

# BOOK OF PROCEEDINGS



*XIV International Scientific Agriculture Symposium  
"Agrosym 2023"  
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## QUALITY CHARACTERIZATION OF BLACKBERRY FRUITS (*Rubus* subg. *Rubus* Watson) IN DIFFERENT MATURITY STAGES

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### Abstract

The European blackberry (*Rubus* subg. *Rubus* Watson) is an important berry in Serbia and is positioned immediately after the raspberry and strawberry in terms of economic significance. The two most grown cultivars of blackberry in Serbia are ‘Loch Ness’ and ‘Čačanska Bestrna,’ each with their own unique characteristics and benefits. The choice of cultivar depends on the specific needs and preferences of the grower, as well as the growing conditions and market demand in their region. The aim of this study was to compare the fruit quality of the ‘Loch Ness’ and ‘Čačanska Bestrna’ blackberry cultivars during different maturity stages (fully ripe and overripe). The study found that both cultivars had higher values of soluble solids content (10.80° Brix), total anthocyanins (118.09 mg cyn-3-glu 100 g<sup>-1</sup> FW), weight (7.64 g), length (26.57 mm), and fruit shape index (1.48) when sampled at the overripe maturity stage. On the other hand, an increase in total phenolic content (601.56 mg 100 g<sup>-1</sup> FW), antioxidant capacity (3.81 μmol Trolox 100 g<sup>-1</sup> FW), and fruit width (18.30 mm) was determined in fully ripe blackberry fruits. Considering that blackberry fruits produced in Serbia are mainly sold on domestic and foreign markets in a frozen state due to the low level of processing capacity, these research findings will be useful in modern food technology. The trend in food technology is directed towards extending the shelf life and increasing the content of nutrients in blackberry fruits.

**Keywords:** *blackberry, maturity stage, pomological traits, antioxidants*

### Introduction

The European blackberry (*Rubus* subg. *Rubus* Watson) is an important berry in Serbia, positioned immediately after raspberry and strawberry (Nikolić and Milivojević, 2015) due to its economic significance. Blackberry production began after World War II, but production level did not significantly increase, as was the case with raspberry. Milutinović et al. (1999) reported that around 12,000 t of blackberry was produced in Serbia in 1997. The production tripled by 2005, amounting to 28,000 t on a total area of about 5,000 ha (Nikolić et al., 2012). According to Strik et al. (2007), Serbia is among the four leading global producers of blackberries, accounting for 69% of the European and 17.82% of the world's production. Small fruits, such as blackberries, have exceptional nutritional potential due to their high content of natural antioxidant compounds (Ding et al., 2006; Tulipani et al., 2008), which exhibit anti-inflammatory, antiviral, antimicrobial, and antioxidant activities (Reyes-Carmona et al., 2005). Berries are a rich source of a wide range of phenolics, including anthocyanins, flavonols, flavanols, and other flavonoids (Mikulic-Petkovsek et al., 2012; Mitic et al., 2014), which are responsible for the high free radical scavenging capacity measured by *in vitro* and *in vivo* assays (Kaume et al., 2012; Slatnar et al., 2012). Blackberries stand out among commercially important small fruits for their highest antioxidant capacity (Pantelidis et al., 2007), but the chemical composition of blackberries can vary depending on cultivar, growing conditions, ripeness stage, and other factors (Kaume et al., 2012). The appearance and internal

quality of fruit are the most important traits for fresh market and processing, and they are formed by important changes in the activities of a series of metabolic pathways during the ripening process. Fruit ripening is a biochemical process that changes physical and chemical characteristics. As a general rule, berries become softer, darker, and sweeter during the ripening process. Information on physical and chemical changes during fruit development is available for many fruit species (Kaume et al., 2012). The objective of the present research was to investigate changes in the physico-chemical properties of blackberry fruit of ‘Čačanska Bestrna’ and ‘Loch Ness’ at two maturity stages.

