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ASSESSMENT OF THE BIOCHEMICAL QUALITIES AND COLOUR PARAMETERS OF FRESH AND FROZEN FRUITS OF HIGHBUSH BLUEBERRIES GROWN IN BULGARIA

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Abstract

In recent decades, the attention of scientists has been focused on the fruits of Vaccinium L. due to their significant potential to be used in the food, pharmaceutical, and cosmetic industries. In the present experiment, the biochemical composition of fruits of four introduced highbush blueberry varieties (Bluecrop, Bluegold, Spartan, Toro) grown in a demonstration plantation of RIMSA-Troyan was analyzed. The changes in the biochemical composition of fresh and frozen fruits after 1-year of storage at - 18°C (in refrigerated conditions) were compared. The fresh fruits of Bluegold are distinguished by a higher content of total and inverted sugar, ascorbic acid, tannins, anthocyanins, and pectin than the other studied varieties. In Bluecrop fruits, after one year of storage under refrigerated conditions, higher content of dry matter weight, ascorbic acid, tannins, and pectin were reported. The best results in terms of colour brightness were reported for fruits of the Spartan variety (22.68).

Key words: *blueberry, chemical composition, colour parameters, storage, Vaccinium corymbosum L.*

INTRODUCTION

In conditions of a quite polluted environment and irrational nutrition, humanity pays attention to the connection between the fruit diet and the preservation of human health. Fresh fruits have always had an essential role in the traditional diet and healthy menu of people. They are used for fresh consumption and processing, such as freezing, production of fruit juices and preserves, drying, and as an additive in sugar products. Blueberry fruits consist of balanced amount of sugars (invert and sucrose), organic acids, vitamins, tannins, pectin, anthocyanins, polyphenols, etc., which are used in the food, pharmaceutical and medical industries (Hung et al., 2004; Puupponen-Pimiä et al., 2005; Kalt et al., 2007; Szajdek & Borowska, 2008; Petronelli et al., 2009; Gilbert et al., 2014; Lin et al., 2016; Correia et al., 2017; Shi et al., 2017; Dare et al., 2022; Ferrão et al. 2022; Felipe et al., 2022, Bai et al., 2023; Antal, 2024). Their biochemical composition and sensory evaluation are not constant, but they change depending on the variety, ripeness stage, abundance of the harvest, applied agrotechnical events, the influence of soil and climatic conditions in the growing region, altitude, etc. (Skupień, 2006; Bryla & Strik, 2007; Leposavić, 2014; Gilbert et al., 2015; Solovchenko et al., 2019; Spinardi, 2019; Niedbała et al., 2022).

The present study aims to evaluate the biochemical composition (qualitative characteristics) and colour parameters in fresh and frozen (-18°C) fruits of the genus *Vaccinium*.

MATERIALS AND METHODS

Fruits of four highbush blueberry varieties (Bluecrop - standard, Bluegold, Spartan, and Toro) were gathered from a demonstration plantation of RIMSA over three years.

The planting scheme is 3.00 m x 1.50 m, as inter and intra-row spacings are with natural grass cover. The area around the stems is maintained in black fallow using tillage. The collection plantation is located 474 m above sea level, on an eastern exposure slope. Drip irrigation was provided during the vegetation.

The heaviest rainfall in the period from March to July in the region was registered in the 2020 vegetation season (346 l/m^2), whereas the lowest amount was in 2021 (232.1 l/m^2). In

2022, the vegetation sum of average monthly rainfall (from March to July) was 253.3 l/m^2. In the last two years of the experimental period, the lowest reported vegetation precipitation amount for the last five years was reported (Atanasova, 2021).

The average monthly temperature sum from March to July does not differ significantly in the research period and is from 13.9°C (2020) to 14.3°C (2022).

The relative humidity for vegetation season from March to July within the three years is in the range of 69-81%, as the lowest was in 2022.

The varieties included in the study are distinguished by growth habitus, fruit ripening period, and resistance to abiotic and biotic stress factors.

