciness have made berries one of the world's most popular types of fruits. Given the suitability of the fruits to be consumed either fresh or processed and increasing demands in the world market, berry fruits have been very significant for the Serbian economy.

Berries are poor in sodium but rich in potassium, magnesium, phosphorus, manganese, iron, and zinc (Bagdatlioglu et al., 2010; Guedes et al., 2013; Hakala et al, 2003; Khan et al., 2010), and therefore an excellent food choice for people with hypertension. The presence of copper, nickel, and chromium has been detected in raspberries, blueberries, and strawberries (Grembecka and Szefer, 2013). Other than minerals, berries contain vitamins, and fibers (Zhao, 2007), and are exceptionally rich in polyphenolic compounds (Szajdek & Borowska, 2008; Zhao, 2007). These bioactive compounds participate in the prevention of cardiovascular and neurodegenerative diseases, diabetes, and obesity due to their highly expressed capacity to scavenge free radicals (Coates et al., 2007; Mattioli et al., 2020; Mullen et al., 2002; Paredes-López et al., 2010). Anticancer, antimicrobial, and anti-inflammatory properties were observed *in vitro* and *in vivo* (Nile & Park, 2014). According to Miyake et al. (2012), vision preservation during retinal inflammation is achieved by consuming anthocyanin-rich bilberry extracts. The most significant health benefits of berry fruits are assigned to the presence of polyphenolic compounds. Phenolic acids, anthocyanins, flavonols, and tannins also contribute to the quality, nutritional value, color, and flavor of the fruits (Delgado-Vargas et al., 2000).

Considering such a positive effect on human health, this study aimed to provide a comprehensive analysis and an update on individual phenolic compounds in strawberries, blackberries, and blueberries.

Experimental

Chemicals

Purified water (18 MΩcm), prepared by a MicroMed purification system (TKA Wasseraufbereitungssysteme GmbH, Niederelbert, Germany) was used to prepare all samples and standards. Methanol and acetonitrile (HPLC grade) were obtained from J.T. Baker (Deventer, The Netherlands), and hydrochloric and formic acids were purchased from Merck® (Darmstadt,

Germany). Kaempferol, malvidin-3-O-glucoside chloride, coumaric acid, ferulic acid, caffeic acid, rutin, quercetin (HPLC grade), delphinidin-3-O-glucoside chloride, pelargonidin-3-O-glucoside chloride and ellagic were purchased from Sigma Aldrich (Steinheim, Germany). Cyanidin-3-O-glucoside chloride and quercetin-3-O-β-glucoside (HPLC grade) were from ChromaDex (Irvine, CA, USA).

Samples

Three cultivars of strawberry fruits, five cultivars of blackberry fruits, and three cultivars of blueberry fruits were collected in parts of Western and Southern Serbia (Table 1). Approximately 500 g of each cultivar was collected and stored in the freezer at -18°C.

Table 1. Cultivars and geographic origin of berry fruits

Fruit	Cultivar	Sample designation	Harvesting period	Geographic origin
Strawberry	Čačanska rana	S1	May	Western Serbia
	Alba	S2	May	Western Serbia
	Senga Sengana	S3	June	Southern Serbia
Blackberry	Čačanska bestrna	Bc1	August	Western Serbia
	Triple Crown	Bc2	August	Western Serbia
	Čačanska bestrna	Bc3	August	Southern Serbia
	Thorn free	Bc4	August	Southern Serbia
	Wild blackberry	Bc5	August	Southern Serbia
Blueberry	Draper	B1	July	Western Serbia
	Huron	B2	July	Western Serbia
	Wild blueberry (bilberry)	B3	August	Southern Serbia

Preparation of extracts

Ultrasound-assisted extraction has been recently reported as a more efficient method for the extraction of anthocyanins and flavonols from berry fruits than conventional solvent extraction (Ivanović et al., 2014). Ultrasound-assisted extraction with acidified 80% methanol (0.01%, v/v HCl) was used to prepare anthocyanin and flavonol-containing extracts from berry samples. Frozen berry fruits were milled in the blender to obtain puree, which was subsequently used for extraction. Briefly, 2.0000 g ± 0.0001 g of each berry sample was weighed, and the flask with a sample was placed into the ultrasonic bath and sonicated for 15 min at 25 °C. After sonication, the

extracts were joined, and the solvent was removed by vacuum evaporation. The extracts were stored in the fridge before analysis.