### Materials and methods

Blackberry (*Rubus* subg. *Rubus* Watson) fruits of two cultivars (‘Čačanska Bestrna’ and ‘Loch Ness’) were harvested at two different stages of ripeness, namely fully ripe and over ripe. Fully ripe (optimally ripe) fruits were black, glossy and easily picked from the branches (Karaklajić-Stajić et al., 2017). Over ripe fruits were black, without the characteristic gloss and fell from the branches at the slightest touch. The investigation was conducted over a two-year period (2017–2018) in experimental planting at Gornja Gorevnica (43°53' N latitude, 20°20' E longitude, 290 m altitude) near Čačak, Western Serbia. Blackberries were planted at a spacing of 3.0 m between the rows and 1.5 m within the row, and trained to a three-wire trellis. Fertilisation, weed control and irrigation practices standard for the region were provided during both seasons. 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. The soil in the blackberry planting was vertisol, moderately supplied with organic matter (2.92%) and poor in N (0.11%); soil pH in KCl 0.01 mol L<sup>-1</sup> was 4.98. The contents of available soil P and K were 4.64 and 29.23 mg 100 g<sup>-1</sup>, respectively. After harvest, 25 fruits of each cultivars and ripeness stage (n=25) in four replicates were randomly selected (400 fruits in total) and used for determination of the most important physical and chemical properties. Fruit weight was measured using a Mettler balance (Toledo, Switzerland) with accuracy of ±0.01 g, and the data were expressed in g per fruit. Fruit dimensions (mm) (length, breadth, and fruit shape index) were determined in the samples by ‘Inox’ vernier scale (±0.05 mm accuracy). The shape index of the fruit was determined based on the length/fruit width ratio. Total soluble solids content (SSC) was determined by a digital refractometer (Carl Zeiss, Jena, Germany) at 20°C, and data were expressed in °Brix. Total phenolic content (TPC), were determined using a modified Folin-Ciocalteu method (Singleton et al., 1999; Liu et al., 2002) and the results were expressed as g of gallic acid equivalents per 100 g fresh weight of the sample (g GAE 100<sup>-1</sup> g FW). Total anthocyanins content (TAC) was measured by the previously described pH-differential method (Torre and Barritt, 1977; Liu et al., 2002). Pigment content was calculated as milligrams of cyanidin-3-glucoside per 100 g fresh weight (mg cyn-3-glu 100 g<sup>-1</sup> FW). Antioxidant capacity (AC) was determined using the DPPH method reported by Brand-Williams et al. (1995) with modifications (Sánchez-Moreno et al., 1998), and expressed in µmol TE 100 g<sup>-1</sup> FW. The data were presented as two-year mean value ± standard error of mean (SE). Differences between means were compared by Duncan’s Multiple Range tests in a two-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

Analysis of variance showed a significant effect of ripeness stage on the fruit breadth, SSC, and TPC, and AC, whereas the cultivar significantly affected the fruit length and breadth, SSC, TPC and TAC (Tables 1 and 2). On the other hand, the interaction effect of variability factors significantly affected the breadth and shape index of fruit (Figure 1).

Table 1. Influence of ripeness stage, and cultivar on the physical properties of blackberry fruits

Treatment	Fruit weight (g)	Length (mm)	Breadth (mm)	Shape index
<b>Ripeness stage (A)</b>				
Fully ripe	7.56±0.15 a	26.48±0.20 a	18.30±0.42 a	1.44±0.03 a
Overripe	7.64±0.22 a	26.57±0.21 a	18.19±0.60 b	1.48±0.05 a
<b>Cultivar (B)</b>				
‘Loch Ness’	7.79±0.17 a	26.23±0.15 b	17.55±0.58 b	1.50±0.05 a
‘Čačanska Bestrna’	7.41±0.18 a	26.82±0.17 a	18.85±0.20 a	1.42±0.01 a
<b>ANOVA</b>				
A	ns	ns	*	ns
B	ns	*	*	ns
A×B	ns	ns	*	*

Values within each column followed by the same small letter are not significantly different at  $p \leq 0.05$  by Duncan’s Multiple Range tests. \*Significant differences; n.s. – not significant differences for  $P \leq 0.05$  according to F test.

