

## EVALUATION OF BLUEBERRY FRUITS AND JUICE OF SOME CULTIVARS GROWN IN PITEȘTI - MĂRĂCINENI, ROMANIA

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### Abstract

*Highbush blueberry is a popular berry with an attractive flavour and colour. It provides health benefits due to an important number of bioactive compounds with antioxidant, antitumor, antimutagenic, and antidiabetic effects, as well as to its ability to prevent cardiovascular disease. Blueberry juice is one of the most widespread blueberry products and it is demanded by consumers since it is tasty and it preserves most of the nutrients. The aim of this study is the evaluation of the blueberry fruits and juice of some cultivars grown at the Research Institute for Fruit Growing Pitești - Mărăcineni, Romania. Since heat processing affects the nutritional and sensory properties, blueberry juices have been prepared by cold pressing the fruit. In order to inhibit the development of the fermentation microbiota and improve the organoleptic characteristics of blueberry juices, the samples of each juice were prepared in a 1:1 (w:w) mixture with acacia honey. Total tannin content (TTC) and total flavonoid content (TFC) were periodically determined for all juices. The results revealed very significant effects of the three factors (genotype, storage period and sweetener) on TTC and TFC, with acacia honey showing the lowest effect size.*

**Key words:** highbush blueberry, total tannins content (TTC), total flavonoids content (TFC.)

### INTRODUCTION

Analysis of public health shows an alarming increase in the number of people who suffer from or are prone to cardiovascular diseases, cancer, disorders of lipid metabolism (increased cholesterol and blood sugar, weight gain), allergic diseases, or various nervous system diseases (Hera et al., 2023; Mazilu et al., 2022). Nutritionists strongly recommend a diet rich in fruits and vegetables so as to prevent premature aging, metabolic diseases, and even to fight obesity (Lavéffe et al., 2020). Since fruits and vegetables are seasonal and perishable, they are transformed into functional foods so that they can keep their nutritional value closer to that of fresh products while being available beyond the harvest season (Slavin and Lloyd, 2012).

Among these, the blueberry juice is one of the most widespread blueberry products, and it is demanded by consumers since it is tasty and it preserves most of the nutrients (Tobar-Bolaños et al., 2021).

Blueberries are a valuable crop that can thrive in acidic, poorly drained sandy soils, which may otherwise be considered unsuitable for agricultural production. North America is the primary producer of blueberries.

Cultivated blueberries belong to the *Cyanococcus* section of the *Vaccinium* genus in the heath family *Ericaceae* (Ballington et al., 1997). Species within this section are often referred to as the "true" or cluster-fruited blueberries (Camp, 1945). Wild *Cyanococcus* species are exclusively found in North America (Hancock and Draper, 1989).

Blueberry species are commonly classified as lowbush, highbush, and rabbiteye types based on their stature. Lowbush plants are rhizomatous, with stems ranging from 0.3 m to 0.6 m. Highbush plants are crown-forming shrubs and they are generally 1.8-2.5 m tall. Rabbiteye plants are also crown-forming shrubs; normally, they are 2-4 m tall (Ballington et al., 1997). Among major fruit crops, blueberries have been domesticated recently, during the 20th century.

The berries that cannot be sold on fresh markets are used to make juices, which decreases waste and allows the obtaining of some blueberry products that can still maintain the flavour and nutrients of the fruit (Bates et al., 2001).

The blueberry juice is a popular product demanded by consumers for its appealing taste and ability to preserve the nutrients from fresh fruit (Hotchkiss et al., 2021). Due to its high level of anthocyanins and other antioxidant compounds like polyphenols, vitamins C and E, the blueberry juice is recommended to maintain human health (Nindo et al., 2005; Tobar-Bolaños et al., 2021). It can be made from fresh or frozen berries, using a juice squeezer. Since blueberries have a balanced composition in sugars and acids (Hera et al., 2023), the preparation of blueberry juices does not require the dilution with water or the addition of sugar or other sweeteners. The abundance of blueberry juice in bioactive phenolic compounds is correlated with the synthesis of secondary metabolites by the plant, and with the processing juice technology (Brambilla et al., 2008).

The impact of juice processing on fresh berry bioactive compounds content has been studied and the results showed a detrimental effect of the processing technology of juice explained through oxidative degradation because of tissue disorganization (Lee et al., 2002; Skrede et al., 2000). It has been proven that the steam-blanching of berries before milling is effective in improving stability and in the recovery of the phenolic bioactive compounds (Rossi et al., 2003). It seems that blanching acts by inactivating the oxidative enzymes of the processed fruits and by physically improving the permeability of the pigmented pericarp cells (Pizzocaro et al., 1988). The juice needs to be clarified, filtered, and pasteurized to ensure that it becomes a microbiologically stable food product with an appealing colour, a quality highly valued by consumers (Girard and Sinha, 2006; Siddiq et al., 2018; Song et al., 2018).

