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Influence of Growing Region and Storage Time on Phenolic Profile of Cornelian Cherry Jam and Fruit

A. Begic-Akagic[1)], P. Drkenda[1)], A. Vranac[1)], P. Orazem[2)] and M. Hudina[2)]
([1)]University of Sarajevo, Faculty of Agricultural and Food Sciences, Sarajevo, Bosnia and Herzegovina and [2)]University of Ljubljana, Biotechnical Faculty, Department of Agronomy, Ljubljana, Slovenia)

Summary

The paper reports the profile of individual organic acids and phenolic content in fresh fruit and jam from three populations of cornelian cherry (*Cornus mas* L.) wild genotypes originating from Bosnia and Herzegovina. Immediately after harvest, jams were cooked at maximum 80 °C from the corresponding fruit. The jam was sampled in five replications at three different storage times for each region: immediately after processing, and then after four and six months of storage, for individual organic acids and phenolics by high-performance liquid chromatography (HPLC). The phenolic compounds found in the fresh fruit were also found in the jams – however, in significantly lower amounts in jam. The three most abundant phenolic compounds found in cornelian cherry fruit as well as in the corresponding jams were procyanidin B1, quercetin 3-*O*-robinobioside and peonidin 3-*O*-glucoside. Jam contained dramatically lower levels of phenolic compounds compared to fresh fruit. The most stable phenolic compounds were flavonols where the smallest losses were recorded. The levels of flavan 3-ols, hydroxycinnamic acid, flavonols and anthocyanins declined more during jam processing than during storage, with a significant influence of the growing region.

Key words. *Cornus mas* L. – organic acids – HPLC-MS – flavonols – elevation

Introduction

Recent worldwide consumer interest in healthy food has increased the demand for alternative, high quality fruit such as cornelian cherry (*Cornus mas* L.), which is a good source of natural antioxidant substances, such as vitamin C, anthocyanins, flavonols, and phenolics (Güleryüz et al. 1998; Klimenko 2004; Dragovic-Uzelac et al. 2007; Pantelidis et al. 2007; Tural and Koca 2008; Rop et al. 2010; Ersoy et al. 2011). Seeram et al. (2002) reported the following anthocyanins: delphinidin 3-*O*-galactopyranoside, cyanidin 3-*O*-galactopyranoside, and pelargonidin 3-*O*-galactopyranoside. According to Pantelidis et al. (2007) a native population of cornelian cherry contained up to 3-fold higher amount of vitamin C compared to commercial cultivars of other small fruits. The species is highly tolerant to diverse abiotic and biotic conditions. It is rarely attacked by pests and diseases, and it can be grown without the use of pesticides (Ercisli et al. 2011). The cornelian cherry fruit is also valuable raw material for processing into many high quality products, such as syrups, juices, jams (Brindza et al. 2007), spirits and other traditional products (Tesevic et al. 2009). The concentrations of organic acids are important factors influencing the organoleptic properties of fruit and their jams (Lee 1993). An organic acid level is sometimes useful to determine the percent of fruit in fruit products (Coppola and Starr 1986). Acids are components of fruits that do not change much during processing and storage than other components such as pigments and flavour compounds (Evans et al. 1983).

The antioxidant content of processed fruit products is largely dependent on the antioxidant content of fresh fruit, the type of process as well as the storage duration and its conditions (Nicoli et al. 1999; Markowski and Plocharski 2006; Sacchetti et al. 2008; Kopjar et al. 2009; Howard et al. 2010; Levaj et al. 2010; Begic-Akagic et al. 2011; Poiana et al. 2011). Jayaprakasam et al. (2006) suggest that consumption of cornelian cherry or other fruits containing anthocyanins has the potential to reduce the risk of diabetes and obesity.

The majority of the cornelian cherry production is consumed as processed food, and the native population is an extremely rich source of antioxidants, therefore we studied the profile and stability of organic acids and phenolic compounds in fresh fruit and jams (stored up to 6 months) obtained from a native cornelian cherry population (three growing regions) originated from Bosnia and Herzegovina. Another aim of this study was to determine the influence of the growing region as well as the storage time on the phenolics in cornelian cherry jam.

Materials and Methods

Fruit material

Samples of a native cornelian cherry population (40 kg per region), dark red in colour, were collected in Bosnia and Herzegovina from the following regions: Visegrad (altitude = 389 m), Drvar (altitude = 700 m) and Gorazde (altitude = 345 m). The selected regions are the most important and famous for uncultivated cornelian cherry production in Bosnia and Herzegovina. In addition, the Gorazde region is an organic certificated region for wild fruits. Soil and climate characteristics of selected regions are as follows: Gorazde region has an average annual air temperatures of 10.0 °C and annual precipitation of 750–1000 mm, the soil is high cambisols-moderately developed soils with lower horizons; Drvar region has an average annual air temperatures of 11.0 °C and annual precipitation of 1250–1500 mm, the soil is red soil rich in carbonates and that area lacks surface water; Visegrad region has an average annual air temperatures of 9.5 °C and annual precipitation of 850–1000 mm, the soil is distric cambisols.

