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# Impact of freezing and freeze-drying processes on changes in phytochemicals and antioxidant capacity of blackberry fruit

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**Abstract**

Blackberry (*Rubus* subg. *Rubus* Watson) is highly valued for its nutritional properties, which may be linked to its high content of primary and secondary metabolites. This study evaluates the effect of freezing and freeze-drying processes on the phytochemical content and antioxidant capacity of the 'Čačanska Bestrna' blackberry cultivar, comparing these changes with the quality of fresh blackberry fruit. Primary metabolites, including soluble solids content (SSC), total and reduced sugars (TS, RS), total sucrose (TS), and total acids (TA) content, were determined in freeze-dryed, frozen and fresh blackberry fruits. Among the secondary metabolites, the content of total phenolics (TPC), and total anthocyanins (TAC) was analyzed as phytochemicals responsible for the antioxidant capacity of the fruit, which was also determined using the DPPH method. The results showed that the applied preservation methods positively affected the examined fruit parameters. Freeze-dried blackberry fruits showed superior levels of SSC (90.63%), TS (52.25%), RS (45.83%), and TA (6.10%) compared to frozen and fresh fruits. TPC values did not demonstrate significant variations between the two preservation methods and fresh fruit. In this regard, further research should be focused on increasing the efficiency of preserving beneficial phytonutrients in final blackberry products that are essential for human health, thereby ensuring better quality and high nutritional value.

Key words: blackberry, freezing, lyophilization, sugars, acids, phenols

## INTRODUCTION

Blackberries are recognized as significant sources of natural antioxidants. These fruits have long been acknowledged for abundant quantities of phenolic compounds that have the substantial health benefits to humans (Marjanovic et al., 2021). In Serbia, blackberry is considered one of the economically important berry fruit species (Nikolić & Milivojević, 2015). Over the past decade, Serbia has ranked among the top four global producers of blackberries, with a 69% share of total European production and 18% of the total global production (Strik et al., 2014). It ranks fourth in the world in blackberry production, following the USA, Mexico, and China. The most prevalent cultivars in global commercial blackberry plantings are 'Čačanska Bestrna', 'Thornfree,' 'Loch Ness,' and 'Chester Thornless' (Milivojević, 2022). Currently, 'Čačanska Bestrna', developed at the Fruit Research Institute in Čačak, is the dominant cultivar in Serbian blackberry plantings, occupying 60% of the total planting area, despite the introduction of several other cultivars. Beyond its adaptability to diverse agro-ecological conditions, 'Čačanska Bestrna' is valued for its high yield and large, nutritionally rich fruits that align with the primary objectives of blackberry breeding programs (Clark & Finn, 2011). Stanisavljević et al. (1999) emphasized that this cultivar exhibits remarkable yield potential, which is a critical factor in ensuring the profitability of blackberry cultivation.

The visual quality of fruit, encompassing attributes such as shape, color, and size, is a crucial determinant of consumer acceptance. Similar to other soft fruits, the limited seasonal availability and short shelf life of fresh blackberries often lead to their preservation through methods like freezing, freeze-drying, canning, or processing into jams, jellies, and juices. These techniques help extend the storage life of the fruit, ensure availability in various markets, and meet consumer demand (Veberic et al., 2014). Freezing, in particular, is considered one of the least destructive preservation methods for phenolic compounds in berries, making it a recommended pre-treatment for berry processing (Wu et al., 2010). On the other hand, the freeze-drying method, which eliminates liquid water, operates in an oxygen-free environment (when conducted under vacuum), and utilizes low temperatures, is considered the optimal choice for dehydrating fruits and vegetables (Bhatta et al., 2020). This process helps preserve the content of valuable biocompounds in the final products. Literature data regarding the impact of freezing and freeze-drying on the chemical properties of berries is limited, with most studies focusing on the overall content of certain compound groups (e.g., phenols and anthocyanins). Given this, there is a need for comprehensive research on the nutritional changes in blackberry fruit induced by freezing and freeze-drying preservation methods.