HPLC analysis

A model 1200 (Agilent Technologies, Santa Clara, California, USA) was used for HPLC analysis. The analytical column was C18 Zorbax Eclipse XDB-C18, 5µm, 4.6×150 mm (Agilent Technologies, Santa Clara, California, USA). To identify and determine individual anthocyanins, Agilent-1200 series HPLC with the UV-Vis photodiode array detector (DAD) was used. The column was thermostated at 25 °C. Chromatographic separation was performed in an Agilent-Eclipse XDB C-18 4.6×150 mm column. The mobile phase consisted of aqueous 5% formic acid (eluent A) and 80% acetonitrile/5% formic acid (eluent B). The elution program used was as follows: from 0 to 10 min 0% B, from 10 to 28 min gradually increases 0-25% B, from 28 to 30 min 25% B, from 30 to 35 min gradually increases 25-50% B, from 35 to 40 min gradually increases 50-80% B, and for the last 5 min gradually decreases 80-0% B.

Results and Discussion

Identification of individual polyphenolics was conducted by comparing UV/Vis spectra and retention times of compounds to available standards and literature data. The quantification of phenolic acids was performed based on calibration standards for caffeic, ferulic, and *p*-coumaric acid standards in the concentration interval from 0.1 mg/ml to 1 mg/ml, and for ellagic acid in the concentration interval from 0.2 mg/ml to 1 mg/ml.

The quantification of quercetin (Que), kaempferol (Kaem), and rutin (Rut) were performed based on calibration curves for standards of Que, Kaem, and Rut in the concentration interval from 0.1 mg/ml to 1 mg/ml. Quercetin-glucoside (Que-glu) and quercetin-galactoside (Que-gal) were calculated as Que-equivalents, while kaempferol-glucoside (Kaem-glu) was calculated as Kaem-equivalent.

Quantifications of cyanidin-3-*O*-glucoside (Cy-glu), delphinidin-3-*O*-glucoside (Delp-glu), and malvidin-3-*O*-galactoside (Mal-gal) were performed based on calibration curves for the standards of Cy-glu, Delp-glu, and Mal-gal in the concentration interval from 0.1 mg/ml to 1 mg/ml. Cyanidin-3-O-rutinoside (Cy-rut), cyanidin-3-*O*-malonylglucoside (Cy-malglu), and

cyanidin-3-*O*-galactoside (Cy-gal) were calculated as cy-glu equivalents. Delphinidin-3-*O*-galactoside (Delp-gal) and delphinidin-3-O-arabinoside (Delp-ara) were calculated as Delp-glu equivalents. Petunidin-3-*O*-arabinoside (Pet-ara), petunidin-3-*O*-galactoside (Pet-gal), and peonidin-3-*O*-galactoside (Peo-gal) were calculated based on the calibration curve for Cy-glu (Nyman and Kumpulainen, 2001). Pelargonidin-3-O-glucoside (Pg-glu) and pelargonidin-3-*O*-rutinoside (Pg-rut) were calculated based on the calibration curve for the Pg-glu standard in the concentration interval from 0.1 mg/ml to 1 mg/ml. All the measurements were performed in triplicate.

The content of phenolic acids

The content of phenolic acids in analyzed berry fruits has been given in Table 2. In strawberry samples, caffeic, *p*-coumaric, ferulic, and ellagic acids were identified and quantified. Obtained results for caffeic, *p*-coumaric, and ferulic acid were lower than the literature data (Häkkinen & Törrönen, 2000; Jakobek et al., 2007; Matilla et al., 2006). On the other hand, the content of ellagic acid was higher than that one of Croatian strawberries (Jakobek et al., 2007).

In blackberry samples, *p*-coumaric, ferulic, and ellagic acid were detected, and caffeic acid was detected in one sample of blackberries only (Bc5). The content of ellagic acid in blackberries in our study was lower than reported by Đurić et al. (2014). The main cause may be acidic hydrolysis (2 hours, 85 ^{o}C) applied in the mentioned study (Đurić et al., 2014). Namely, the main ellagitannin compounds are sanguiin H-6 and lambertianin C. Ellagitannins can be hydrolyzed with acids to release hexahydroxydiphenoyl units, which spontaneously form ellagic acid. Therefore, the quantification of ellagic acid is highly dependent on the hydrolysis procedure (Bobinaite et al., 2012). It has been shown that ellagic acid exhibits anticancer properties in living organisms by modifying the metabolism of toxins absorbed from the environment (Vattem & Shetty, 2005). After oral application, hepatoprotective effect against CCl_4 *in vitro* and *in vivo* has been observed (Singh et al., 1999).