The harvesting time of cornelian cherry from Gorazde was 10th September 2010, from Visegrad 17th September 2010 and from Drvar the 30th September 2010. All fruit were harvested at the optimum maturity stage for the jam making process (dark red colour of skin and more than 15 % of total soluble solids as well as soluble solid/acid ratio above 7). After harvest, the samples were immediately transferred to the Laboratory of Food Technology at the Faculty of Agriculture and Food Science in Sarajevo and used for jam processing. Fruit and stone weight were measured by using electronic weights with an accuracy of 0.01 g. The fruit flesh ratio was calculated using a formula: fruit flesh ratio = (fruit weight − fruit stone) × 100/ fruit weight). The method for the determination of titratable acidity utilizes sodium hydroxide (0.1 mol l^{-1} NaOH) to neutralise all the titratable protons at the end point of pH 8.2. The calculation expresses the titratable acidity as the percentage of malic acid. Soluble solids content was measured with Abbe refractometer (Euromex, Holland). Reducing sugar content was determined according to the Luff-Schoorl method (Lees 1975). From each location, three samples of fresh fruit (3 kg per sample) were taken, frozen and kept at −20 °C until analyzed.

Preparation of cornelian cherry jam

Cornelian cherry jams were prepared in the laboratory, according to the traditional procedure, by boiling in an open pan with manual stirring. For the jam preparation, the fruit was puréed and then cooked under atmospheric pressure with the addition of sucrose (60 g per 100 g of jam). Soluble solids of the fruit ranged between 16.1 and 17.6 % and titratable acidity between 2.21 and 2.67 %. Fruit content for processing was 40 g per 100 g of jam. The jams were cooked until the final product reached 60 % soluble solids (measured by a refractometer − Euromex, Holland). Time of cooking was 30 min at maximum 80 °C. The fruit contained enough pectin and a sufficient acid level; therefore, jam was prepared without the addition of pectin and acid. Five replications at three different storage times for each region were made. The jams were hot-filled into sterilized glass jars, cooled to room temperature (24 °C) and stored at the same temperature for 6 months. The samples were taken on the day of processing (0), after four (4) and after six (6) months of storage and kept at −20 °C until analyzed.

Organic acids extraction and analysis

For the analysis of individual organic acids and phenolic compounds in fresh fruit as well as in the corresponding jam, the samples were transferred on dry ice to the laboratory of Chair for Fruit Growing of Department of Agronomy at the Biotechnical Faculty, Ljubljana, Slovenia.

Ten grams of each sample (jam or fresh fruit) were brought to 40 ml of total volume with bidistilled water and homogenized for 1 min at 24 °C with a T-25 Ultra-Turrax (IKA − Labortechnik, Staufen, Germany). Samples were left to extract for half an hour at 24 °C and centrifuged at 10,000 rpm for 7 min at 5 °C (Eppendort 5810 R Centrifuge, Hamburg, Germany). The supernatant was used for analysis after filtration through a 0.45 μm cellulose filter (Macherey − Nagel, Düren, Germany) into vials.

An analysis of individual organic acid content (malic, citric, tartaric, quinic, shikimic and fumaric acids) was performed by a Thermo Separation Products high performance liquid chromatography (HPLC). For the separation of organic acids, the Razex ROA-Organic Acid H+ (300 × 7.8 mm; BioRad, USA) column with particle size 8 μm was used with the temperature maintained at 65 °C at a flow rate of 0.6 ml min^{-1}. Injection volume was 20 μl. The mobile phase (isocratic system) was 4 mM H_2SO_4 aqueous solution. The ultraviolet detector was set at 210 nm.

The method described by Hudina and Stampar (2006) was utilized. Malic, tartaric and citric acid content levels were expressed in g kg^{-1} of FW; shikimic, quinic and fumaric acid content levels were expressed in mg kg^{-1} of FW. Total acids were calculated as the sum of individual acids.

Individual phenolic compounds extraction and analysis

The extraction of individual phenolic compounds consisted of 10 g of fruit or jams, which was homogenized with a 10 ml extraction solution (methanol containing 3 % formic acid and 1 % of 2.6-di-tert-butyl-4-methylphenol (BHT) to prevent degradation of the phenolic compounds) in an ultrasonic ice bath for 1 h before centrifuging at 10,000 rpm for 7 min at 5 °C. The supernatant was filtered through the Chromafil AO-45/25 polyamide filter

(Macherey-Nagel, Düren, Germany) into a vial. The HPLC analysis was performed with the Surveyor system with a diode array detector (DAD) controlled by a Crom-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, Torrence, CA) with particle size of 3 μm maintained at 25 °C. According to Bakhshi and Arakawa (2006) the phenolic compounds (chlorogenic acid, procyanidin B1, procyanidin B2) were analyzed at 280 nm, flavonols (quercetin 3-*O*-galactoside, quercetin 3-*O*-rutinoside, quercetin 3-*O*-glucoside, quercetin 3-*O*-xyloside, quercetin 3-*O*-robinobiozide (expressed as equivalent of quercetin 3-*O*-rutinoside), kaempferol 3-*O*-glucoside and quercetin 3-*O*-rhamnoside) were analyzed at 350 nm, and anthocyanins (cyanidin 3-*O*-galactoside, cyanidin 3-*O*-rutinoside and peonidin 3-glucoside (both expressed in equivalents of cyanidin 3-*O*-galactoside)) were analyzed at 530 nm and identified by their retention times 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}.