Ripe fruits were picked by hand and put in polythene boxes. The following biochemical indicators of fresh and frozen fruits were observed (after storage one year of storage at -18°C in laboratory freezer, model Indesit):

- dry matter (DM) (%), total soluble solids (TSS) and sugars (total, invert and sucrose %) had been made, according to the methods described by Donchev et al., 2001;
- titratable acidity (malic acid %), ascorbic acid (mg %) and tannins substances (%) had been made according to the method of Donchev et al., 2000, using 1: 4 H2SO4, 0.1 N KM4O4 and indigo carmine as indicator for tannins quantification;
- total anthocyanins (mg %) was made using the method of Fuleki & Francis (1968);
- pectin according to the method of Melitz (Donchev et al., 2000) has been performed, using 0.1 N NaOH, CH3 COOH, CaCl 2, AgNO3;
- total polyphenols (mg/100 g) has been made using the method of Folin-Ciocalteu, (Singleton & Rossi, 1965).

Colour parmeteres

The indicators were reported according to the CIE Lab system. The colour coordinates L, a, and b were measured: (L) – colour brightness; (+a) - red; (-a) – green; (+ b) – yellow; (- b) – blue. The colour analysis has been made with Color meter CM-200S.

The colour tone value or dominant wavelength is represented by the ratio a/b.

The data were statistically processed by the software product MS Excel – 2010.

RESULTS AND DISCUSSIONS

Tables 1, 2, and 3 present the results for the main biochemical composition of four blueberry varieties, including dry weight matter, total soluble solids, total sugars, inverted sugar, sucrose, malic acid, ascorbic acid, tannins, anthocyanins, pectin and total polyphenols. Our study shows differences in some quality indicators among varieties.

Table 1. Biochemical composition of fruits of some highbush blueberry varieties, 2022 harvest

Indicators / Varieties	D.M (%)	TSS (%)	Total sugars (%)	Inverted sugar (%)	Sucrose (%)	Acids (as malic) (%)	Ascorbic acid (mg %)	Tannins (%)	Total anthocyanins (mg/%)	Pectin (%)	Total polyphenols mg/100 g
Bluecrop	18.74	14.00	8.90	8.90	0	0.20	17.60	0.219	20.32	1.02	229.73
Bluegold	17.38	12.50	16.70	14.30	2.28	0.27	21.12	0.263	49.03	1.51	197.31
Spartan	14.63	10.00	12.10	11.45	0.62	0.27	17.60	0.197	42.26	0.39	149.19
Toro	16.10	9.50	8.35	5.70	2.52	0.27	12.32	0.197	27.42	0.72	166.88
Mean	16.71	11.50	11.51	10.09	1.36	0.25	17.16	0.22	34.76	0.91	185.78
SE	0.88	1.06	1.92	1.83	0.62	0.02	1.81	0.02	6.60	0.24	17.70
St Dev	1.76	2.12	3.83	3.66	1.24	0.04	3.63	0.03	13.19	0.48	35.41
CV	10.52	18.45	33.30	36.32	91.28	13.86	21.14	14.21	37.96	52.26	19.06
Amplitude min - max	14.63-18.74	9.5-14	8.35-16.7	5.7-14.3	0-2.52	0.2-0.27	12.32-21.12	0.197-0.263	20.32-49.03	0.39-1.51	149.19-229.73

The comparative analysis shows that the dry matter weight in the fresh fruits is from 14.63% (Spartan) to 18.74% (for Bluecrop), with a low coefficient of variation of 10.52%.

The highest content of TSS was reported for Bluecrop with 14.00% and Bluegold with 12.50%. The average value is 11.50%, with an average coefficient of variation of 18.45%.

The content of carbohydrates in blueberry fruits includes various types of sugars (total, inverted sugar, and sucrose), which are quickly absorbed by the human body and are an indispensable energy source (Mondeshka, 2005).