The objective of this study was to determine variations in the content of primary and secondary metabolites among fresh, freeze-dried and frozen fruit of blackberries.

## MATERIAL AND METHODS

### Plant material and experimental design

Blackberry (*Rubus* subg. *Rubus* Watson) fruits of -cultivar 'Čačanska Bestrna' were harvested at fully ripe stages. Fully ripe (optimally ripe) fruits were black, glossy and easily picked from the branches. The investigation was conducted in 2018 in experimental orchard established in 2006 at Gornja Gorevnica (43°53' N latitude, 20°20' E longitude, 290 m altitude) near Čačak, in Western Serbia. Blackberries were planted at a spacing of 3.0 m in the row and 1.5 m within the row and trained to a three-wire trellis. The experiment was laid out in a randomised block design with four replications. Fertilisation, weed control and irrigation practices were provided regularly during the vegetative season. Weather conditions in Čačak were characterised by the mean growing season temperature of 17.0° C and total rainfall of 340.4 mm for the long-term averages (Figure 1).

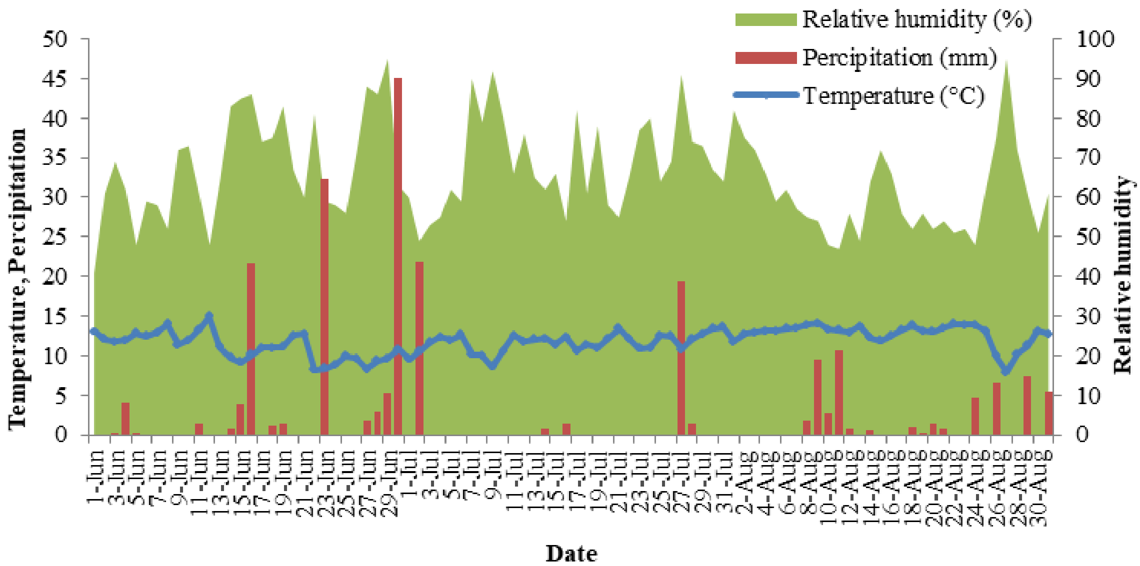


Figure 1. Variation of temperature, precipitation, and relative humidity from 1 June to 30 August 2018

The soil in the blackberry orchard was vertisol, moderately supplied with organic matter (2.92%) and poor in N (0.11%); soil pH in KCl 0.01 mol $L^{-1}$ was 4.98. The contents of available soil P and K were 4.64 and 29.23 mg 100 $g^{-1}$, respectively. In agro-ecological conditions in the Republic of Serbia, blackberries perform best in warm pre-mountainous areas with high levels of air humidity during the growing season, particularly at the fruit maturation stage (Nikolić and Milivojević, 2015).