Caffeic and ferulic acid were detected in blueberry samples. The content of ferulic acid is following literature data (Jakobek et al., 2007; Mattila et al., 2006). *p*-Coumaric and ellagic acid were not detected in blueberry samples, and a similar was noted in a study by Häkkinen and Törrönen (2000).

Table 2. Content of phenolic acids in analyzed samples (mg/kg)

Sample	Caffeic acid		*p*-Coumaric acid		Ferulic acid		Ellagic acid	
	c±SD	RSD (%)	c±SD	RSD (%)	c±SD	RSD (%)	c±SD	RSD (%)
S1	0.741±0.001	0.13	3.47±0.05	1.44	1.95±0.02	1.02	125±3	2.40
S2	0.893±0.003	0.34	1.50±0.03	2.00	1.16±0.01	0.86	65±1	1.54
S3	1.127±0.007	0.62	2.00±0.02	1.00	1.68±0.03	1.78	69±1	1.45
Bc1	n.d.	-	4.28±0.07	1.63	1.30±0.01	0.77	76±1	1.31
Bc2	n.d.	-	3.19±0.03	0.94	1.36±0.05	3.68	85±2	2.35
Bc3	n.d.	-	2.81±0.01	0.35	1.74±0.07	4.02	73±1	1.37
Bc4	n.d.	-	2.27±0.01	0.44	1.92±0.03	1.56	97±1	1.03
Bc5	0.981±0.003	0.31	2.83±0.06	2.12	2.15±0.06	2.79	227±5	2.20
B1	9.21±0.05	0.54	n.d.	-	4.50±0.03	0.67	n.d.	-
B2	9.12±0.09	0.99	n.d.	-	20.7±0.3	1.45	n.d.	-
B3	14.37±0.08	0.56	n.d.	-	26.2±0.5	1.91	n.d.	-

n.d. – not detected

The content of flavonols

As can be seen from Table 3, six flavonols have been identified in analyzed samples: Que, Que-glu, Que-gal, Kaem, Kaem-glu, and Rut, with glycosides of Que being more abundant than glycosides of Kaem. The content of Que in Serbian berries was higher than the one reported for Finnish berries (Häkkinen et al., 1999). According to Mikulic-Petkovsek et al. (2012), glycosides of Que represent 46-100% of total flavonols in different types of berries. Efficient extraction of flavonols from apple samples has been achieved by applying methanol and acidified methanol (Rupashinge et al., 2011). Furthermore, the extraction of flavonols from onion by ethanol, 70% methanol, and subcritical water has been described in the literature (Kim et al., 2014; Ko et al., 2011; Numata & Tanaka, 2011).

Rutin was detected in blackberry and blueberry fruits but not in strawberry samples. There is not much data on the presence of Rut in berry fruits. Obtained results for the content of Rut in blackberries were significantly lower than the available data (Okatan, 2020).

The content of anthocyanins

Four anthocyanins were identified in strawberries: Pg-glu, Pg-rut, Cy-glu, and Cy-malglu (Table 4). The most abundant anthocyanin in all samples was Pg-glu (73.8-80.8%), which agrees with the literature data (da Silva et al., 2007). The content of the other anthocyanins depended on the cultivar of strawberries.

Cy-glu, Cy-rut, and Cy-malglu were found in blackberries (Table 5). The most abundant anthocyanin in all samples was Cy-glu, exceeding 85% in Bc3 and Bc4 and exceeding 90% in Bc1, Bc2, and Bc5 of total anthocyanin content. The presence of these anthocyanins was also detected in blackberries from Chile, France, North Macedonia, and the USA (Fang-Chiang & Wrolstad, 2005).