The variation in the content of total sugars is significant. The highest content is reported for Bluegold with 16.70%, and Toro with 8.35%, whereas the lowest content is observed in Bluecrop with 8.90%. The average value of the indicator for varieties is 11.50%, with an average coefficient of variation of 33.30%. A similar coefficient of variation (36.32%) was also reported for inverted sugar. Bluegold has the highest content with 14.30%, whereas Toro shows the lowest with 5.70%. The average indicator among varieties is 10.09%. No sucrose was reported in Bluecrop, as it is from 0.62% (Spartan) to 2.52% (Toro), with a very high coefficient of variation.

In agreement with the present results, Leposavić, (2014) analyzed the main biochemical composition of the fruit of five blueberry genotypes (Bluecrop (control), Duke, Reka, Nui, and Ozarkblue) and found the highest amount of soluble dry matter, total and reducing sugars in the fruit of Ozarkblue, least of all in those of Nui.

It is a well-known fact that fruit acids are associated with accelerating the metabolism and increasing the defenses of the human body. In the present paper, they are represented by malic and ascorbic acid (Mondeshka, 2005). The established organic acids were in close values among the varieties in the range of 0.2-0.27%. The highest ascorbic acid content was found in Bluegold (21.12 mg/%), whereas the lowest was in Toro (12.32 mg/%). The average value of the indicator is 17.16%, with an average coefficient of variation of 21.14%.

The astringent taste of blueberry fruits is due to the content of tannins, which have anti-inflammatory properties for colds (Mondeshka, 2005). The largest amount was registered in Bluegold (0.263%) and Bluecrop (0.219%) varieties (VC-14.21%).

The highest anthocyanin values were observed in Bluegold (49.03 mg/%) and Spartan (42.26 mg/%), whereas the lowest were in Bluecrop (20.32 mg/%). The average content of anthocyanins is 34.76 mg/%, with a very high coefficient of variation (37.96%).

The highest pectin level was reported in Bluegold (1.51%), followed by Bluecrop (1.02%), and the lowest was in Spartan (0.39%).

Total polyphenol values are from 149.19 mg/100 g (Spartan) to 229.73 mg/100 g (Bluecrop). The average value of the indicator is 185.78%, with an average coefficient of variation of 19.06%.

Similar to our results were obtained by Skupień (2006), who compared the main biochemical composition of four highbush blueberry cultivars (Spartan, Bluecrop, Jersey, and Blueray) grown in an orchard in the Szczecin region. The author found that the fruits of Bluecrop had the highest total content of polyphenols, and those of Blueray were distinguished by the highest amount of total acids. The lowest total content of total polyphenols was analyzed in Jersey berries.

Frozen Fruits

Freezing fruits is one of the most practical methods of preservation and storage of their valuable biochemical components. The species and varietal specificity, agrotechnical practices, duration, and storage conditions are of primary importance for their preservation (Lohachoompol et al., 2004; Poiana et al., 2010; Celli et al., 2015; Stamenković et al., 2019; Žlabur et al., 2021; Wang et al., 2024;)

The changes that occurred in the biochemical composition of blueberry fruits after one year of storage at -18°C are presented in Table 2

The dry matter weight of the frozen fruits is from 15.84% (Toro) to 19.39% (Bluecrop), with a low coefficient of variation (CV-8.91%). More significant differences were observed compared to fresh fruit in the Spartan variety with approximately four units more in frozen fruit.

Table 2. Biochemical composition of frozen fruits of some highbush blueberry varieties, after 1 year of storage at -18°C

Indicators / Varieties in 2022	D.M (%)	TSS (%)	Total sugars (%)	Inverted sugar (%)	Sucrose (%)	Acids (as malic) (%)	Ascorbic acid (mg %)	Tannins (%)	Total anthocyanins (mg/%)	Pectin (%)	Total polyphenols mg/100 g
Bluecrop	19.39	13	3.70	3.70	0	0.13	10.56	0.109	4.52	0.66	247.37
Bluegold	16.73	12	2.85	2.85	0	0.13	10.56	0.088	13.55	0.42	162.75
Spartan	18.13	13.50	4.05	4.05	0	0.20	8.80	0.088	1.94	0.20	238.24
Toro	15.84	11.50	3.20	3.20	0	0.27	8.80	0.066	1.29	0.20	336.99
Mean	17.52	12.50	3.45	3.45	-	0.18	9.68	0.09	5.33	0.37	246.34
SE	0.78	0.46	0.27	0.27	-	0.03	0.51	0.01	2.83	0.11	35.67
St Dev	1.56	0.91	0.53	0.53	-	0.07	1.02	0.02	5.66	0.22	71.35
CV	8.91	7.30	15.38	15.38	-	36.72	10.50	20.01	106.25	59.30	28.96
Amplitude min - max	15.84-19,39	11.5-13.5	2.85-4.05	2.85-4.05	-	0.13-0.27	8.8-10.56	0.066-0.109	1.29-13.55	0.2-0.66	162.75-336.99