After harvest, blackberry fruits were transported to the laboratory and randomly assigned to three treatment groups: 1) the harvest treatment, where fresh fruits were immediately analysed; 2) fruits stored in a freezer at -20°C for 30 days; and 3) fruits subjected to freeze-drying at a temperature of up to 70°C (Aktas et al., 2007). Four replications were conducted for each treatment, with each replication consisting of 100 blackberry fruits. All fruits were collected on the same sampling date.

Chemical parameters, including soluble solids content (SSC), total sugars (TS), reducing sugars (RS), titratable acidity (TA), and sucrose content (SC), were measured at commercial maturity stages. Each fruit sample consisted of 25 fruits was pooled to obtain a composite sample and then analyzed for SSC by a digital refractometer (Carl Zeiss, Jena, Germany) at 20°C, and the data were expressed in percentages. The content of TS (%) and RS (%) was determined volumetrically, using the Luff-Schoorl method (Egan et al., 1981). The SC was calculated by multiplying the difference between the TS and RS contents by the 0.95 coefficient. The TA was measured by neutralization to pH 8.2 with 0.1 N NaOH and the data were presented as % of malic acid.

The total phenolic content (TPC) was determined using a modified Folin-Ciocalteu colorimetric method (Singleton et al., 1999) and the results were expressed as milligrams of gallic acid equivalents per 100 g of fresh weight (mg GAE 100 $g^{-1}$ FW). The ground sample (4.0 g) was stirred vigorously with 40 mL of extraction solution consisting of methanol and distilled water (80% v/v) and was kept for 2 hours in the dark at room temperature. The mixture was centrifuged in two sequential times for 15 min at 3500 rpm, and the supernatant was filtered through a 0.45 μm Minisart filter before analysis. A 40 μL of fruit extracts or gallic acid standard solution was mixed with 3.16 mL of distilled water where 200 μL of Folin-Ciocalteu reagent was added and allowed to stand for 8 min before 600 μL of 20% $Na_2CO_3$ solution was added. The solution was well mixed and absorbance at 765 nm against an appropriate blank was determined after 2 hours. Data are reported as means for at least three replications. Due to significant differences in water content between the fresh, frozen, and lyophilized samples, a direct comparison of the TPC across these sample types was not feasible. Therefore, the results are presented separately for each sample type, with an emphasis on the specific characteristics of each processing method.

The total anthocyanin content (TAC) of the aqueous extracts was determined using the previously described pH-differential method (Torre & Barritt, 1977; Liu et al., 2002). Briefly, 20 g of ground fruit was blended with 40 mL of extracting solvent (95% ethanol/1.5 N HCl, 85:15). The extract was collected by filtration with an additional 30 ml of solvent washing. The residue was soaked with 70 mL of extracting solvent, and the extract was collected after 2h. The total extracts were pooled and brought up to 200 mL. A UV/VIS spectrophotometer (PU 8740 UV/VIS, England) and a 1-cm path length disposable cell were used for spectral measurements at 510 and 700 nm. Pigment content was calculated as milligrams of cyanidin-3-glucoside per 100 g of fresh weight (mg cyn-3-glu 100 g $FW^{-1}$) using an extinction coefficient of 26,900 L/cm/mol and molecular weight of 449.2 g/mol. Given the substantial differences in water content between the fresh, frozen, and lyophilized samples, it was not possible to make a direct comparison of TPC, AC, TS, RS, SSC, and TA across these sample types. As a result, the data for each parameter are presented individually for each sample type, highlighting the unique characteristics associated with each processing method.