A large number of individual anthocyanins was detected in blueberries: Delp-gal, Delp-glu, Delp-ara, Cy-gal, Cy-glu, Cy-ara, Pet-ara, Pet-gal, Peo-gal, and Mal-gal. Serbian blueberries manifest a high level of similarity to Slovenian and South Korean blueberries regarding anthocyanin profile (Bae et al., 2015; Trošt et al., 2008). Delp-ara was not detected in sample B1. Glycosides of delphinidin were the most abundant in all samples (33.41-39.05%), followed by glycosides of cyanidin (26.08-28.01%).

According to obtained results, it is evident that each of the berry fruits possessed a very specific anthocyanin profile. Glycosides of pelargonidin were found only in strawberries, while the glycosides of delphinidin, malvidin, peonidin, and petunidin were found only in blueberries. Blackberries contained glycosides of cyanidin exclusively. Specific differences among samples were also observed regarding sugar moieties. Rutinosides and malonylglucosides were found in strawberries and blackberries, while arabinosides and galactosides were exclusively found in blueberries.

The beneficial effect of anthocyanins on human health was the subject of many studies. Delphinidin exhibits an antimicrobial effect against *Staphylococcus aureus* bacteria and acts as an antiphlogistic (anti-inflammatory) and immunosuppressant (Roewer & Broschet, 2013a; Roewer & Broschet, 2013b).

Pelargonidin-3-glucoside, cyanidin-3-glucoside, and delphinidin-3-glucoside, as well as their aglycones, exhibit a strong antioxidant effect in the liposomal system and reduce the formation of malonyl-aldehyde, which occurs as a result of UVB radiation. At the same time,

pelargonidin is the most effective in scavenging hydroxyl radicals, while delphinidin is more effective in inhibiting lipid peroxidation and scavenging superoxide radicals (Tsuda et al., 1996). Anthocyanins exert an anti-angiogenic effect. Angiogenesis represents the growth of new blood vessels from existing capillaries or veins, and endothelial cells play a crucial role in this physiological process. However, disruption of the balance in the body can lead to angiogenesis in cases where it is not desirable (the occurrence of cancer, cardiovascular diseases, retinopathy, and nephropathy caused by diabetes) (Xue et al., 2010). Blueberry, strawberry, and cranberry extracts, rich in anthocyanins, have a suppressive effect on the vascular endothelial growth of human keratinocytes, which is caused by the action of the multifunctional cytokine tumor necrosis factor (TNF) (Roy et al., 2002).

Anthocyanins also have a beneficial effect on the cardiovascular system, exerting a vasorelaxant effect (Bell & Gochenaur, 2006). The antidiabetic effect of pelargonidin-3-galactoside, cyanidin-3-glucoside, and delphinidin-3-glucoside is reflected in the promotion of insulin secretion, reduction of blood glucose levels, and prevention of insulin resistance (Li et al., 2015).

Table 3. Content of flavonols (mg/kg) in analyzed samples

Sample	Que		Que-glu		Que-gal		Kaem		Kaem-glu		Rut	
	c±SD	RSD (%)	c±SD	RSD (%)	c±SD	RSD (%)	c±SD	RSD (%)	c±SD	RSD (%)	c±SD	RSD (%)
S1	21.26±0.5	2.35	3.16±0.03	0.95	32.1±0.1	0.31	3.01±0.01	0.33	3.30±0.02	0.61	n.d.	-
S2	14.37±0.2	1.39	3.33±0.05	1.50	21.9±0.3	1.37	1.77±0.02	1.13	2.58±0.07	2.71	n.d.	-
S3	12.90±0.7	5.43	2.48±0.04	1.61	23.2±0.5	2.16	1.84±0.03	1.63	2.34±0.05	2.14	n.d.	-
Bc1	11.59±0.02	0.43	1.55±0.02	1.29	24.4±0.3	1.23	1.72±0.06	3.49	1.76±0.01	0.57	2.59±0.03	1.16
Bc2	10.14±0.1	0.99	2.44±0.01	0.41	26.8±0.7	2.61	1.69±0.05	2.96	1.95±0.03	1.54	5.61±0.05	0.89
Bc3	8.95±0.01	0.89	1.68±0.01	0.60	24.3±0.2	0.82	1.55±0.03	1.94	1.94±0.03	1.55	4.09±0.07	1.71
Bc4	8.09±0.01	0.12	1.76±0.03	1.70	29.8±0.3	1.01	1.66±0.04	2.41	3.08±0.07	2.27	2.42±0.04	1.65
Bc5	10.45±0.02	0.19	15.72±0.05	0.32	40.1±0.5	1.25	2.74±0.05	1.82	2.37±0.06	2.53	16.4±0.3	1.83
B1	13.99±0.5	3.57	2.32±0.07	3.02	29.8±0.6	2.01	1.58±0.01	0.63	3.05±0.02	0.66	9.2±0.03	0.33
B2	16.47±0.3	1.82	2.65±0.03	1.13	35.8±0.3	0.84	1.82±0.01	0.55	3.30±0.04	1.21	7.2±0.01	0.14
B3	16.17±0.3	1.86	2.69±0.08	2.97	38.6±0.7	1.81	1.86±0.02	1.08	3.40±0.01	0.29	31.1±0.9	2.89

