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Phenolic content and bioactivity of two sour cherry cultivars and their products

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Abstract: Bioactive compounds are produced as secondary metabolites in plants. Positive correlation between presence of bioactive compounds and health benefits of plants have been reported in many studies. Sour cherry contains high content of bioactive compounds, mostly polyphenols and anthocyanins. They are mostly consumed in fresh state, but are also used to produce jam, jelly, marmalade, juice, and syrup. Aim of this study was to evaluate total phenolic content, anthocyanins, as well as the antioxidant activity in two sour cherry cultivars and their products, jams and juice, prepared using traditional recipes. Total phenolic content was determined using Folin-Ciocalteu method and antioxidant activity was assessed using ABTS radical cation decolorization assay. pH-Differential method was used to determine anthocyanin content. Marasca cultivar had higher content of phenols, anthocyanins and antioxidant activity than Oblačinska cultivar. Processing of sour cherries had a greater impact on the reduction of anthocyanin content but did not have significant effect on antioxidant activity.

INTRODUCTION

Fruits are natural sources of bioactive phytochemicals that are produced in plants as secondary metabolites. Numerous studies have shown the protective effect and positive influence of fruit consumption on human health (Jakobek Šeruga, Medvidović-Kosanović, et al., 2007; Shashirekha, Mallikarjuna, and Rajarathnam 2015). Beneficial effects of fruit have been attributed to the presence of the bioactive compounds. Sour cherries (Prunus cerasus L.) are rich in bioactive compounds, and they contain high levels of phenolic compounds and anthocyanins (Khoo, Clausen, Pedersen et al., 2011; Serradilla, Fotirić Akšić, Manganaris et al., 2017). Several studies have demonstrated that sour cherries contain significant levels of anthocyanins that have strong antioxidant and anti-inflammatory activity with a real impact on human health (Khoo et al., 2011; Blando, Gerardi and Nicoletti 2004; He and Giusti 2009; Levaj, Dragović-Uzelac, Delonga et al., 2010). It has been shown that consumption of sweet or sour cherries may reduce the risk of cancer, inflammatory diseases including arthritis, and muscle soreness, (Prvulović, Popović, Malenčić et al., 2012) cardiovascular diseases, osteoporosis as well neurodegenerative diseases and diabetes mellitus (Kim and Padilla-Zakour, 2004; Viljevac Vuletić, Dugalić, Mihaljević et al., 2017).

Sour cherry is mostly used to produce jam, jelly, stewed fruit, marmalade, and syrup in the food industry (Corts, Rodrigues, Ortiz Marcide et al., 2008). The production of cherries in the continental part of Bosnia and Herzegovina is mainly related to the cultivation of the Oblačinska cultivar, which is the most demanded variety because of its high quality (Mitić, Obradović, Kostić et al., 2012) and high anthocyanin content. In Herzegovina, due to different climates, Maraska is the most grown sour cherry cultivar. The sour cherry Marasca is a rich source of organic and inorganic bioactive compounds (Pedisić, Levaj, Dragović-Uzelac et al., 2007). Marasca had higher polyphenolic content and higher antioxidant activity compared to other sour cherry cultivars. Both cultivars showed industrial potential for processing that can be used in the production of various functional beverages (Repajić, Bursać Kovačević, Putnik. et al., 2015).

The aim of this study was to evaluate fresh samples, jams and juices of two different sour cherry cultivars, Oblačinska and Marasca regarding the amount of total phenolic content, anthocyanins and antioxidant activity.

MATERIALS AND METHODS

Chemicals

Compound 2,2´ - azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) was purchased from Sigma-Aldrich (Germany) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) from Across organics (USA). Potassium chloride was purchased from Lach:ner (Czech Republic). All other chemicals were purchased from Semikem (BiH).

Cherry samples

Fruits of two sour cherry (Prunus cerasus L.) cultivars Oblačinska and Marasca were collected in May 2019, in the area of Mostar, Bosnia and Herzegovina. Immediately after harvesting, samples were frozen at -20oC until analysis. All fruits were harvested at the optimum maturity stage for the technology of jam and juice production.