The gradient method used was as described by Marks et al. (2007). All phenolic compounds presented in our results were identified by an HPLC-Finnigan MS detector and an LCQ Deca XP MAX (Thermo Finigan, San Jose, CA) instrument with electrospray interface (ESI) operating in negative ion mode. The analyses were performed using full-scan, data dependent MS^2 scanning from *m/z* 115 to 2000. Column and chromatographic conditions were identical to those used for the HPLC-DAD analyses. Quantification of individual phenolic compounds was achieved according to the concentrations of the corresponding external standard and was expressed in mg kg^{-1} of FW.

Standards and chemicals

Malic acid was purchased from Merck (Darmstadt, Germany), quinic acid from Sigma (St. Louis, MO, USA) and citric, fumaric, tartaric and shikimic acids were purchased from Fluka (Buchs, Switzerland) to quantify the organic acids. The following standards were used for quantification of these individual polyphenolic compounds: chlorogenic acid (5-caffeoylquinic acid) from Sigma (St. Louis, MO, USA) and procyanidin B1, procyanidin B2 from Fluka (Buchs, Switzerland), for quantification of flavonols, quercetin 3-*O*-rhamnoside, quercetin 3-*O*-glucoside, quercetin 3-*O*-galactoside, quercetin 3-*O*-xyloside, and kaempferol 3-*O*-glucoside from Fluka. To quantify anthocyanins, cyanidin 3-*O*-galactoside was purchased from Fluka (Buchs, Switzerland). BHT was obtained 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. Bidistilled water purified in a Milli-Q water purification system by Millipore (Bedford, MA, USA) was used. Organic acid standards were prepared in bidistilled water and polyphenol standards in methanol.

Statistical analysis

For determining the influence of geographical region on the bio-chemical properties of cornelian cherries, one-way analysis of variance (ANOVA) was used. Two-way analysis of variance (MANOVA) was used to determine the effect of storage time and growing region on cornelian cherry jam, and the determined differences were tested by the LSD test at a significance level of 0.05 (using the SPSS 16 program).

Results and Discussion

Physical and chemical properties of cornelian cherry fruit

Some basic information of the analyzed physical and chemical properties of cornelian cherry fruits are presented in Table 1. Tural and Koca (2008) reported that fruit weight of cornelian cherry ranges from 0.39 g to 1.03 g. The values obtained in our study were somewhat higher to those reports. Our results for stone weight are in agreement with Güleryüz et al. (1998), who reported a stone weight of 0.316–0.530 g. The soluble solid contents of cornelian cherry genotypes were recorded in range of 12.50–21.17 % (Güleryüz et al. 1998; Pantelidis et al. 2007; Tural and Koca 2008; Yilmaz et al. 2009). Titratable acidity in cornelian cherry was also reported by Klimenko (2004), Tural and Koca (2008) and Yilmaz et al. (2009) and ranged between 1.10 and 3.89 %. Soluble solids and titratable acidity data of our cornelian cherry are comparable with these studies. Many factors affect soluble solids and acidity in fruit species including the cultivar, altitude, environmental conditions, etc. (Güleryüz et al. 1998; Ercisli and Orhan 2007). Reducing sugar values were in agreement with those published by Didin et al. (2000), who reported that the reducing sugar content was in range of 6.98–11.56 g per 100 g of cornelian cherry.

Organic acids of cornelian cherry genotypes and the corresponding jams

Content of total and individual organic acids in cornelian cherry fresh fruit are shown in Table 2. The levels of total and individual organic acids (except for shikimic acid) in fresh cornelian cherries were affected by growing region (Table 2). Generally, malic acid was the most abundant acid in cornelian cherry fruit, followed by tartaric and citric acid, respectively. Quinic, shikimic and fumaric were determined at very low levels. The fruit from Visegrad had higher levels of tartaric, citric and fumaric acid than fruit from the other two regions. Cornelian cherry population from Visegrad contained a significantly higher total acid content than that from Drvar, while no signifi-

Table 1. Some basic physical and chemical properties of cornelian cherry fruits. Each mean is the average of 30 replicates.

Parameters	Visegrad (389 m)	Gorazde (345 m)	Drvar (700 m)
Fruit weight (g)	2.30 ± 0.35 c	2.54 ± 0.33 b	2.59 ± 0.57 a
Stone weight (g)	0.51 ± 0.09 a	0.51 ± 0.11 a	0.41 ± 0.14 b
Fruit flesh ratio (%)	77.46 ± 3.30 c	79.80 ± 3.97 b	83.96 ± 3.25 a
Soluble solids (%)	16.10 ± 0.13 c	17.55 ± 0.09 a	17.10 ± 0.42 b
Titratable acidity (%)	2.21 ± 0.33 a	2.67 ± 0.09 a	2.48 ± 0.11 a
Reducing sugar (g kg^{-1})	105.10 ± 3.24 b	101.20 ± 0.83 b	117.50 ± 6.10 a

Different letters in rows from a to c for each parameter indicate significantly different values among growing regions at $P < 0.05$.

Table 2. Average organic acids content (g kg^{-1} FW; for quinic, shikimic and fumaric acid in mg kg^{-1}) ± standard error in cornelian cherry fruit from three regions of Bosnia and Herzegovina. Each mean is the average of 5 replicates.

