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EVALUATION OF ANTHOCYANIN CHANGES IN BLUEBERRIES AND IN BLUEBERRY JAM AFTER THE PROCESSING AND STORAGE

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ABSTRACT

Blueberry (*Vaccinium corymbosum* L.) is worldwide famous as the healthy and desirable fruit. The most valuable nutritional components of fruits are polyphenols, which include anthocyanins. The aim of the study was to assess the content of anthocyanin dyes in selected varieties of blueberry fruit. We evaluated the changes in the content of colorants that occur after treatment for fruit jam and its subsequent storage at 21°C under the light. Varieties Ramcocas, Record, Iranka, Nelson, Pemberton, Jersey and Coville were observed. Content of anthocyanins was determined spectrophotometrically. In fresh fruits anthocyanin content ranged from 9.878 g kg^{-1} of dry matter (Jersey variety) to 18.555 g kg^{-1} of dry matter (Nelson variety). After treatment there was found a decrease in the anthocyanins content, in the product's content were determined in the amount 1.645 g kg^{-1} of dry matter (Jersey variety) to 3.476 g kg^{-1} of dry matter (variety Ramcocas). The decrease was due to decomposition of anthocyans at high temperatures in processed products and also by the replacement of dry matter by sucrose in the product. Mean color decrease in blueberry jam was 84.5%. After storage of the product, there were found further degradations of colorants, evaluated at 34.9%. The content of anthocyanin in jam was found to be 1.089 g kg^{-1} of dry matter (Jersey variety) to 2.199 g kg^{-1} of dry matter (Ramcocas variety).

Keywords: blueberry; anthocyanin; jam

INTRODUCTION

Blueberries are fruits native to the North America. The different parts of the plants have been used in the treatment, but also in the preparation of foods. As useful plant, the blueberries have started to be cultivated only in the 20th century. In the world the leadership in their growing has USA, in Europe are grown mainly in Germany and Poland. In Slovakia blueberries can be found mainly in the northern regions of Orava and Turiec **(Šimala, 2007)**.

As reported **Ochmian et al. (2009)** berries of blueberries are unique in appearance, taste and flavoring properties as well as nutritionally important substances. Their important nutritional properties are attributed mainly to phenolic substances **(Vollmanová et al., 2009; Krikorian et al., 2010)**. As reported **Burdulis et al. (2007), Riihinen et al. (2008) and Habánová et al. (2013)** blueberry is a rich source of polyphenols and antioxidants, including tannins, phenolic acids, flavonols and anthocyanins. Blueberries in comparison with other kinds of small fruits contain a wide range of anthocyanins. In the fruit dominate malvinidin and delphinidin, in lower concentrations are represented peonidin, cyanidin and petunidin. Phenolics of the colorants are attached to the glucoside, galactoside or arabinoside **(Nicoue et al., 2007; Scalzo et al., 2009)**. Fruits are also a good source of fiber, and are very low in fat and sodium. They contain vitamins C and A, iron, magnesium and potassium. These compounds have high antioxidant activity and strongly support the natural defenses of the human body **(Petii and Scully, 2009)**.

For high health-promoting active components are fruit used in supportive treatment of cardiovascular, neurodegenerative, oncological and other diseases of civilization **(Basu et al. 2010; Michalská et al., 2010; Mateos et al., 2012; Navas et al., 2012)**.

Fresh fruits are characterized by short shelflife. An important part of the production of blueberries is therefore to put them on the market in processed fruit products. Popular product is blueberry jam. Color of the jam is one of the fundamental attributes f the acceptability of the product among consumers **(Connor et al, 2002; Ścibisz and Mitek, 2009)**. In production the primary objective is minimizing the loss of the original content of anthocyanins. It is generally known that anthocyanins are unstable due to processing and storage. The stability is affected by temperature, pH, water activity, presence of carbohydrates and enzymatic activity **(Lee and Wrolstad, 2006)**.