The highest level of refractometric solids is found in Spartan (13.50%) and Bluecrop (13.00%). Their increase is more significant again in Spartan (13.50%), compared to their amount in fresh fruit. The average value of the indicator is 12.50%. A significant decrease is reported in the total sugars of frozen fruit, compared to the fresh ones, which are from 2.85% (Bluegold) to 4.05% (Spartan) (CV-15.38%).

A decrease in the amount of inverted sugar was registered and no sucrose was detected for all frozen fruits.

No significant changes were observed in the values of organic acids compared to those of fresh fruit. A decrease in the ascorbic acid content was reported, compared to those in fresh fruit, which was from 8.80 mg/% (for Spartan and Toro) to 10.56 mg/% (for Bluecrop and Bluegold), with a coefficient of variation of 10.50%.

A strong decrease in the content of tannins compared to the starting raw material was reported, with a high coefficient of variation - 20.01%. The lowest amount is reported for Toro with 0.066%, and the highest for Bluecrop with 0.109%.

The decrease of anthocyanins in frozen fruits is several times and with a very high coefficient of variation, with the lowest values reported for Toro - 1.29 mg/%, Spartan - 1.94 mg/%, and Bluecrop - 4.52 mg/%, and the highest is that of Bluegold - 13.55 mg/%.

A decrease in the pectin level was observed, as it was most pronounced in Bluegold with 0.42%, compared to fresh fruit. Its content is from 0.20% (Spartan and Toro) to 0.66% (Bluecrop).

Regarding the total polyphenols, a decrease in their amount in the frozen fruit was observed only in Bluegold (162.75 mg/100 g), compared to fresh fruit. The most significant increase was found in Toro (336.99 mg/100 g) and Spartan (238.24 mg/100 g), compared to the starting material (VC - 28.96%). The average value of total polyphenols in the varieties is 246.34 mg/100 g.

Comparing the brightness of the color of the blueberry varieties studied, the highest value was reported for the fruits of the Spartan variety (22.68) and the lowest for Bluegold (8.87). The average value for the varieties is 16.78 (Table 3).

The lowest values for the red colour component were measured in Toro (0.50) and the highest in Bluegold (5.97).

For the established blue colour component, the best results were reported for the Bluegold variety and the lowest values for the Bluecrop.

The quality indicator of colour tone has the highest value for fruits of the Spartan variety (15.09).

Table 3. Colour parameters of frozen fruits of different highbush blueberry varieties

Colour parameters / Varieties	L	a	b	a/b
Bluecrop	19.15	1.54	- 1.60	-5.24
Bluegold	8.87	5.97	- 0.11	-60.46
Patriot	15.94	5.05	- 0.93	-0.31
Spartan	22.68	4.20	- 0.61	15.09
Toro	17.28	0.50	- 0.85	-0.75
Mean	16.78	3.45	-0.82	-10.33
SE	2.28	1.04	0.24	12.99
St Dev	5.10	2.34	0.54	29.06

CONCLUSIONS

The obtained results of the biochemical analyses show that the valuable components in the fruits of highbush blueberries are preserved to a significant extent even after freezing at -18 ^{0}C for one year. The reported differences in biochemical composition and colour parameters are probably due to the diverse soil and climatic conditions during the vegetation combined with the biological characteristics of the varieties within the experimental period.

Fresh Bluegold berries had a higher content of total and inverted sugar, ascorbic acid, tannins, anthocyanins, and pectin of all the varieties studied.