During the harvest of blackberry fruits at different stages of ripeness, higher average values of weight, length, and fruit shape index were observed in overripe fruits (7.64 g, 26.57 mm, and 1.48, respectively), while there were no statistically significant differences in fruit weight and length.

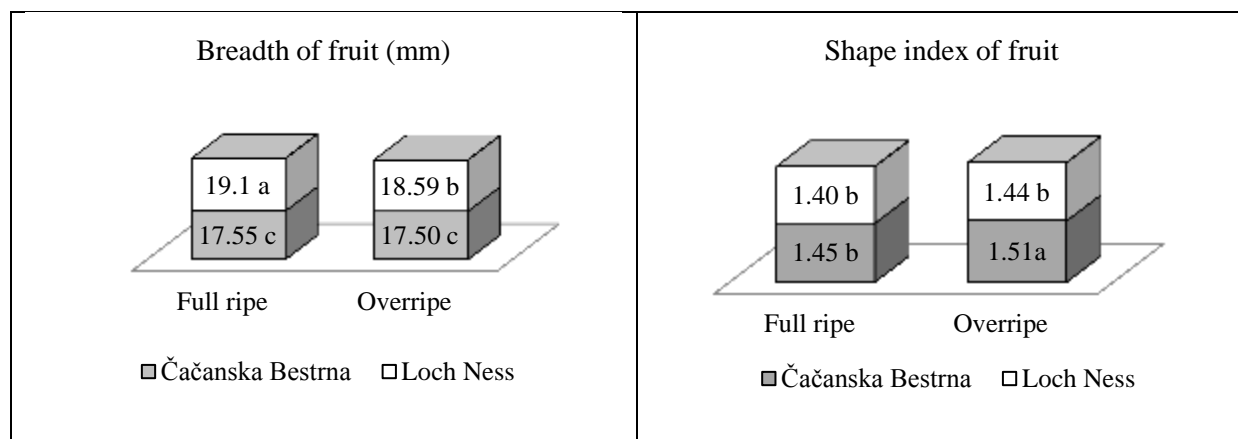


Figure 1. Interaction effects of ripeness stage/cultivar on the breadth of fruit and fruit shape index

When observed by cultivars, significantly higher values of fruit dimensions, length (26.82 mm) and breadth (18.85 mm) were determined in the cultivar ‘Čačanska Bestrna’. The significance of differences in the interaction between ripeness stage and cultivar was only established for fruit width and fruit shape index (Figure 1). The largest width was found in fruits sampled at physiological ripeness in the ‘Loch Ness’ cultivar (19.10 mm), while the highest fruit shape index values (1.51) were recorded in overripe fruits of the ‘Čačanska Bestrna’ cultivar. Numerous authors state that this characteristic depends on the cultivar as



well as environmental conditions, cultivation practices, and so on (Eyduran et al., 2008). Unlike raspberries, Nikolić and Milivojević (2010) mention that the fruits of most cultivated blackberry cultivars are larger, with an average weight ranging from 3.5 to 7.5 g, and there are even larger cultivars, such as the domestic cultivar ‘Čačanska Bestrna’. According to Stanisavljević (1999), the fruits of the ‘Čačanska Bestrna’ cultivar have the same shape as the parent cultivar ‘Black Satin’, which corresponds to an elongated-cylindrical shape. The fruit shape index values calculated in this study ( $>1$ ) are consistent with the results obtained by Stanisavljević (1999) and Milivojević et al. (2010) for the blackberry fruit.