As reported **Haffner et al. (2003), Kim and Zakour (2004)** among the most important factors that affect the quality of blueberry jam are quality and ripeness of the raw material, product composition, boiling time and method of preservation and also the storage conditions of the finished product.

The aim of the study was to assess the content of anthocyanins in different varieties of blueberry and observing their stability after processing for jam and after its subsequent storage at 21°C under the light.

MATERIAL A METHODOLOGY

In this work, 7 blueberry varieties were evaluated, that originated from research station in Kriva na Orava. The

research station is located at an altitude of 700 mamsl., the area has an average annual temperature of 6°C, average annual rainfall is 800-900 mm, the soil is sandy-loam with pH 3.8. Acidic pH is an important factor in the successful cultivation of these crops.

The experiment included early varieties: Ramcocas and Record, medium early varieties: Iranka, Nelson, Pemberton, Jersey and late variety: Coville. Blueberry fruits were harvested at the stage of full coloring. Collecting of fruit was hand made.

Jam was prepared by classic procedure, the product was concentrated by addition of sucrose and boiled at common atmospheric pressure. The addition of sugar was determined by the difference of the required soluble dry matter of the finished product and raw materials. Pectin preparation was used and to adjust the acidity, citric acid was added. Boiling of jam was fast, with a maximum duration of three minutes. The finished product was filled into glass containers. The product was stored for 6 months at 21°C in the light.

The content of anthocyanins was determined spectrophotometrically. Homogenised samples were extracted in ethanol solution with the addition of 0.01% hydrochloric acid. Repeated extraction of samples to complete discoloration went under hot conditions. The content of anthocyanins was then determined by measuring the absorbance of the UV-VIS spectrophotometer Jenway at a wavelength of 543 nm.

Dry matter content of all samples was assessed by gravimetric method. Homogenized samples were dried to constant weight at 105°C in laboratory oven WTC Binder. Analyses were performed in triplicate.

The results were processed by the statistical program Statistica. Effect of variety on the content of anthocyanins was observed by the one-way analysis of variance, the effect of treatment on changes in the content of anthocyanins by two-factor analysis of variance and the differences among varieties were tested by the Fisher test.

RESULTS AND DISCUSSION

The total content of anthocyanins observed in the varieties ranged from 1.932 g kg^{-1} to 3.945 g kg^{-1} of fresh matter. Fresh fruit varieties by decreasing values of colorants can be ordered as follows: Ramcocas > Nelson > Coville > Iranka > Pemberton > Record > Jersey.

Our results correspond with the results of the work **Wu et al. (2006**), who report that the content of anthocyanin in blueberries ranges from 2.5 to 4.95 g kg^{-1}. Similar results reached **Cho et al. (2004),** who reports the content of anthocyans in the range of 1.43 to 8.22 g kg^{-1}. The measured values were compared to the average values from 0.45 to 1.25 g kg^{-1}, indicated by **Ścibisz and Mitek (2007)** much higher. **Stevenson and Scalzo (2012)** observed content of anthocyanins in cultural varieties of blueberries grown in different countries e.g. New Zealand, Canada, China, USA, Poland, Italy. They found that the locality of cultivation has extremely great influence on the content of anthocyans. Four varieties varying widely in reporting parameter, authors identified a variety Duke, in which the colorant content ranged from 1.01–2.16 g kg^{-1}, Elliott (1.52–2.61 g kg^{-1}), Climax (0.99–2.56 g kg^{-1}) and Powderblue (1.65–2.43 g kg^{-1}).

On the basis of the average values of the monitored localities they tried to identify varieties with the highest content of anthocyanin pigments. Among the 10 best performing varieties were included varieties Rubel (2.9 g kg^{-1}), Darrow (2.87 g kg^{-1}), Elliot (2.61 g kg^{-1}), Northland (2.38 g kg^{-1}), Duke (1.89 g kg^{-1}), Jersey (1.85 g kg^{-1}), Caroline Blue (1.54 g kg^{-1}), Nui (1.49 g kg^{-1}), Hortblue Poppins (1.39 g kg^{-1}) and Blue Moon (1.39 g kg^{-1}).