After one year of storage, Bluecrop fruits had a higher content of dry matter weight, ascorbic acid, tannins, and pectin, whereas the Spartan fruits had a higher content of refractometric solids, total and inverted sugar.

Biologically active substances, ascorbic acid, and anthocyanins are best preserved in Toro and Bluegold fruits, respectively, whereas total polyphenols are in Toro.

The Spartan variety is distinguished by the highest values for brightness and color tone of the fruits, and for the red and blue color components those of Bluegold stand out.

The comprehensive analysis of fresh and frozen blueberry fruit can be used in future studies to incorporate health-promoting compounds of fruit into healthy foods, with potential benefits for human health.

REFERENCES

Antal T. (2024). The effect of refrigeration and room temperature storage conditions on the physico-chemical characteristics of hybrid and freeze-dried blueberries. *Journal of Agriculture and Food Research*, *16*, 101083.

Atanasova, S. (2021). Behavior of *in vitro* raspberry plants grown under *in vivo* conditions. PhD thesis, 48–56.

Bai X., Zhou L., Zhou L., Cang S., Liu Y., Liu R., Liu J., Feng X., & Fan R. (2023). The research progress of extraction, purification and analysis methods of phenolic compounds from blueberry: a comprehensive review. *Molecules*. 28(8): 3610.

Bryla, D.R. & Strik, B.C. (2007). Effects of cultivar and plant spacing on the seasonal water requirements of highbush blueberry. *J. Amer. Soc. Hort. Sci.* 132:270–277.

Celli, G.B., Ghanem, A., & Brooks, M.S.-L. (2015). Influence of freezing process and frozen storage on the quality of fruits and fruit products. *Food Rev. Int. 32*, 280–304.

Correia, R., Grace, M. H., Esposito, D., & Lila, M. A. (2017). Wild blueberry polyphenol-protein food ingredients produced by three drying methods: Comparative physico-chemical properties, phytochemical content, and stability during storage. Food Chemistry, 235, 76–85.

Dare, A.P., Günther, C.S., Grey, A.C., Guo, G., Demarais, N.J., Cordiner, S., McGhie, T.K., Boldingh, H., Hunt, M., Deng, C., et al. (2022). Resolving the developmental distribution patterns of polyphenols and related primary metabolites in bilberry (*Vaccinium myrtillus*) fruit. *Food Chem.*, *374*, 131703.

Donchev, Hr., Zlateva D, & Pashova S. (2000). Food science, laboratory exercise guide. Part Two, Varna, BG: University of Economics Publishing House, 61-63.

Donchev, Hr., Zlateva D, Pashova S., & Ivanov A. (2001). *Food science, laboratory exercise guide*. Part First, Varna, BG: University of Economics Publishing House, 103-106.

Felipe, L., Ferrão, V., Sater, H., Lyrene, P., Amadeu, R.R., Sims, Ch.A., Tieman, D.M., & Munoz, P.R. (2022). Terpene volatiles mediate the chemical basis of blueberry aroma and consumer acceptability. *Food Research International*, Vol. 158, 111468.

Ferrão, L. F. V., Sater, H., Lyrene, P., Amadeu, R. R., Sims, C. A., Tieman, D. M., & Munoz, P. R. (2022). Terpene volatiles mediate the chemical basis of blueberry aroma and consumer

acceptability. *Food Research International*, *158*, 111468.

Fuleki T., & Francis F.J. (1968). *Journal of food science, 33*(5), 471.

Gilbert, JL., Olmstead, JW., Colquhoun TA., et al. (2014). Consumer-assisted selection of blueberry fruit quality traits. *HortScience*, *49*: 864–73.

Gilbert, J.L., Guthart, M.J., Gezan, S.A., Carvalho, M.P. de Schwieterman, M.L., & Colquhoun, T.A. (2015). Identifying breeding priorities for blueberry flavor using biochemical, sensory, and genotype by environment analyses, *PLoS ONE*, 10 (9).