Antioxidant capacity (AC) was determined using the DPPH method reported by Brand-Williams et al. (1995) with modifications (Sánchez-Moreno et al., 1998). The scavenging activity percentage (AA%) was determined according to Mensor et al. (2001):

$$AA\% = 100 - \left[ \frac{(Abs_{sample} - Abs_{blank}) x100}{Abs_{control}} \right]$$

## Statistical analysis

The data were presented as mean ± standard error. Differences between means were compared by Duncan's Multiple Range tests in a one-way analysis of variance (ANOVA) using the MSTAT-C statistical computer package (Michigan State University, East Lansing, MI, USA). The significance of differences at a 5% level among means was determined.

## RESULTS AND DISCUSSION

The results of the SSC, TS, RS, SC, and TA tests for fresh, frozen, and freeze-dried blackberry fruits of the ‘Čačanska Bestrna’ cultivar are presented in Table 1. Significant differences in the values of these fruit quality parameters were observed across all fruit categories. The SSC, TS, RS, SC, and TA of the fruit ranged from 9.25 to 90.63%, from 5.28 to 52.25%, from 4.98 to 45.83%, from 0.28 to 9.57%, and from 1.29 to 6.10% of malic acid, respectively. The highest SSC was recorded in the freeze-dried fruit, which is expected given the applied preservation method, while no statistically significant differences were found between the SSC values of fresh and freeze fruits. The highest TA content was noted in the freeze-dried blackberry fruits, compared to fresh and freeze fruits, which had similar values.

Table 1. SSC, TS, RS, SC, and TA of fresh, freeze, freeze-dried blackberry ‘Čačanska Bestrna’

| Parameter | Blackberry ‘Čačanska Bestrna’ | | | |
|---|---|---|---|---|
| | Fresh fruit | Freeze fruit | Freeze-dried fruit | |
| SSC (%) | 9.25±0.57 b | 10.04±0.60 b | 90.63±5.11 a | * |
| TS (%) | 5.28±0.62 c | 6.21±0.70 b | 52.25±3.55 a | * |
| RS (%) | 4.98±0.45 b | 5.13±0.42 b | 45.83±3.09 a | * |
| SC | 0.28±0.03 b | 0.33±0.02 b | 9.57±0.55 a | * |
| TA (% of malic acid) | 1.29±0.03 b | 1.33±0.03 b | 6.10±0.05 a | * |

Values within each row followed by the same small letter are not significantly different at $p \leq 0.05$ by LSD test; ns - non-significant differences.

Veberic et al. (2014) observed that the impact of processing and various storage methods on the primary and secondary metabolites (primarily anthocyanins) in blackberries has been widely studied, particularly in juiced, pureed, and canned fruits. The sensory quality attributes and nutritional value of blackberry fruits, as well as those of other fruits, play a crucial role in consumer satisfaction and influence subsequent consumption. Taste is primarily associated with water-soluble compounds. Sweetness is mainly attributed to mono- and disaccharides, rather than other compounds (Ruiz-Aceituno et al., 2018). Sourness is consistently linked to organic acids and pH levels. The compounds responsible for taste are generally water-soluble and non-volatile (such as carbohydrates and organic acids), unlike those responsible for aroma, which are typically unstable (Shepherd, 2002). Organic acids are vital for stabilizing ascorbic acid and anthocyanins, which is why they are key to the formation of fruit color and enhancing the storage life of both fresh and processed fruits (Enaru et al., 2021). Beyond their role in fruit coloration and storage extension, organic acids are also significant in fruit processing because they influence the gelling properties of pectin (Cordenunsi et al., 2003). As shown by Kopjar et al. (2012), specific sugars, such as glucose, can have a positive effect on anthocyanin content in blackberry juice by preventing their degradation during storage. In berries, as well as in other fruits, anthocyanins begin to accumulate at the onset of ripening, when the activity of certain enzymes in the phenylpropanoid pathway increases and continue to build up during maturation (Slatnar et al., 2012a). The evolution of anthocyanins parallels the accumulation of sugars, although no direct relationship has been established (Cadot et al., 2011).