In our work, we used the best variety Jersey, in which we analyzed the content of anthocyanins in the amount of 1.93 g kg^{-1}, the result is comparable with findings of **Stevenson and Scalzo (2012)**.

To objectively comparison of fresh fruit and jam we converted anthocyanins to dry matter content. Dry matter of fresh blueberry fruit was 22.6% (Ramcocas) to 17.8% (Iranka). The highest content of anthocyanins, calculated per the dry matter content was determined at Nelson 18.56 g kg^{-1} and the lowest was measured at variety Jersey, 9.88 g kg^{-1}.

By decreasing amounts of anthocyanins in fresh fruits calculated per dry matter, varieties can be ordered as follows: Nelson > Ramcocas > Iranka > Pemberton > Coville > Record > Jersey (Fig. 1).

One-way analysis of variance showed that the variety has a statistically significant effect on the anthocyanin content ($P < 0.01$). By use of Fisher's LSD test ($P < 0.05$) were observed relative differences in the content of colorants in different varieties. Fresh fruit varieties created 5 homogeneous groups, which differed in the content of the monitored components (Table 1).

Variety Nelson showed statistically significantly the highest content of anthocyanins and variety Jersey significantly the lowest. Most balanced group of varieties in anthocyanin content was determined group, which consists of variety Coville, Pemberton, Iranka and Ramcocas, among which is found no statistically significant difference in the content of anthocyanins in fresh fruits.

Evaluation of anthocyanin content of blueberry jam showed the highest content in sample from variety Ramcocas 2.294 g kg^{-1} and the lowest in sample of the variety Jersey 1.127 g kg^{-1}. By decreasing of colorants content in blueberry jam, varieties can be ordered as follows: Ramcocas > Pemberton (1.907 g kg^{-1}) > Nelson (1.815 g kg^{-1}) > Iranka (1.591 g kg^{-1}) > Record (1.524 g kg^{-1}) > Coville (1.154 g kg^{-1}) > Jersey.

Ścibisz and Mitek (2007) monitored the content of anthocyanins in blueberry jam prepared from a variety Bluecop. In the fresh fruit was determined the content of colorants in the amount of 0.946 g kg^{-1}, in jam with high sugar content 0.32 g kg^{-1}, and jam with low sugar and sugar free only 0.28 g kg^{-1}, that are several times lower than the value we found in our work.

Bluecop variety was observed by **Ehlenfeldt and Prior (2001)** who found the content of anthocyanins in jam 1.82 g kg^{-1}, this value is comparable with our findings. **Rdboten et al. (2005)** compared the color of blueberry jam made from fruit of blueberries *Vaccinium myrtillus* L. and *Vaccinium corymbosum* L. of varieties Berkley and Bluecop from northern Norway.