Hung, H.C., Joshipura, K.J., & Jiang, R. (2004). Fruit and vegetable intake and risk of major chronic disease. *J. Natl. Cancer Inst*., *96*, 1577–1584.

Kalt, W., Joseph, J. A., & Shukitt-Hale, B. (2007). Blueberries and human health. A review of the current research. *Journal of the American Pomological Society*, *61*: 151–160.

Leposavić A., (2014) Pomological characteristics of newly introduced varieties of highbush blueberry (*Vaccinium corymbosum* L.). PhD Thesis, 109-117.

Lin, D., Xiao M., Zhao J., Li, Z, Xing, B., Li, X., Kong, M., Li, L., Zhang, Q., Liu, Y., et al. (2016). An Overview of Plant Phenolic Compounds and Their Importance in Human Nutrition and Management of Type 2 Diabetes. *Molecules*, *21*(10):1374.

Lohachoompol, V., Srzednicki, G., & Craske, J. (2004). The change of total anthocyanins in blueberries and their antioxidant effect after drying and freezing. *J. Biomed. Biotechnol.*, 248–252.

Mondeshka (2005). Medicinal fruit plants, Sofia, BG: Zemizdat, 72-91.

Niedbała, G., Kurek, J., Świderski, B., Wojciechowski, T., Antoniuk, I., & Bobran, K. (2022). Prediction of Blueberry (*Vaccinium corymbosum* L.) Yield Based on Artificial Intelligence Methods. *Agriculture*, *12*(12), 2089.

Petronelli, A., Pannitteri, G., & Testa, U. (2009). Triterpenoids as new promising anticancer drugs. *Anti-Cancer Drug*. 20:880-92.

Poiana, M.A., Moigradean, D., Raba, D., Alda, L.M., & Popa, M. (2010). The effect of long-term frozen storage on the nutraceutical compounds, antioxidant properties and color indices of different kinds of berries. *J. Food Agric. Environ.*, *8*, 54–58.

Puupponen-Pimiä, R., Nohynek, L., Alakomi, H.L., Oksman-Caldentey, K.M. (2005). The action of berry phenolics against human intestinal pathogens. *BioFactors*, *23*, 243–251.

Shi, M., Loftus, H., McAinch, A.J., & Su, X.Q. (2017). Blueberry as a source of bioactive compounds for the treatment of obesity, type 2 diabetes and chronic inflammation. *J. Funct. Foods*, *30*, 16–29.

Singleton, V.L. & Rossi, J.A., Jr. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture,* 16, 144–158.

Skupień, K. (2006). Chemical composition of selected cultivars of highbush blueberry fruit (*Vaccinium corymbosum* L.). *Folia horticulturae*, 18(2), 47–56.

Solovchenko, A., Yahia, E. M., &Chen, Ch. (2019). Postharvest physiology and biochemistry of fruits and vegetables, Chapter 11 – Pigments, 225-252.

Stamenković, Z., Pavkov, I., Radojčin, M., Horecki, A.T., Kešelj, K., Kovačević, D.B. & Putnik, P. (2019). Convective drying of fresh and frozen raspberries and change of their physical and nutritive properties. *Foods*, *8*, 251.

Szajdek, A. & Borowska, E.J. (2008). Bioactive compounds and health-promoting properties of berry fruits: a review. *Plant Foods Hum. Nutr.*, *63*, 147–156.

Žlabur, JŠ., Mikulec, N., Dožđor, L., Duralija, B., Galić, A., &Voća S. (2021). Preservation of biologically active compounds and nutritional potential of quick-frozen berry fruits of the genus *Rubus*. *Processes*. 9(11):1940.

Spinardi, A., Cola, G., Gardana, C.S., & Mignani, I. (2019). Variation of anthocyanin content and profile throughout fruit development and ripening of highbush blueberry cultivars crown at two different altitudes. *Front Plant Sci.,* Sep 4; 10:1045.

Wang, C., Zhang, S., Han, Y., Li, L., Ban, Z., & Lu, P. (2024). γ irradiation mediated the inhibitory effect of Ca2+ signals on softening of blueberry fruit during cold storage. *Food Bioscience*, 57, 103513.