Table 2. TPC, TAC, and AC of fresh, freeze, freeze-dried blackberry ‘Čačanska Bestrna’

| **Parameter** | Blackberry ‘Čačanska Bestrna’ | | | |
|---|---|---|---|---|
| | Fresh fruit | Freeze fruit | Freeze-dried fruit | |
| TPC (mg GAE 100 $g^{-1}$ FW) | 805.17±15.55 a | 819.60±13.62 a | 874.96±12.22 a | ns |
| TAC (mg cyn-3-glu 100 $g^{-1}$ FW) | 74.72±1.65 c | 101.40±1.80 b | 119.81±2.85 a | * |
| AC (AA%) | 2.72±0.08 a | 2.35±0.09 b | 2.49±0.04 b | * |

Values within each row followed by the same small letter are not significantly different at $p \leq 0.05$ by LSD test; ns - non-significant differences.

Among commercially important small fruits, blackberries are notable for their exceptionally high AC, which is strictly correlated to the presence of efficient oxygen radical scavengers, such as phenolic compounds (Pantelidis et al., 2007). However, Kaume et al. (2012) highlighted significant variations in the chemical composition of blackberries, which are influenced by several factors including cultivar, growing conditions, and ripeness. Furthermore, Skrovankova et al. (2015) reported that berries with intense coloration are particularly rich in polyphenols, including anthocyanins, flavonol glycosides, and hydroxycinnamic acids, which are known for their health-promoting properties. These variations underscore the complexity of blackberry composition and the impact of various factors on their nutritional and bioactive profiles. The TPC, TAC, and AC values of fresh, frozen, and lyophilized fruits of the blackberry cultivar 'Čačanska Bestrna' are presented in Table 2. The TPC ranged from 805.17±15.55 to 874.96±12.22 mg GAE 100 $g^{-1}$ FW, the TAC ranged from 74.72±1.65 to 119.81±2.85 mg cyn-3-glu 100 $g^{-1}$ FW, and the AC of the fruit ranged from 2.35±0.09 to 2.72±0.08%. No significant differences were observed in the TPC among fresh, frozen, and lyophilized fruits of the blackberry cultivar 'Čačanska Bestrna'. However, significant differences were confirmed in terms of the TAC and AC of the blackberry fruit. The highest TAC was elevated significantly in lyophilized fruits, while the lowest value was found in fresh blackberries. A similar result for blackberries has also been reported by Kolniak-Ostek et al. (2015) and Mikulic-Petkovsek et al. (2012). Lyophilization tends to preserve anthocyanins more effectively compared to freezing, due to reduced oxidation and less damage to cell structures. This preservation method minimizes the exposure of the fruit to oxidative conditions and enzymatic activity, which are the primary factors responsible for anthocyanin degradation (Marszałek et al., 2017). The low temperatures and vacuum environment used in the lyophilization process prevent the formation of ice crystals, which could otherwise disrupt cell integrity, allowing for a higher retention of anthocyanins (Enaru et al., 2021). Additionally, other phenolic compounds, such as flavonoids and tannins, may be more stable under the influence of preservation treatments and provide further protection against degradation. These compounds are less susceptible to the conditions that affect anthocyanins, such as oxidative stress and enzymatic breakdown. The overall stability of phenolic compounds in lyophilized blackberries may, therefore, be attributed to both the protective effect of the treatment itself and the inherent stability of certain phenolic classes under low-temperature conditions. On the other hand, the highest AC values were observed in fresh blackberries, while the lowest were found in frozen fruits. There were no significant differences between frozen and lyophilized fruits, so it should be noted that both preservation methods caused a significant reduction in AC compared to fresh fruit. According to De Torres et al. (2010), lyophilized skins retain their volatile and phenolic composition when compared to the original skins. Additionally, Wojdylo et al. (2009), who analyzed phenolic compounds (such as anthocyanins, flavanols, hydroxycinnamic acids, and flavonols) in strawberries dehydrated using different methods, found that the highest concentrations of phenolic compounds were recorded in the freeze-dried and vacuum-microwave dried samples.