Organic acids	Growing region		
	Visegrad (389 m)	Gorazde (345 m)	Drvar (700 m)
Malic acid (g kg^{-1})	43.7 ± 0.73 a	40.1 ± 1.66 ab	35.2 ± 2.27 b
Tartaric acid (g kg^{-1})	2.8 ± 0.37 a	1.7 ± 0.23 b	1.1 ± 0.21 c
Citric acid (g kg^{-1})	1.8 ± 0.31 a	0.5 ± 0.20 b	0.33 ± 0.18 b
Quinic acid (mg kg^{-1})	51.0 ± 1.43 a	51.0 ± 3.95 a	41.9 ± 3.35 b
Shikimic acid (mg kg^{-1})	18.1 ± 1.54 a	17.8 ± 1.72 a	17.3 ± 1.86 a
Fumaric acid (mg kg^{-1})	8.0 ± 0.22 c	10.8 ± 0.73 b	12.9 ± 0.68 a
Total acids	48.4 ± 1.07 a	42.4 ± 1.63 ab	36.6 ± 2.48 b

Different letters in rows from a to c for each organic acids indicate significantly different values among growing regions at $P < 0.05$.

cant differences were found compared to the fruit from Gorazde. It was expected that cornelian cherry from Drvar had a lower total organic acid content than cornelian cherries from the other two locations because of a higher annual precipitation (1250–1500 mm) and higher annual temperature. Malic acid, the most abundant acid in cornelian cherry fruit, is the main substrate, along with carbohydrates, used in respiration (Kovács and Djedjro 1994), which is more intensive at higher temperatures. In previous studies with cornelian cherry fruit, total acids varied from 11.0 to 25.3 g kg^{-1} (Tural and Koca 2008), from 22.1 to 46.9 g kg^{-1} (Güleryüz et al. 1998), and from 13.0 to 19.0 g kg^{-1} (Klimenko 2004). Our results were in agreement with these values, because many factors affect acidity in fruit species including the altitude, stage of maturity, various genotypes as well as environmental conditions, etc. (Güleryüz et al. 1998; Leeuwen et al. 2004; Ercisli and Orhan 2007; Panico et al. 2009).

The same individual organic acids found in cornelian cherry fruit were also found in the jams, but in lower quantities (a decrease of up to 44 % compared to fresh fruit). Since the jams contained 400 g of fruit per 1,000 g of jam, it was expected that the acid content would be approximately half the value recorded in fresh fruit. We found that the content of tartaric, shikimic and fumaric acids in the jams was affected by the growing region. Storage time affected the content of malic, citric, tartaric and shikimic acids, while interaction was only significant for shikimic acid (Table 3). Total acid content found in the jam ranged between 24.3 and 29.3 g kg^{-1}, and it significantly increased over 4 months of storage. The lowest level was detected immediately after processing in all three growing regions. Conversely, Vidhya and Narain (2011), found that acid content decreased to 0.51 % at 30 days and 1.55 % and 2.5 % at 60 and 90 days of jam storage, respectively. On the other hand, Begic-Akagic (2001) found that an increase of about 20 % of total acidity during the six months of storage period at 4 °C in low-calorie blackberry jam, similar to our results. To the best of our knowledge, no data exists regarding the influence of processing and storage on organic acid content in cornelian cherry jams.

Individual phenolic compounds of Cornelian cherry genotypes and the corresponding jams

In Table 4 we present the phenolic compounds, flavonols and anthocyanins in fresh cornelian cherry. In the fresh

Table 3. Average organic acids content (g kg^{-1} FW; for quinic, shikimic and fumaric acid in mg kg^{-1}) ± standard error of jam from cornelian cherry fruit from three growing regions of Bosnia and Herzegovina during storage. Each mean is the average of 5 replicates.

Region	Visegrad (389 m)				Gorazde (345 m)				Drvar (700 m)						
Storage	0 m.	4 m.	6 m.	R	0 m.	4 m.	6 m.	R	0 m.	4 m.	6 m.	R	R	S	RxS
Malic	20.2 ±	23.3 ±	20.4 ±	*x*	20.3 ±	22.7 ±	22.1 ±	*x*	20.0 ±	20.1 ±	20.3 ±	*x*	*ns*	*ns*	*ns*
acid	0.09 a	2.18 a	0.72 a		0.69 a	0.42 a	2.83 a		0.61 a	0.30 a	0.62 a				
Tartaric	2.6 ±	3.1 ±	2.9 ±	*x*	2.5 ±	3.2 ±	3.1 ±	*x*	2.1 ±	2.8 ±	2.7 ±	*y*	***	***	*ns*
acid	0.08 b	0.07 a	0.08 a		0.16 b	0.14 a	0.22 a		0.09 b	0.11b a	0.10 a				
Citric	2.4 ±	2.7 ±	2.8 ±	*x*	2.1 ±	2.8 ±	2.8 ±	*x*	2.0 ±	2.6 ±	2.4 ±	*x*	*ns*	***	*ns*
acid	0.08 b	0.26 a	0.09 a		0.11 b	0.01 a	0.14 a		0.05 b	0.06b a	0.23 a				
Quinic	94.4 ±	91.3 ±	91.8 ±	*x*	88.6 ±	96.7 ±	95.9 ±	*x*	90.6 ±	91.4 ±	90.8 ±	*x*	*ns*	*ns*	*ns*
acid	0.76 a	4.12 a	1.85 a		5.36 a	1.05 a	0.37 a		1.46 a	1.44 a	1.37 a				
Shikimic	75.4 ±	76.4 ±	72.7 ±	*y*	107.4 ±	116.6 ±	112.5 ±	*x*	66.0 ±	72.1 ±	67.3 ±	*y*	***	*	*
acid	0.61 a	0.53 a	0.84 a		0.96 a	4.76 a	11.91 a		2.33 a	1.67 a	3.98 a				
Fumaric	34.6 ±	35.8 ±	32.8 ±	*y*	36.9 ±	40.9 ±	40.2 ±	*x*	28.8 ±	30.6 ±	30.2 ±	*z*	***	*ns*	*ns*
acid	0.51 a	1.52 a	1.08 a		2.20 a	1.02 a	2.44 a		0.72 a	1.59 a	0.93 a				
Total	25.3 ±	29.3 ±	26.2 ±	*x*	25.1 ±	28.9 ±	28.2 ±	*x*	24.3 ±	25.6 ±	25.6 ±	*x*	*ns*	*ns*	*ns*
acids	0.14 b	1.87 a	0.88 a		0.93 b	0.58 a	3.20 a		0.68 b	0.45a	0.50 a				