Jam preparation

To prepare jams 350 g of fruits and 120 g of sugar were mixed and stirred until boiling. Fruits were cooked under atmospheric pressure for 20 minutes. Jams were prepared in three series and filled into hot glass jars. They were allowed to cool at room temperature and stored in dark until analysis. Jams were analyzed within one week.

Juice preparation

Juices were prepared in three series by mixing 300 g of fruits, 100 g of sugar and 200 mL of water and cooked for 15 minutes. Juices were filtrated after they were cooked and filled into hot glass bottles. Samples were stored in dark at room temperature and analyzed within one week.

Extraction procedure

The amount of 200 g of frozen sour cherries from each cultivar was homogenized using a food blender. 1 g of homogenized sample was extracted with 10 mL of acidified methanol (1% HCl) for 60 min at 200 rpm at room temperature using an orbital shaker (Agitador orbital, Optic Ivymen System). All samples were analyzed the same day when they were prepared. Jams were extracted using the same procedure.

Determination of total phenolic (TF) content

Total phenolic content was determined with the Folin-Ciocalteu colorimetric method described by Singleton, Orthofer, and Lamuela-Raventos (1999) and as previously described by Kazazic, Djapo and Ademovic (2016). Standard curve of gallic acid was used for the evaluation of results. For sample measurement, 100 µL of the extract was added to 5 mL (1/10 dilution) of Folin Ciocalteu phenol reagent and 900 µL of distilled water. After 5 min, 4 mL of 15% sodium carbonate (Na2CO3) was added. Following the incubation at room temperature for 120 minutes the absorbance at 765 nm was measured.

Data are presented as average values of three measurements for each sample (R2=0.998 for fresh samples, R2=0.999 for juices and R2=0.999 for jams).

The content of TF was expressed as mg of gallic equivalent (GAE) per 100 g of fruits fresh weight (FW) for fresh samples and jams. For juices the content of TF was expressed as mg of gallic equivalent (GAE) per L of juice.

Determination of total anthocyanin (TA) content

Total anthocyanin content was determined using the pH-differential method (Zhishen, Mengcheng and Jianming 1999). Two solutions of fruit samples were prepared, one with 0.5 mL of extracts in 2 mL of potassium chloride buffer (0.025 M, pH 1.0) and other with 0.5 mL of extracts in 2 mL of sodium acetate buffer (0.4 M, pH 4.5). The absorbance was measured at both 510 nm and 700 nm with a spectrophotometer (Genesys 20 thermo spectronic) after 20 min. The total anthocyanin content is expressed as mg of cyanidin-3-glucoside equivalent (CGE) per 100 g of fruits fresh weight (FW) for fresh samples and jams. For juices the content of anthocyanin was expressed as mg of cyanidin-3-glucoside equivalent (CGE) per L of juice.

Determination of antioxidant activity (AA) using the ABTS method

Determination of the antioxidant activity with ABTS• (Re, Pellegrini, Proteggente et al., 1999) was done as described in work by Kazazic et al. (2016). ABTS cation radical (ABTS•) was made by dissolving 19.5 mg of ABTS and 3.3 mg of potassium persulfate in 7 mL of distilled water and allowing free radical generationin the dark at room temperature for 12-16 h. Then ABTS radical solution was diluted in ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. Then 40 µL of the sample was mixed with 4 mL of diluted ABTS radical solution in the cuvette and absorbance was measured at 734 nm after 6 minutes. All the solutions were prepared on the same day of the experiment. The results were expressed as mmol of Trolox (TE) equivalent per kilogram for fresh samples and jams, and as mmol of Trolox (TE) equivalent per L in juices (R2=0.9942 for fresh samples, R2=0.9939 for juices and R2=0.9947 for jams).

Statistical analysis

All measurements are expressed as mean ± standard deviations. The statistical differences are considered significant at $p < 0.05$. All the analyses were done in triplicates.

RESULTS AND DISCUSSION

Quantitative content of investigated bioactive compounds (total phenols and total anthocyanins) and antioxidant activity in fresh sour cherries, jams and juices are presented in Tables 1, 2 and 3.