## CONCLUSION

The Republic of Serbia occupies a prominent place among the world's blackberry producers, with the majority of production being exported in frozen form, while the processing sector remains significantly smaller. Accordingly, a comparative analysis of changes in chemical attributes between fresh, frozen, and lyophilized blackberry fruits was conducted. This assessment revealed that the lyophilization enhanced significantly the content of SSC, TS, RS, SC, TA and TAC fruit compared to freezing treatment, while freezing of blackberry fruits showed pronounced effect to enhance of TS and TAC in comparison to fresh fruit. The obtained results indicate the potential to maintain and even improve the chemical fruit composition during freezing and freeze-drying processes.

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## REFERENCES

Aktas, T., Fujii, S., Kawano, Y., & Yamamoto, S. (2007). Effects of pretreatments of sliced vegetables with trehalose on drying and quality of dried products. *Food and Bioproducts Processing*, 85(3), 178-183. DOI: 10.1205/fbp07037

Bhatta, S., Stevanovic Janezic, T., & Ratti, C. (2020). Freeze-drying of plant-based foods. *Foods*, 9(1), 87. DOI: 10.3390/foods9010087

Brand-Williams, W, Cuvelier, M. E., & Berset, C. L. W. T. (1995). Use of a free radical method to evaluate antioxidant activity. *Food Science and Technology*, 28, 25-30. DOI: 10.1016/S0023-6438(95)80008-5

Clark, J. R., & Finn, C. E. (2011). Blackberry breeding and genetics. *Fruit, Vegetable and Cereal Science and Biotechnology*, 5, 27-43.

Cordenunsi, B. R., Nascimento, J. D., & Lajolo, F. M. (2003). Physico-chemical changes related to quality of five strawberry fruit cultivars during cool-storage. *Food Chemistry*, 83(2), 167-173. DOI: 10.1016/S0308-8146(03)00059-1

De Torres, C., Diaz-Maroto, M. C., Hermosin-Gutierrez., I., & Perez-Coello, M. S. (2010). Effect of freeze-drying and oven-drying on volatiles and phenolics composition of grape skin. *Analytica Chimica Acta*, 660, 177-182. DOI: 10.1016/j.aca.2009.10.005

Egan, H., Kirk, R., & Sawyer, R. (1981). The Luff Schoorl method. Sugars and Preserves. Pearson's Chemical Analysis of Foods, 8, 152-153.

Enaru, B., Drețcanu, G., Pop, T. D., Stănilă, A., & Diaconeasa, Z. (2021). Anthocyanins: factors affecting their stability and degradation. *Antioxidants*, 10(12), 1967. DOI: 10.3390/antiox10121967

Kolniak-Ostek, J., Kucharska, A.Z., Sokól-Letowska, A., & Fecka, I. (2015). Characterization of phenolic compounds of thorny and thornless. *Journal of Agricultural and Food Chemistry*, 63, 3012-3021. DOI: 10.1021/jf5039794

Liu, M., Li, X. Q., Weber, C., Lee, C. Y., Brown, J., & Liu, R. H. (2002). Antioxidant and antiproliferative activities of raspberries. Journal of Agricultural and Food Chemistry, 50, 2926-2930. DOI: 10.1021/jf0111209

Marjanovic, A., Djedjibegovic, J., Lugusic, A., Sober, M., & Saso, L. (2021). Multivariate analysis of polyphenolic content and *in vitro* antioxidant capacity of wild and cultivated berries from Bosnia and Herzegovina. *Scientific Reports*, 11(1), 1-10. DOI: 10.1038/s41598-021-98896-8

Marszałek, K., Woźniak, Ł., Kruszewski, B., & Skąpska, S. (2017). The effect of high pressure techniques on the stability of anthocyanins in fruit and vegetables. *International Journal of Molecular Sciences*, 18(2), 277. DOI: 10.3390/ijms18020277