n.d.-not detected

Table 4. Content of individual anthocyanins (mg/kg) in strawberry samples

Sample	Pg-glu		Pg-rut		Cy-glu		Cy-malglu	
	c±SD	RSD (%)	c_{sr}±SD	RSD (%)	c_{sr}±SD	RSD (%)	c_{sr}±SD	RSD (%)
S1	76±1	1.31	4.08±0.03	0.73	2.31±0.04	1.73	11.6±0.1	0.86
S2	84±2	2.38	6.05±0.05	0.83	9.77±0.03	0.31	14.0±0.2	1.43
S3	135±5	3.70	6.51±0.05	0.77	12.4±0.1	0.80	13.3±0.1	0.75

Table 5. Content of individual anthocyanins (mg/kg) in blackberry samples

Sample	Cy-glu		Cy-rut		Cy-malglu	
	c±SD	RSD (%)	c±SD	RSD (%)	c±SD	RSD (%)
Bc1	338±8	2.37	22.9±0.2	0.87	10.84±0.05	0.46
Bc2	336±5	1.49	20.2±0.4	1.98	12.8±0.1	0.78
Bc3	202±4	1.98	13.3±0.1	0.75	9.8±0.1	1.02
Bc4	246±4	1.63	16.7±0.3	1.80	23.8±0.3	1.26
Bc5	438±7	1.60	14.3±0.1	0.70	12.21±0.07	0.57

Table 6. Content of individual anthocyanins (mg/kg) in blueberry samples

Sample	Delp-gal		Delp-glu		Delp-ara		Cy-glu		Cy-gal	
	c±SD	RSD (%)	c_{sr}±SD	RSD (%)	c_{sr}±SD	RSD (%)	c_{sr}±SD	RSD (%)	c_{sr}±SD	RSD (%)
B1	67±3	4.48	23.4±0.3	1.28	n.d.	-	24.2±0.5	2.07	29±1	3.45
B2	127±4	3.15	128±2	1.56	69±3	4.35	41±2	4.88	149±4	2.68
B3	157±2	1.27	192±3	1.57	57±1	1.75	160±6	3.75	90±1	1.11

n.d.-not detected

Table 6. Content of individual anthocyanins (mg/kg) in blueberry samples (continued)

Sample	Cy-ara		Pet-ara		Pet-gal		Peo-gal		Mal-gal	
	c±SD	RSD (%)	c±SD	RSD (%)	c±SD	RSD (%)	c±SD	RSD (%)	c±SD	RSD (%)
B1	21.3±0.1	0.47	14.1±0.2	1.42	20±1	5.00	36±1	2.78	35.6±0,2	0.56
B2	26.4±0.1	0.38	111±3	2.70	96±2	2.08	56±1	1.78	26.2±0.1	0.38
B3	80±2	2.50	151±2	1.32	172±3	1.74	71±2	2.82	48±1	2.08

Statistical analysis

Statistical multivariate method - principal component analysis (PCA) was used for the classification of phenolic acids, flavonols, and anthocyanins according to their contents in berry samples. Cluster analysis (CA) was used to classify samples based on the individual polyphenolics content. PCA and CA were performed using a statistical package running on a computer (Statistica 8.0, StatSoft, Tulsa, Oklahoma, USA).

Two significant principal components are extracted based on the Kaiser criterion. The first principal component (PC1) (with an eigenvalue of 13.99) explained 58.28% of the variance, and the second principal component (PC2) (with an eigenvalue of 4.14) explained 17.25% of the variance. The first two PCs are sufficient to explain 75.53% of the pattern variation.