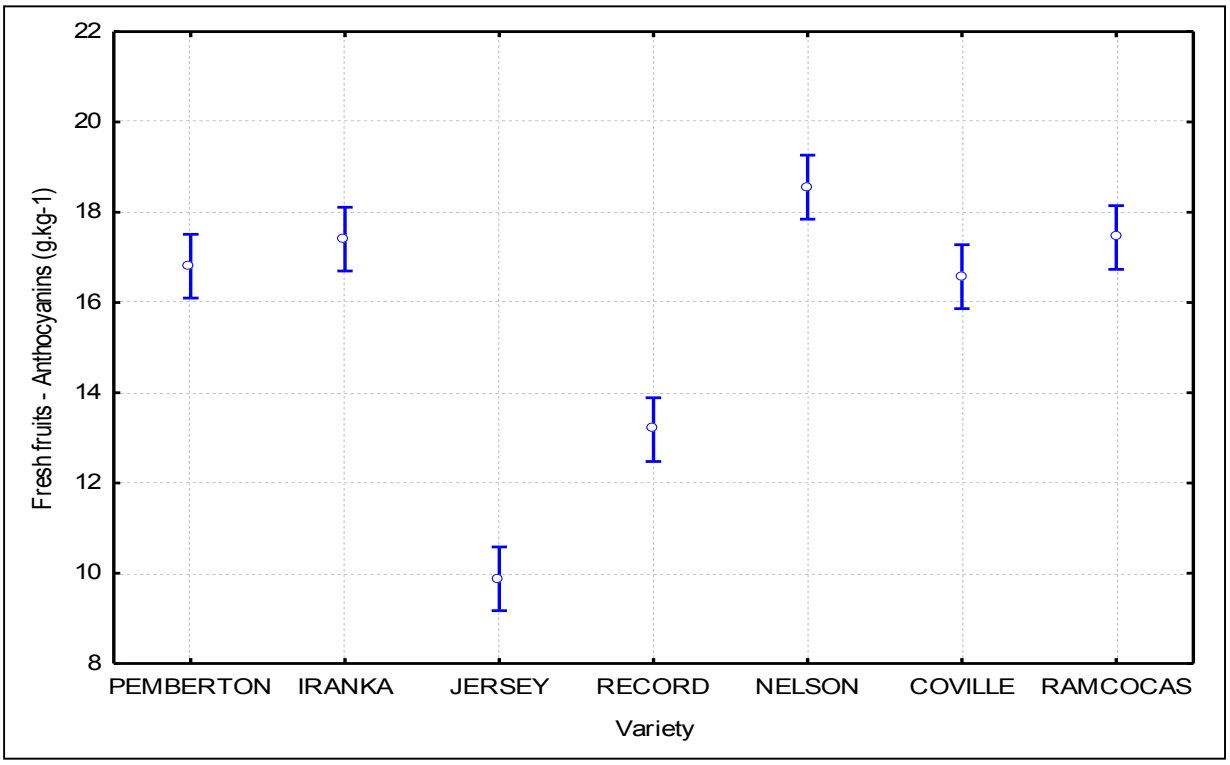

Figure 1 Comparison of average content of anthocyanins (g kg^{-1} dry matter) in fresh blueberry fruits

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Table 1 Mean content of anthocyanins (g kg^{-1} dry matter) in fresh blueberry fruits and blueberry jams

Fresh fruit		**Jam**	
variety	Anthocyanins (g kg^{-1} dry matter)	variety	Anthocyanins (g kg^{-1} dry matter)
Jersey	9.8781[a]	Jersey	1.6452[a]
Record	13.1826[b]	Coville	1.7861[b]
Coville	16.5724[c]	Record	2.1616[c]
Pemberton	16.8033[c]	Iranka	2.3298[d]
Iranka	17.4041[c]	Pemberton	2.6748[e]
Ramcocas	17.4393[c]	Nelson	2.8579[f]
Nelson	18.5553[d]	Ramcocas	3.4756[g]

They found a higher color in jams made from fruits of wild blueberries than in cultural varieties of fruits, but also point to the high nutritional and health quality jam made from various types and forms of blueberries.

Schmidt et al. (2005) observed antioxidant activity, polyphenol content and the content of anthocyanins in cultural fruits of large-fruited blueberries (*Vaccinium corymbosum* L.) and in wild fruit (*Vaccinium angustifolium* Ait.) in various products such as frozen fruits, jams, juices, concentrates, dried fruits and freeze dried. In both they found a decrease in selected parameters, in case it was used high temperature at processing technology. Similar findings state **Oliveira et al. (2010),** who show that the decomposition of anthocyans is running at 70°C.

Wrolstadt et al. (2005) in their work report decreases of anthocyanins compared blueberry jam to fresh fruit of 81-84%. Similar observations reached **Šavikin et al. (2009)**, who report a decrease in jam anthocyans up to 85%. **Pinto et al. (2007) and Brownmiller et al. (2008)** state that anthocyanins are very sensitive to temperature, and the combination of temperature - time can cause a significant loss of colorants in the finished product. Loss of monomeric anthocyans is mostly result of a consequence of polymers or condensation reactions between anthocyanins and procyanidins in the preparation of jams and also during storage of finished products.