Different letters in rows from a to c for each organic acid indicate significantly different values among storage times at $p < 0.05$.
Different letters in rows from *x* to *z* for each organic acids indicate significantly different values among regions at $P < 0.05$.
R – region; S – storage; RxS – interaction between region and storage. ns – not significant; * – significant differences at p-value below 0.05; ** – significant differences at p-value below 0.01; *** – significant differences at p-value below 0.001.
m. – months

fruit, procyanidin B1 was present at the highest concentrations among the identified phenolic compounds, followed by procyanidin B2, while chlorogenic acid was determined in the lowest concentrations. A significant effect of growing region on individual phenolic compounds was evident for fresh fruit. Significantly lower levels of procyanidin B1 and procyanidin B2 were recorded in cornelian cherry fruit from Visegrad, whereas no differences were found between fruit from Gorazde and Drvar. The chlorogenic acid level in fruit from Drvar was the highest compared to the other two locations.

We also found significant differences among the fruit from different geographical regions in all individual flavonols analyzed, except for quercetin 3-*O*-xyloside. Among flavonols, quercetin 3-*O*-robinobioside was present at the highest levels in cornelian cherry fruit. The fruit from Visegrad contained the lowest content of quercetin 3-*O*-robinobioside, quercetin 3-*O*-rutinoside, quercetin 3-*O*-glucoside and quercetin 3-*O*-rhamnoside compared to fruit from the other two locations, while no significant differences was found between these.

Peonidin 3-*O*-glucoside was the major anthocyanin identified in cornelian cherry fruit (Table 4), followed by cyanidin 3-*O*-galactoside while cyanidin 3-*O*-rutinoside was found in low concentrations. According to Tural and Koca (2008), the main pigment found in cornelian cherry was pelargonidin 3-*O*-glucoside, followed by cyanidin 3-*O*-glucoside and cyanidin 3-*O*-rutinoside, while Seeram et al. (2002) found a mixture of three compounds (delphinidin 3-*O*-galactopyranoside, cyanidin 3-*O*-galactopyranoside and pelargonidin 3-*O*-galactopyranoside). A significant effect of growing region on individual anthocyanins was evident for cornelian cherry fruit, except for the case of cyanidin 3-*O*-galactoside (Table 4). These results are in accordance with the previous study of the accumulation of individual anthocyanins in the sour cherry cv. 'Marasca' grown in two Dalmatian geographical regions (Pedisić et al. 2010). Significantly lower levels of peonidin 3-*O*-glucoside and cyanidin 3-*O*-rutinoside were recorded in cornelian cherries from Visegrad compared to other regions. In the case of cyanidin 3-*O*-galactoside, there were no differences between locations (Table 4). Since the synthesis of flavonoids, especially anthocyanins, is known to be stimulated by light (Macheix et al. 1990), differences in individual anthocyanin compounds in cornelian cherry at different locations would be expected. The reason for differences in phenolic content among the analysed regions could be that the cornelian cherry population from Gorazde and Drvar were better exposed to sun than Visegrad. The other reason could be the type of soil because Leeuwen et al. (2004) reported that the impacts of climate and soil were greater than the impact of cultivar and genotype on berry anthocyanin concentration. Our results may have occurred because of

Table 4. Average individual phenolics content (mg kg^{-1}) ± standard error in cornelian cherry fruit from three regions of Bosnia and Herzegovina. Each mean is the average of 5 replicates.