Table 1: The content of total phenols (TF), total anthocyanins (TA) and antioxidant activity (AA) by ABTS method in sour cherries

Cultivar	TF, mg GA/100 g FW	TA, mg CGE/100g FW	AA, mmol TE/kg FW
Marasca	340.80±23.23^{b}	79.7±0.35^{b}	29.03±1.49^{b}
Oblačinska	235.81±68.10^{a}	65.3±0.76^{a}	23.66±0.96^{a}

*Values with different letters in the same column are significantly different at p<0.05

Table 2: The content of total phenols (TF), total anthocyanins (TA) and antioxidant activity (AA) using ABTS method in sour cherries jams

Cultivar	TF, mg GA/100 g FW	TA, mg CGE/100g FW	AA, mmol TE/kg FW
Marasca	366.34±29.78^{b}	32.26±2.52^{b}	28.90±1.80^{b}
Oblačinska	306.67±12.57^{a}	21.10±1.85^{a}	23.71±2.20^{a}

*Values with different letters in the same column are significantly different at p<0.05

Table 3: The content of total phenols (TF), total anthocyanins (TA) and antioxidant activity (AA) determined by ABTS method in sour cherries juice

Cultivar	TF, mg GA/L FW	TA, mg CGE/L FW	AA, mmol TE/L FW
Marasca	514.48±83.70^{b}	48.78±3.28^{b}	42.03±0.25^{b}
Oblačinska	365.59±15.17^{a}	34.77±0.95^{a}	30.55±0.07^{a}

*Values with different letters in the same column are significantly different at p<0.05

The total phenolic content in the sour cherries was 235.81 mg (GA)/100 g FW in the Oblačinska cultivar and lower than reported by Khoo et al. (2011), and Mitić et al. (2012). Sour cherry Marasca fruit had higher total phenolic content (340.80 mg (GA)/100 g FW) than Oblačinska which is in accordance with Viljevac Vuletić et.al. (2017). Čoga, Jurkić, Zeman et al. (2017) reported values for total phenolic content in Marasca fresh fruits from 391.4 mg (GA)/100 g to 691.7 mg (GA)/100 g. Variation of total phenolic content in Marasca cultivar was due to significant difference in chemical composition for fruits of Marasca cultivar depending on climatic conditions (Čoga et al., 2017).

For jam samples total phenolic content was 306.67 mg (GA)/100 g FW for the Oblačinska cultivar and 366.34 mg (GA)/100 g FW for the Marasca cultivar which is in accordance with values reported by Kim and Padilla-Zakour (2004). Levaj et al. (2010) pointed that sour cherry Marasca fruits and jams contained the higher level of phenolic compounds than sour cherry Oblačinska which is similar to our results. The study by Poiana, Moigradean, Dogaru et al. (2011) showed that changes in total phenolic compounds in sour cherries were less pronounced compared to strawberry and cherries during thermal processing and storage. Vukoja, Pichler and Kopjar (2019) pointed that jams with a higher concentration of added sugar have a higher concentration of total phenols, comparable to the results of our study.

The total phenolic content in juices ranged from 365.59 mg (GA)/L FW for the Oblačinska cultivar and 514.48 mg (GA)/L FW for the Marasca cultivar.

The concentration of total anthocyanins in sour cherries was 65.3±0.76 mg CGE/100 g FW for the Oblačinska cultivar and 79.7±0.35 mg CGE/100 g FW for the Marasca cultivar. These results are in accordance with the anthocyanin content determined by Blando et al. (2004) (27.8 - 80.4 mg (CGE)/100 g FW). Viljevac Vuletić et al. (2017) investigated the impact of season, location, and cultivar influence on bioactive compounds of sour cherries and reported that total anthocyanin content was higher in Marasca cultivar compared to Oblačinska. Anthocyanin concentrations reported by Vijevac Vuletić et al. (2017) were higher than anthocyanin concentrations in this study due to the difference in extraction method used. Anthocyanins are vacuola pigments and ultrasound extraction which was used by Viljevac Vuletić et al. (2017) enhances transfer from the sample to the solvent improving the extraction of anthocyanins (Rodrigues, Fernandes, Sosua de Brito et al., 2015).
Total anthocyanin content in jam samples ranged from 21.10±1.85 mg (CGE)/100 g FW for Oblačinska cultivar and 32.26±2.52 mg (CGE)/100 g FW for Marasca cultivar. Anthocyanin losses are probably due to complex formation with other compounds during jam processing, namely sugars and degradation products of ascorbic acid (Bursać Kovačević, Levaj and Dragović-Uzelac 2009).