Cy-ara, Cy-gal, Delp-glu, Delp-gal, Delp-ara, Pet-gal, Pet-ara, Peo-gal, caffeic, and ferulic acid were located on the positive side of PC1, and the zero-values of PC2. Mentioned anthocyanins were found in blueberries only. Furthermore, ferulic and caffeic acids were the only phenolic acids found in blueberry samples. Glycosides of pelargonidin (found only in strawberries) were located on the negative sides of both PC1 and PC2 (third quadrant). Strawberries were also characterized by the absence of Rut, which was located in the first quadrant of the diagram (positive sides of PC1 and PC2). Cy-rut was found only in blackberries, and the highest content of Cy-glu was detected in blackberry samples. Both Cy-rut and cy-glu were located in the second quadrant of the diagram (Figure 1).

The cluster analysis was applied to the analyzed berry samples using Ward's method with Euclidian distances as the criterion for forming clusters. Three separate clusters were obtained at $(D_{link}/D_{max}) \times 100<50$. (Figure 2). The samples of blackberries were grouped in the first cluster. The samples of blueberries B2 and B3 were joined in the second cluster. The third cluster included strawberry samples S1, S2, S3, and one blueberry sample B1. The blueberry sample B1 is characterized by lower Que, Que-gal, and anthocyanin content than samples B2 and B3. Furthermore, the content of ferulic acid in B1 is much lower than in samples B2 and B3 and is closer to the ferulic acid level in strawberry samples. PCA analysis of the samples provided a very similar grouping obtained based on CA (Figure 3). It can be concluded that the dominant factor for grouping the samples was the type of anthocyanins in berry fruits, a direct consequence of the genotype of the plant.

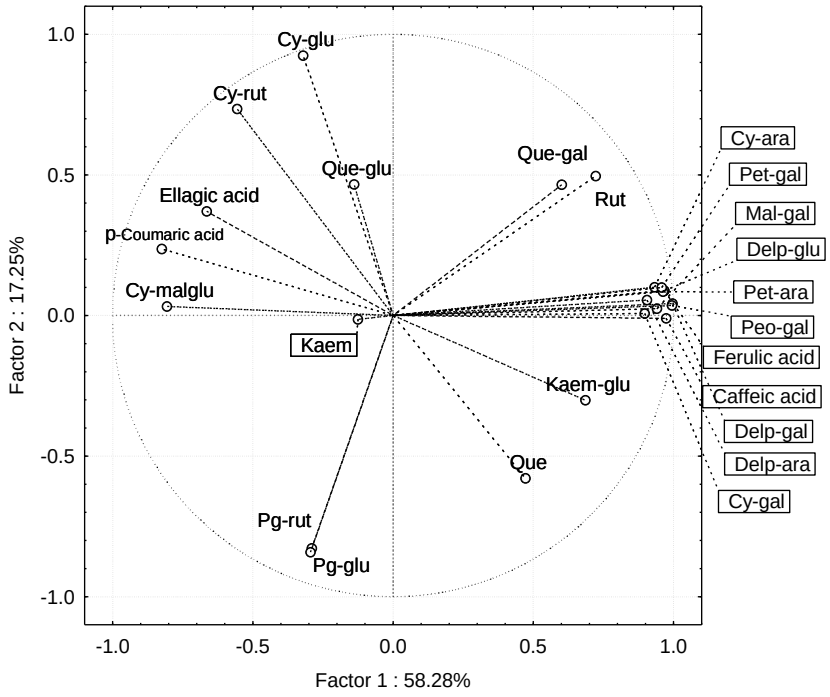

Figure 1. Principal component analysis of individual polyphenolic compounds in berry samples