Growing region	Visegrad (389 m)	Gorazde (345 m)	Drvar (700 m)
Procyanidin B1	678.81 ± 5.14 b	946.82 ± 6.58 a	944.92 ± 8.39 a
Procyanidin B2	89.80 ± 2.33 b	186.63 ± 5.20 a	166.58 ± 4.57 a
Chlorogenic acid	26.03 ± 2.68 c	65.36 ± 2.05 b	142.46 ± 4.35 a
Q-robinobioside	1.80 ± 0.32 b	5.81 ± 0.36 a	5.02 ± 0.23 a
Q-galactoside	1.04 ± 0.13 c	2.44 ± 0.32 b	3.24 ± 0.18 a
Kaempferol-3-glucoside	0.89 ± 0.07 c	2.17 ± 0.13 b	3.19 ± 0.24 a
Q-rutinoside	1.35 ± 0.12 b	2.66 ± 0.21 a	2.87 ± 0.26 a
Q-glucoside	0.50 ± 0.03 b	2.13 ± 0.28 a	2.07 ± 0.14 a
Q-rhamnoside	0.75 ± 0.04 b	1.33 ± 0.05 a	1.68 ± 0.08 a
Q-xyloside	0.35 ± 0.01 a	0.47 ± 0.01 a	0.56 ± 0.09 a
Peonidin 3-*O*-glucoside	178.99 ± 7.54 c	502.31 ± 9.21 b	696.13 ± 6.51 a
Cyanidin 3-*O*-galactoside	208.77 ± 9.21 a	296.94 ± 7.86 a	290.16 ± 8.21 a
Cyanidin 3-*O*-rutinoside	2.08 ± 0.31 c	10.95 ± 0.78 b	47.41 ± 2.06 a

Different letters in rows from a to c for each phenolic compound indicate significantly different values among growing region at $P < 0.05$.

Q-robinobioside = quercetin 3-*O*-robinobioside; Q-galactoside = quercetin 3-*O*-galactoside; Q-rutinoside = quercetin 3-*O*-rutinoside; Q-glucoside = quercetin 3-*O*-glucoside; Q-rhamnoside = quercetin 3-*O*-rhamnoside; Q-xyloside = quercetin 3-*O*-xyloside

differences between day and night temperatures, which can affect the anthocyanin accumulation in some fruit (Tomás-Barberán and Espín 2001) as well as temperatures because Drvar region had a higher average annual temperature than the other two locations. The same phenolic compounds that were found in the fruit were also found in the jam (Table 5), but in much lower quantities (a decrease of up to 99.18 %) compared to fresh fruit. It was expected that the polyphenol content would be approximately half the value observed for fresh fruit, since the jams contained 400 g of fruit per 1,000 g of jam. The other reason for lower polyphenol content in jam could be processing. There have been some studies regarding the effect of processing and storage on phenolics in jams (Viberg et al. 1997; Häkkinen and Törrönen 2000; Zafrilla et al. 2001; Bursać Kovačević et al. 2009; Scibisz and Mitek 2009; Howard et al. 2010; Levaj et al. 2010; Poiana et al. 2011; Rababah et al. 2011), but no data are available on how processing and storage influence the phenolic content of cornelian cherry jam. In our experiment the levels of individual phenolic compounds were unaffected by storage time or interaction (Table 5). Our results are in disagreement with those of Howard et al. (2010), who reported that the levels of individual procyanidins were affected by storage time and storage temperature in blueberry jam.

The jams produced from fruit grown in the Visegrad region had significantly higher procyanidin B1 content than jam produced from fruit grown in Gorazde. Average individual phenolics content in the Visegrad slightly increased during the storage time but the difference was not significant. No significant differences were detected in relation to Drvar. The levels of chlorogenic acid were significantly higher in jams produced from fruit grown in Drvar than in jams from fruit produced in the other two regions. Our results for chlorogenic acid content in stored jams were much lower than those reported for blueberry jam (Howard et al. 2010). According to the authors, the chlorogenic acid levels of blueberry jam stored for 6 months were 587.5 mg kg^{-1} of FW (Howard et al. 2010) and 305.0 mg kg^{-1} (Scibisz and Mitek 2009). A high level of chlorogenic acid content is observed for jams produced from a variety of small fruit, such as sour cherry, strawberry, raspberry and wild blueberry (8.15, 9.60, 8.15 and 11.90 mg kg^{-1}, respectively) (Levaj et al. 2010).

Levels of individual flavonol compounds in jam (except for quercetin 3-*O*-glucoside and quercetin 3-*O*-xyloside) were affected by the fruit growing region but not by the storage time and their interaction (Table 5). The most unstable flavonol compounds in cornelian cherry jam were quercetin 3-*O*-glucoside, followed by quercetin 3-*O*-rhamnoside and quercetin 3-*O*-galactoside. On the other hand, the most stable flavonols in the jam were quercetin 3-*O*-xyloside (which was only 2.5 times lower than with raw fruit) followed by quercetin 3-*O*-robinobioside, kaempferol 3-*O*-glucoside and quercetin 3-*O*-rutinoside (Table 5). According to Levaj et al. (2010), in sour cherries, mass fractions of quercetin 3-*O*-rutinoside and kaempferol derivate were about 2.36 (cv. 'Marasca') and 2.57 (cv. 'Oblacinska') times lower in the jam than in raw fruit. The same authors reported that a high level of retention was observed for the derivate of flavonols (quercetin and

Table 5. Average individual phenolics content (mg kg^{-1}) ± standard error of jam from cornelian cherry fruit from three growing regions of Bosnia and Herzegovina during storage. Each mean is the average of 5 replicates.