The content of dry matter in the prepared blueberry jam ranged from 63.5 to 71.3%. The lowest values were at Nelson variety and the highest at Pemberton. By

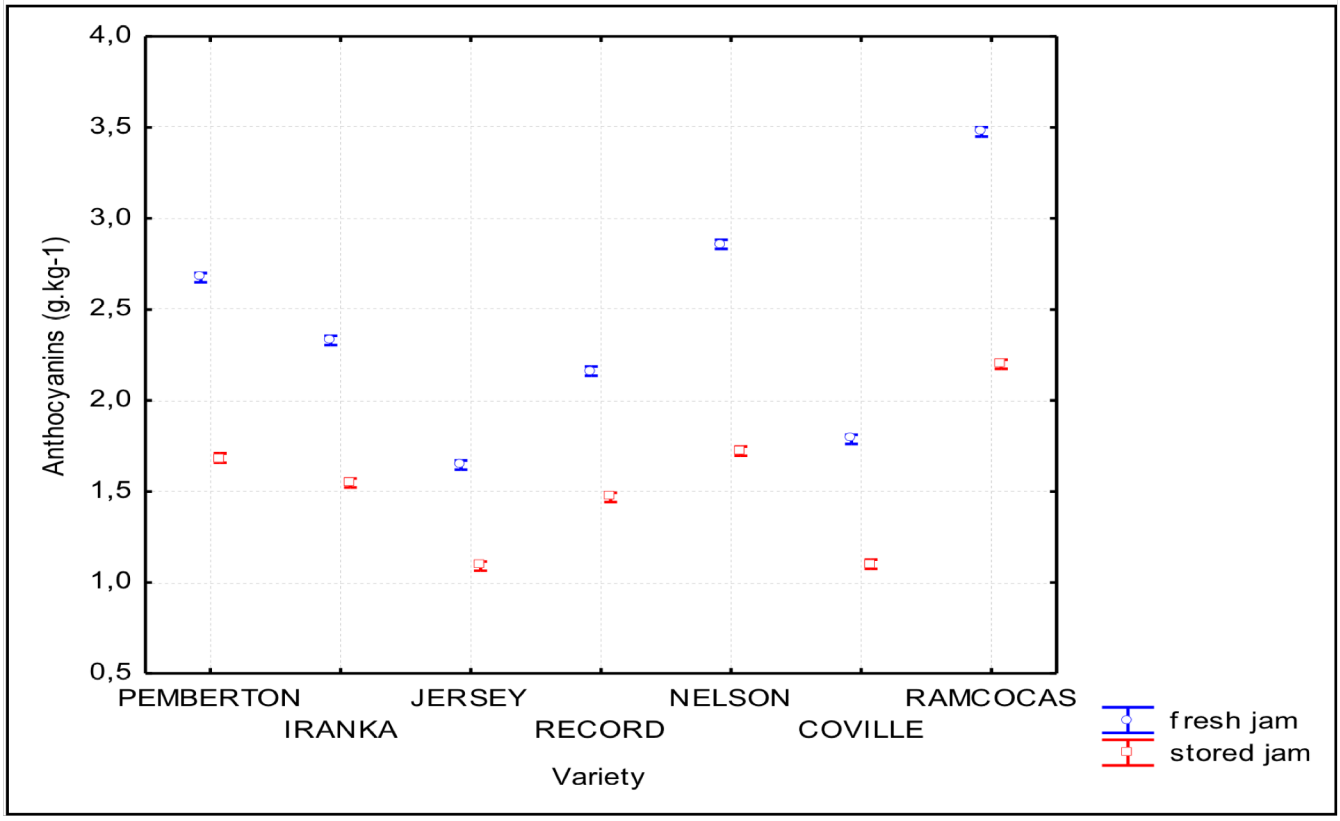

Figure 2 Comparison of mean content of anthocyanins (g kg^{-1} dry matter) in blueberry jam