Table 2. Influence of ripeness stage, and cultivar on the chemical properties of blackberry fruits

Treatment	SSC (°Brix)	TAC (mg cyn-3-glu 100 g <sup>-1</sup> FW)	TPC (g GAE 100 g <sup>-1</sup> FW)	AC (μmol TE 100 g <sup>-1</sup> FW)
<b>Ripeness stage (A)</b>				
Fully ripe	9.64±0.36 b	113.19±5.47 a	601.56±27.62 a	3.81±0.02 a
Overripe	10.80±0.43 a	118.08±10.31 a	581.47±8.48 b	3.76±0.04 b
<b>Cultivar (B)</b>				
‘Loch Ness’	10.93±0.36 a	150.07±12.44 a	657.30±5.14 a	3.77±0.02
‘Čačanska Bestrna’	9.51±0.34 b	81.20±5.03 b	525.73±8.48 b	3.81±0.03
<b>ANOVA</b>				
A	*	ns	*	*
B	*	*	*	ns
A×B	ns	ns	ns	ns

Values within each column followed by the same small letter are not significantly different at  $p \leq 0.05$  by Duncan’s Multiple Range tests. \*Significant differences; n.s. – not significant differences for  $P \leq 0.05$  according to F test.

The results of the chemical properties of the examined blackberry cultivars indicate that overripe fruits of both cultivars exhibited higher levels of SSC (10.80° Brix), and TAC (118.08 mg cyn-3-glu 100 g<sup>-1</sup> FW), while the content of TPC (601.56 g GAE 100 g<sup>-1</sup> FW) and AC (3.81 μmol TE 100 g<sup>-1</sup> FW) were higher in fruits sampled in fully ripe stages. When observed by cultivar, higher values of all examined chemical parameters of the fruit, except for AC, were found in the ‘Loch Ness’ cultivar. The interaction effect of the variability factors (ripeness stage/cultivar) did not have a statistically significant impact on the mentioned blackberry fruit properties. The average values of SSC across all treatments ranged from 9.51 to 10.93° Brix, which were higher than the values reported by Miletić et al. (2006) for the ‘Čačanska Bestrna’ cultivar in eastern Serbia (7.1%), but lower than the values obtained by Stanisavljević (1999) for the conditions in Čačak (10.8%). Phenolic compounds in plants indirectly participate in plant growth processes, all metabolic processes, and also influence the organoleptic and nutritional properties of fruits. Furthermore, their physiological activity in the human body has been proven (Robards et al., 1999). The synthesis and distribution of phenolic compounds are influenced by the complex interaction of external factors (light, temperature, and humidity), internal factors (genetic factors and hormonal status) (Strack, 1997), agroecological factors (Veberič et al., 2014), as well as cultivation conditions and fruit ripening stage (Murillo et al., 2012). The TAC values obtained in this study were higher compared to the results obtained by Stajčić et al. (2012), Ivanovic et al. (2014), and Veberic et al. (2014) for the ‘Čačanska Bestrna’ cultivar. Milivojević et al. (2010) investigated the chemical properties of wild and commercial blackberry cultivars and found that the TPC in the ‘Čačanska Bestrna’ and ‘Loch Ness’ blackberry fruit was 1.74 mg GAE g<sup>-1</sup> FW, which was half of the values obtained in this study but comparable to the results obtained by Stajčić et al. (2012) for the same parameter and cultivar (235.09 mg GAE 100 g<sup>-1</sup> FW). Among the



berry fruit species, blackberry fruit exhibits the highest AC, which is attributed to its high content of phenolic compounds, as reported by Pantelidis et al. (2007). Kähkönen et al. (2003) and Hosseinian et al. (2007) emphasize that the content and composition of anthocyanins in berry fruit species, in addition to genotype (cultivars), are influenced by light intensity, temperature conditions, soil type, moisture level, fertilizer and pesticide usage, and other stress factors. The relationship between AC and the TPC and TAC in blackberry fruit has been confirmed by numerous studies (Sellappan et al., 2002; Reyes-Carmona et al., 2005; Milivojević et al., 2011), indicating that the parameter of ‘total phenols’ can serve as an indicator of AC (Milivojević et al., 2010).

### Conclusion

The results of this study showed that the ripeness stage of blackberry causes significantly increasing of SSC and TAC. The higher values of mentioned parameters have been noted in overripe berries compared to fully ripe fruit. Therefore, our results can be useful for further food processing and human metabolic research studies.

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