Mensor, L. L., Menezes, F. S., Leitao, G. G., Reis, A. S., dos Santos, T. C., & Coube, C. S. (2001). Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytotherapy Research*, 15, 127-130. DOI: 10.1002/ptr.687

Mikulic-Petkovsek, M., Slatnar, A., Stampar, F., & Veberic, R. (2012). HPLC-MSn identification and quantification of flavonol glycosides in 28 wild and cultivated berry species. *Food Chemistry*, 135, 2138-2146. DOI: 10.1016/j.foodchem.2012.06.115

Milivojević, J. (2022). Posebno voćarstvo 3 - Jagodaste voćke. Poljoprivredni fakultet, Univerzitet u Beogradu.

Nikolić, M., & Milivojević. J. (2015). Jagodaste voćke - Tehnologija gajenja. Univerzitet u Beogradu, Poljoprivredni fakultet.

Ruiz-Aceituno, L., Hernandez-Hernandez, O., Kolida, S., Moreno, F. J., & Methven, L. (2018). Sweetness and sensory properties of commercial and novel oligosaccharides of prebiotic potential. *LWT*, 97, 476-482. DOI: 10.1016/j.lwt.2018.07.038

Sánchez-Moreno, C., Larrauri, J. A., & Saura-Calixto, F. (1998). A procedure to measure the antiradical efficiency of polyphenols. *Journal of the Science of Food and Agriculture*, 76, 270-276.

Shepherd, R. (2002). Resistance to changes in diet. *Proceedings of the Nutrition Society*, 61(2), 267-272.

Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, 299, 152-178. DOI: 10.1016/S0076-6879(99)99017-1

Skrovankova, S., Sumczynski, D., Mlcek, J., Jurikova, T., & Sochor, J. (2015). Bioactive compounds and antioxidant activity in different types of berries. *International Journal of Molecular Sciences*, 16(10), 24673-24706. DOI: 10.3390/ijms161024673

Stanisavljević, M. (1999). New Small Fruit Cultivars From Čačak: 1. The new blackberry (*Rubus* sp.) cultivar 'Čačanska Bestrna'. *Acta Horticulturae*, 505, 291-296. DOI: 10.17660/ActaHortic.1999.505.37

Strik, B. C., Clark, J. R., Finn, C. E., & Banados, M. P. (2014). Blackberry cultivation in the world. *Revista Brasileira*, 6, 46-57. DOI: 10.1590/0100-2945-445/13

Torre, L. C., & Barritt, B. H. (1977). Quantitative evaluation of *Rubus* fruit anthocyanin pigments. *Journal of Food Science and Technology*, 42, 488-490. DOI: 10.1111/j.1365-2621.1977.tb01528.x

Veberic, R., Stampar, F., Schmitzer, V., Cunja, V., Zupan, A., Koron, D., & Mikulic-Petkovsek M. (2014). Changes in the contents of anthocyanins and other compounds in blackberry fruits due to freezing and long-term frozen storage. *Journal of Agricultural and Food Chemistry*, 62, 6926-6935. DOI: 10.1021/jf405143w

Wojdylo, A., Figiel, A., & Oszmianski, J. (2009). Effect of drying methods with the application of vacuum-microwave on the phenolic compounds, color and antioxidant activity in strawberry fruits. *Journal of Agricultural and Food Chemistry*, 57, 1337-1343. DOI: 10.1021/jf802507j

Wu, R. Y., Frei, B., Kennedy, J. A., & Zhao, Y. Y. (2010). Effects of refrigerated storage and processing technologies on the bioactive compounds and antioxidant capacities of 'Marion' and 'Evergreen' blackberries. *Journal of Food Science and Technology*, 43, 1253-1264. DOI: 10.1016/j.lwt.2010.04.002