Antioxidant activity depends on the method of extraction and method used for their determination. The ABTS assay was performed to assess the antioxidant activity of sour cherries and their products jams and juices. ABTS is not found naturally, so there is possible criticism that the assay is not directly relevant to any biological function. Model of synthetic radicals has been used frequently in many laboratories around the world for screening and routine determination even though they are not directly related to food. Yet these methods are used to determine the antioxidant activity in most studies due to their simplicity, low cost and repeatability (Milić et al., 2021; Sokol-Letowska et al., 2020; Miguel-Chávez, 2017). ABTS assay can be used to determine the antioxidant capacity of numerous compounds, namely carotenoids, phenolic, and plasma (Re et al., 1999). Antioxidant activity of compounds with redox potential that is lower than that of ABTS can be determined using this assay. Since phenols have redox potential lower than ABTS, this radical is used to determine the antioxidant activity of these molecules (Miguel-Chávez, 2017). The antioxidant activity in the

Marasca cultivar was 29.03±1.49 mmol (TE)/kg which is lower than reported by Dragovic-Uzelac, Levaj, Bursać et al. (2007) (45.36±3.05 mmol TE/kg FW). In Oblačinska cultivar antioxidant activity was found to be 23.66±0.96 mmol (TE)/kg.

Juices and jams represent a noticeable source of antioxidants despite processing. It has been previously reported that the inclusion of sugar and industrial processing of sour cherry does not significantly impact their antioxidant capacity (Kirakosyan, Seymour, Urcuyo Llanes et al., 2009). Anthocyanin losses are compensated by the formation of Maillard products that can contribute to the antioxidant properties (Vukoja et al., 2019).

The correlation between antioxidant activity and total polyphenol content was statistically significant ($r = 0.906$, $p < 0.01$), whereas the correlation between antioxidant activity and total anthocyanin content was non-significant ($r = 0.212$, $p > 0.05$). This can be explained by the high content of melatonin (a phenolic substance), which is a strong antioxidant, in the sour cherry juice (Burkhardt, Tan, Manchester et al., 2001; Reiter, Tan, Leon et al., 2005).

CONCLUSIONS

In conclusion, the content of total phenols and antioxidant activity were well preserved after processing in the final products of sour cherry. The treatment process had a greater impact on the reduction of the anthocyanin content but did not have a significant effect on the reduction of the content of total phenols and antioxidant activity. Results showed that juices and jams possess noticeable content of bioactive compounds with antioxidant activity in the diet.
The obtained results also showed that there are significant differences in the content of investigated bioactive compounds among selected sour cherry varieties in the Herzegovina region. Further investigations are needed to investigate polyphenolic compounds profile and effects of processing of Marasca and Oblačinska sour cherries.

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Summary/Sažetak

Bioaktivne supstance su sekundarni metaboliti proizvedeni u biljkama. Mnoge studije zabilježile su direktnu povezanost između prisustva bioaktivnih supstanci i pozitivnih efekata biljaka na zdravlje. Višnje imaju visok sadržaj bioaktivnih supstanci, uglavnom polifenola i antocijanina. Najčešće se konzumiraju u svježem stanju, ali se koriste za proizvodnju džema, želea, marmalade, sokova i sirupa. Cilj ovog istaživanja bio je utvrditi ukupni sadržaj fenola, antocijanina i antioksidativne aktivnosti dvije sorte višanja, te njihovih proizvoda, džemova i sokova, pripremljenih po tradicionalnoj recepturi. Ukupni sadržaj fenola određen je metodom Folin-Ciocalteu. Za određivanje antioksidativne aktivnosti korišten je ABTS test obezbojenja radikalnih spojeva. Sadržaj antocijanina određen je primjenom pH diferencijalne metode. Sorta Marasca imala je veći sadržaj fenola, antocijanina i antioksidativnu aktivnost u poređenju sa sortom Oblačinska. Procesiranje višanja imalo je veći utjecaj na smanjenje sadržaja antocijana, ali nije imala značajan utjecaj na antioksidativnu aktivnost.