Table 2 Statistical significance of the effect of variety and storage on the content of anthocyanins in blueberry jam by the two-factor analysis of variance

Source of variability	Sum of Squares	df	Mean Square	F
A:storage	8.03703	1	8.03703	422.97**
B:variety	9.37793	6	1.56299	82.26**
Error	0.646046	34	0.0190014	
Total	18.061	41		

** statistically significant at P<0.01; df - degree of freedom; n=42

decreasing levels of anthocyanins in jam after a dry matter conversion, observed varieties can be ordered as follows: Ramcocas > Nelson > Pemberton > Iranka > Record > Coville > Jersey (Table 1). By Fisher's LSD test we found that all jams made from different varieties statistically significantly differed in the content of anthocyanins.

The content of dry matter in the prepared blueberry jam ranged from 63.5% to 71.3%. The lowest amount was at Nelson variety and the highest at Pemberton. By decreasing the levels of anthocyanins in jam after a dry matter conversion, we can classify the varieties in the order: Ramcocas> Nelson> Pemberton> Iranka> Record> Coville> Jersey (Table 1). Fisher's LSD test showed that all jams made from different varieties are statistically significantly different in the content of anthocyanins.

The content of anthocyanins in blueberry jam after 6 months of storage ranged from 0.713 g kg^{-1} of fresh matter in variety Coville to 1.459 g kg^{-1} in variety Ramcocas. After a dry matter calculation, we determined the content of anthocyanins from 1.089 g kg^{-1} of dry matter in the jam of Jersey to 2.199 g kg^{-1} of dry matter in variety of Ramcocas jams (Figure 2). During storage of jam a further reduction of the content of anthocyanins occurs.

Decrease of anthocyanins in jam after storage ranged from 32.12% in jam of Record variety to 39.77% for variety Nelson. The average decrease of anthocyanins in blueberry jam during storage was 35.93%. **Häkkinen et al. (2000)** indicate that during the six months of storage jam contained 87% anthocyanins compared to the original amount of anthocyanins, which are higher than the values we found in our work.

Statistical processing of the data obtained on the content of anthocyans in blueberry jam and blueberry jam after storage showed statistically significant effect of variety from which jam is prepared and storage as well on the content of anthocyanins (Table 2).

Our findings also confirmed **Brownmiller et al. (2008),** who indicated that in jam the content of anthocyanins during storage decreases. They state that the jam without sugar preserves higher values of anthocyans than jam with

added sugar. Losses of anthocyans are smaller in jams stored at low temperatures. **Howard et al. (2010)** observed changes in the content of flavonoids and antioxidant activity in blueberry jam prepared with sugar and without sugar. The primary treatment caused a significant loss of anthocyans, polyphenols, chlorgenic acids, but flavonols were relatively well preserved. Jams were then evaluated after 6 months and it was found that jams, stored at 4°C maintained higher levels of anthocyanins, and have less color changes than jams, which were stored at 25°C.

CONCLUSION

Blueberry is valuable fruit crop, highly evaluated for the nutritional and sensory value. In our work, we evaluated the content of anthocyanins in selected varieties of blueberry (*Vaccinium corymbosum* L.) cultivated at the research station in Kriva na Orava. Fruits were evaluated as fresh and also as blueberry jams prepared, which were assessed immediately after treatment and after 6 - month storage at 21°C in the light. Our aim was to study the changes in anthocyanins that occur after processing and during storage. In terms of the content of anthocyanins in fresh fruits we consider the most valuable medium early variety Nelson.

After processing of fruits for jam we found that the products maintained on average 15.5% of colorants. The content of anthocyanins in blueberry jam ranged from 1.645 to 3.476 g kg^{-1} of dry matter. The highest content was found in the product made from variety Ramcocas. During storage of jam further loss of anthocyanins occurred, the average decrease was evaluated at 35.93%.

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