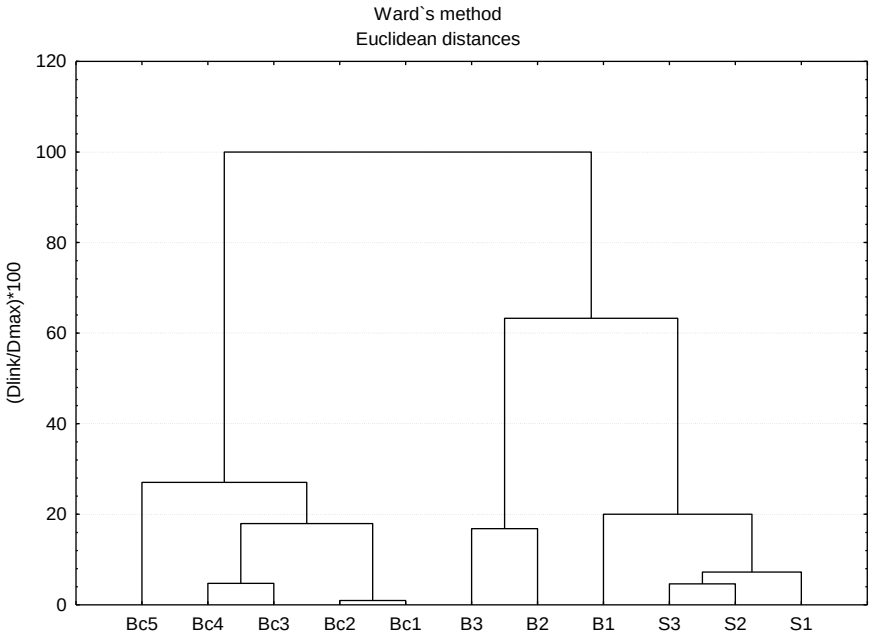

Figure 2. The dendrogram of the cluster analysis of analyzed berries

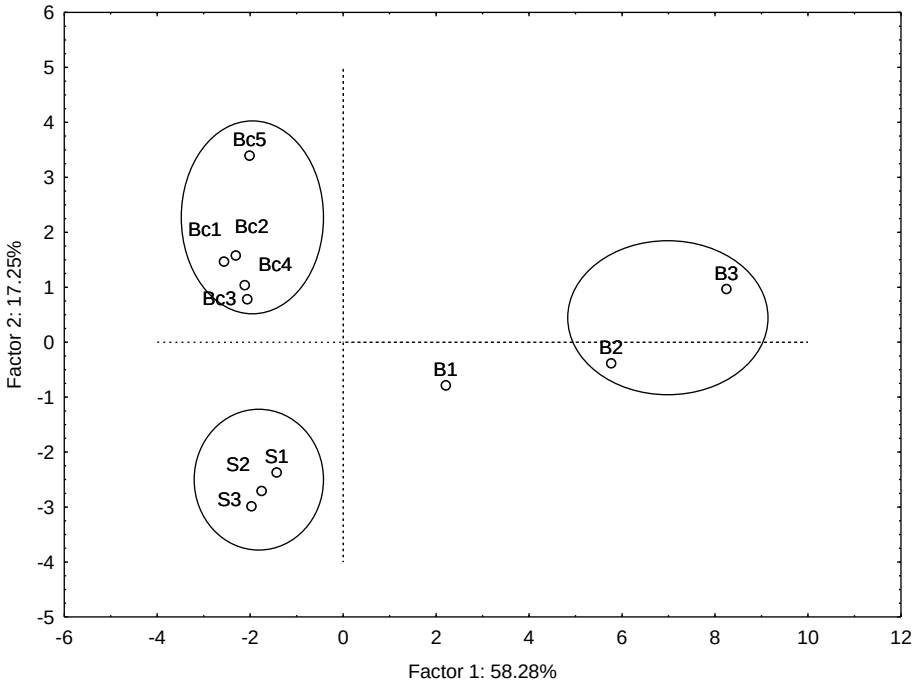

Figure 3. Principal component analysis of analyzed berry samples

Conclusion

Strawberries, blackberries, and blueberries can be considered functional food, regarding the number of bioactive compounds found in these fruits. High similarity in chemical composition and content of phenolic acids, flavonols, and anthocyanins among berries grown in Serbia and the ones grown in other countries has been observed. High amounts of ellagic acid were found in strawberries and blackberries but not in blueberries. The most diverse anthocyanin profile was observed in blueberries, followed by strawberries and blackberries. Nevertheless, certain differences among the cultivars of each fruit have been observed, which can be attributed to the genotype of the plants. The dominant factor for grouping the samples was the type of berry fruit. Based on PCA analysis, individual polyphenolic compounds were grouped in separate quadrants according to the type of berries where those were identified. Cluster analysis of the samples provided a very similar grouping to the one obtained by PCA analysis.

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Conflict-of-Interest Statement

None.

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