Growing region	Visegrad (389 m)				Gorazde (345 m)				Drvar (700 m)						
Storage	0 m.	4 m.	6 m.	R	0 m.	4 m.	6 m.	R	0 m.	4 m.	6 m.	R	R	S	RxS
Procyanidin B1	20.70 ± 2.94 a	21.90 ± 2.20 a	23.20 ± 2.05 a	*x*	21.10 ± 1.09 a	12.20 ± 2.01 a	14.60 ± 2.13 a	*y*	22.70 ± 2.60 a	18.30 ± 1.00 a	16.20 ± 0.78 a	*xy*	*	ns	ns
Procyanidin B2	5.00 ± 0.53 a	6.00 ± 0.32 a	7.60 ± 0.68 a	*x*	8.10 ± 0.92 a	6.80 ± 0.68 a	3.90 ± 0.42 a	*x*	4.80 ± 0.43 a	3.70 ± 0.12 a	3.70 ± 0.08 a	*x*	Ns	ns	ns
Chlorogenic acid	0.77 ± 0.05 a	0.66 ± 0.07 a	0.91 ± 0.09 a	*y*	1.10 ± 0.04 a	0.80 ± 0.14 a	1.10 ± 0.10 a	*y*	1.20 ± 0.09 a	1.50 ± 0.12 a	1.40 ± 0.02 a	*x*	***	ns	ns
Q-robinobioside	0.74 ± 0.07 a	0.84 ± 0.06 a	0.99 ± 0.09 a	*xy*	1.34 ± 0.03 a	0.95 ± 0.06 a	0.94 ± 0.08 a	*x*	0.61 ± 0.09 a	0.70 ± 0.07 a	0.60 ± 0.05 a	*y*	*	*ns*	*ns*
Q-galactoside	0.13 ± 0.02 a	0.14 ± 0.00 a	0.17 ± 0.01 a	*x*	0.09 ± 0.00 a	0.06 ± 0.00 a	0.09 ± 0.00 a	*y*	0.14 ± 0.00 a	0.10 ± 0.00 a	0.12 ± 0.00 a	*xy*	*	*ns*	*ns*
Kaempferol-3-glucoside	0.43 ± 0.04 a	0.44 ± 0.02 a	0.70 ± 0.08 a	*x*	0.29 ± 0.05 a	0.15 ± 0.02 a	0.25 ± 0.03 a	*y*	0.62 ± 0.06 a	0.44 ± 0.04 a	0.53 ± 0.03 a	*x*	***	*ns*	*ns*
Q-rutinoside	0.59 ± 0.06 a	0.58 ± 0.03 a	0.60 ± 0.03 a	*x*	0.40 ± 0.07 a	0.32 ± 0.03 a	0.30 ± 0.03 a	*y*	0.36 ± 0.02 a	0.26 ± 0.04 a	0.45 ± 0.05 a	*y*	*	*ns*	*ns*
Q-glucoside	0.05 ± 0.00 a	0.04 ± 0.00 a	0.06 ± 0.00 a	*x*	0.03 ± 0.00 a	0.01 ± 0.00 a	0.03 ± 0.00 a	*x*	0.05 ± 0.00 a	0.03 ± 0.00 a	0.06 ± 0.00 a	*x*	*ns*	*ns*	*ns*
Q-rhamnoside	0.07 ± 0.00 a	0.06 ± 0.00 a	0.09 ± 0.01 a	*x*	0.05 ± 0.00 a	0.02 ± 0.00 a	0.03 ± 0.00 a	*y*	0.09 ± 0.00 a	0.07 ± 0.00 a	0.08 ± 0.00 a	*x*	***	*ns*	*ns*
Q-xyloside	0.26 ± 0.05 a	0.31 ± 0.02 a	0.41 ± 0.08 a	*x*	0.21 ± 0.03 a	0.23 ± 0.05 a	0.18 ± 0.05 a	*x*	0.19 ± 0.04 a	0.28 ± 0.06 a	0.18 ± 0.02 a	*x*	*ns*	*ns*	*ns*
Peonidin 3-*O*-glucoside	6.86 ± 1.00 a	7.84 ± 1.13 a	6.29 ± 0.54 a	*y*	7.24 ± 0.35 a	4.01 ± 0.55 a	5.46 ± 0.49 a	*y*	11.60 ± 1.82 a	12.60 ± 1.03 a	11.50 ± 0.75 a	*x*	***	*ns*	*ns*
Cyanidin 3-*O*-galactoside	5.90 ± 0.32 a	7.30 ± 0.52 a	5.93 ± 0.47 a	*x*	4.90 ± 0.12 a	2.49 ± 0.37 a	3.00 ± 0.40 a	*y*	6.50 ± 0.02 a	5.00 ± 0.34 a	4.50 ± 0.36 a	*x*	**	*ns*	*ns*
Cyanidin 3-*O*-rutinoside	0.54 ± 0.02 a	2.07 ± 0.56 a	0.54 ± 0.06 a	*x*	0.31 ± 0.01 a	0.18 ± 0.02 b	0.31 ± 0.03 a	*x*	0.80 ± 0.05 a	0.73 ± 0.06 a	0.66 ± 0.06 a	*x*	*ns*	*	*ns*

Different letters in rows from a to b for each phenolic compound indicate significantly different values among storage times at $p < 0.05$. Different letters in rows from *x* to *y* for each phenolic compound indicate significantly different values among regions at $P < 0.05$.

R – region; S – storage; RxS – interaction between region and storage. ns – not significant; * – significant differences at p-value below 0.05; ** – significant differences at p-value below 0.01; *** – significant differences at p-value below 0.001.

Q-robinobioside = quercetin 3-*O*-robinobiozide; Q-galactoside = quercetin 3-*O*-galactoside; Q-rutinoside = quercetin 3-*O*-rutinoside; Q-glucoside = quercetin 3-*O*-glucoside; Q-rhamnoside = quercetin 3-*O*-rhamnoside; Q-xyloside = quercetin 3-*O*-xyloside.

m. – months

kaempferol) and some phenolic acids in all the jams investigated. Howard et al. (2010) found that flavonols were stable not only during processing but also during long-term storage.

Table 5 shows the changes in individual anthocyanins of cornelian cherry jam produced from fruit from the three growing regions over 6 months of storage at room temperature. The statistical analysis showed that the growing region of cornelian cherry fruit did affect the individual anthocyanin compounds in jam, except for cyanidin 3-*O*-rutinoside (Table 5). Jam produced (average over 6 months) from Drvar cornelian cherry contained statistically the highest peonidin 3-*O*-glucoside content compared to fruit from Visegrad and Gorazde in observed year. Significantly higher cyanidin 3-*O*-galactoside levels were recorded in jams produced from Visegrad and Drvar fruit in relation to jam from Gorazde. Our results concerning the levels of individual anthocyanins in stored jam are much lower than these reported in previous studies for strawberry and blueberry (Bursać Kovačević et al. 2009; Howard et al. 2010). During storage, the levels of the individual anthocyanins in all these jams decreased. On the other hand, results reported by Rababah et al. (2011) showed a decrease only in anthocyanins and

pH in apricot and fig jams during 5 months of storage. Our results are also mostly consistent with those of Trošt et al. (2008), who found that cyanidin anthocyanins were most stable during storage of blueberry-aronia nectar, followed by peonidin, petunidin, delphinidin, and malvidin – the only difference being for cyanidin 3-*O*-galactoside. This phenomenon could be explained by the fact that differences in anthocyanin retention were affected by anthocyanin structure as well as the type of sugar attached. According to García-Viguera et al. (1998), during jam processing at atmospheric pressure, anthocyanin losses in the final product varied from 10 % to 80 % when boiling time ranged from 10 min to 15 min or more. On the other hand, the loss of anthocyanins under vacuum pressure conditions was approximately 40 % during 15 min of cooking (García-Viguera and Zafrilla 2001). Boiling time in our processing was about 30 min under atmospheric pressure, which may have caused the greater losses of anthocyanins as well as sugars and the high acid content in the mixture. Losses of peonidin 3-*O*-glucoside, cyanidin 3-*O*-galactoside and cyanidin 3-*O*-rutinoside levels compared to the jams directly after processing were about 0.51–24.61, 40.00 and 15.25 % after 6 months of storage at room temperature. Scibisz and Mitek (2009) reported that six months of blueberry jam storage caused the complete destruction of cyanidin and peonidin derivates. Some studies report that sugar reduces the stability of anthocyanins (Tsai et al. 2005; Hubbermann et al. 2006). According to Krifi and Metche (2000) sugar degradation products, namely furfural and 5-hydroxymethylfurfural, are formed when sugars are heated in an acidic medium. We suspect that the higher sucrose content (600 g per 1,000 g of jam) and total acids in processed jams resulted in a more favorable environment for the formation of sugar degradation products. On the other hand, the loss of anthocyanins in jam can also be affected by the ascorbic acid content. According to Pantelidis et al. (2007) the ascorbic acid content was significantly higher in cornelian cherry fruit than other fruit such as strawberry (460 mg kg^{-1}), orange (310 mg kg^{-1}) and kiwifruit (290–890 mg kg^{-1}).

Conclusion

This investigation demonstrates that cornelian cherries from Bosnia and Herzegovina are a rich source of phenolic compounds. Phenolic compounds followed by anthocyanin compounds were the main polyphenol classes analyzed in the fruit and jams. The three most abundant phenolic compounds found in cornelian cherry fruit and in the corresponding jams were procyanidin B1, quercetin 3-*O*-robinobioside and peonidin 3-*O*-glucoside. The jam processing and the storage period resulted in losses of phenolic compounds, flavonol and anthocyanin compounds compared to fresh fruit. It can also be concluded that the total anthocyanin and phenolic contents of cornelian cherry fruit and jam are significantly affected by the fruit growing region but not by storage time and interaction. The levels of phenolics and flavonols were affected only by the growing region in cornelian cherry fruit. However, in comparison with jam made from cornelian cherry fruit from Drvar and Gorazde, the jam from Visegrad showed the highest stability of phenolics during storage. Further studies are required to fully understand the impact of the jam making process, added sugar and fruits components such as organic acids on the stability of cornelian cherry phenolic compounds during storage.

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Addresses of authors: A. Begic-Akagic, P. Drkenda, A. Vranac, University of Sarajevo, Faculty of Agricultural and Food Sciences, Zmaja od Bosne 8, 71000 Sarajevo, Bosnia and Herzegovina, and P. Orazem and M. Hudina (corresponding author) University of Ljubljana, Biotechnical Faculty, Department of Agronomy, Jamnikarjeva 101, 1000 Ljubljana, Slovenia, e-mail: (corresponding author): metka.hudina@bf.uni-lj.si