It is in our interest to improve knowledge as regards the bioactive compounds in raw fruit and to preserve these compounds in the end-product. To this end, four blueberry cultivars were grown in the same environmental conditions (at the Research Institute for Fruit Growing Pitești - Mărăcineni), and they were

individually processed into juice after an initial blanching step, with a special focus on the cultivar influence upon the phenolic content of the juice. To preserve the organoleptic characteristics and inhibit the fermentation microbiota, simple juices and samples mixed 1:1 (w:w) juice with acacia honey were prepared from the fruits of each variety. Total tannin content (TTC) and total flavonoid content (TFC) were periodically determined for all juices.

## MATERIALS AND METHODS

### Chemicals and Reagents

All the chemicals and reagents were purchased from Merck, Darmstadt, Germany.

### Plant material

The study was carried at the Research Institute for Fruit Growing in Pitești-Mărăcineni (RIFG) in southern Romania, located at 44°54'12" north latitude, and 24°52'18" east longitude, with an altitude of 284 meters.

The study included the following genotypes:

(1) two American cultivars:

- 'Duke' obtained from crossing ('Ivanhoe × Earliblue') with 192-8 (E-30 × E-11),
- 'Northblue' obtained from crossing *Vaccinium corymbosum* with *Vaccinium angustifolium*,

(2) two Romanian advanced selections:

- '4/6' open-pollinated 'Spartan',
- '6/38' obtained from crossing 'Pemberton' with 'Bluecrop'.

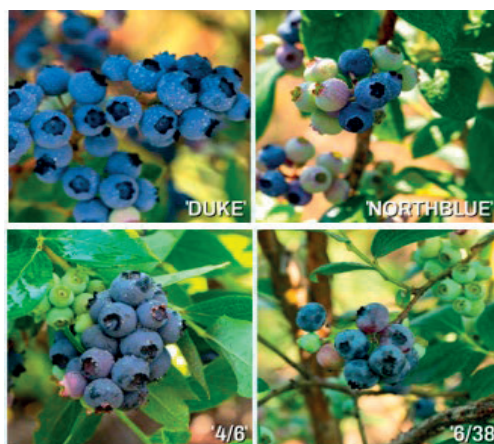


Figure 1. The blueberry genotypes

Acacia honey was produced by bee populations of the *Apis mellifera Carpatica* race in a small

apiary of Southern Romania (Costesti, Arges). Physicochemical analysis of some honey samples (Vijan et al., 2023) showed the acacia honey had a safe moisture content (14.36%) for storage and consumption, an average level of ash (0.14%), free acidity (7.50 mEq/kg) and sugar (63.79 g glucose/100 g), the lowest 5-hydroxymethylfurfural (16.07 mg/kg), phenolic content (90.18 mg GAE/100 g), and flavonoid content (11.83 mg CE/100 g), but a high level of tannins (62.73 mg GAE/100 g).

#### **Fruit quality parameters**

The average fruit weight was determined by weighing a sample of 50 fruits from each repetition at each harvest, using a scale (in grams). After the harvesting work, depending on the ripening period of each genotype, a weighted-average weight was calculated.

The length and diameter of the fruit were determined by measuring the fruit with a digital calliper. The size index of the fruit was calculated as the ratio of these two dimensions (Titirică et al., 2023).

The total soluble solids content was measured using a digital refractometer Haana Instruments 96801, and values were recorded in °Brix.

The fruit firmness was assessed non-destructively using a Bareiss HPE II Fff penetrometer for each sample.

#### **Blueberry juice preparation and treatments**

Fully mature and good-quality blueberries were picked from Mărăcineni, Romania. After washing with distilled water, the fruits were poured into a motorized juice extractor (Midea WJE2802D, Midea, Guiyang, China) for about 10 s to obtain the juice. Afterwards, the juice was filtered by a sterile muslin cloth, necessary to provide uniform consistency. The extracted juice was separated into two, depending on the two treatments to which it was exposed: simple juices obtained without adding honey and juices to which acacia honey was added to improve the organoleptic characteristics of the juice and inhibit the fermentation microbiota.

The blueberry juice samples were kept at 4°C throughout the current study, in hermetically sealed glass containers. The samples were analysed at 5 different time points: I (immediately after obtaining the juice), II (after one month), III (after 3 months), IV (after 4 months), and V (after 6 months).

#### **Blueberry juice biochemical parameters**

The total tannins content (TTC) was measured using the method recommended by Matic et al., 2017. This involved the reaction of tannins with phosphotungstic acid in an alkaline medium, resulting in the formation of a blue-coloured compound. The newly formed compound has maximum absorption at 760 nm. The results were reported as mg gallic acid equivalent (GAE) per 100 g fresh weight (FW). The total flavonoid content (TFC) was measured using the method proposed by Giosanu et al., 2016. Flavonoids reacted with aluminium chloride to form a yellow-orange compound which exhibited maximum absorption at 510 nm. The findings were reported as mg catechin equivalent (CE) per 100 g fresh weight (FW).

#### **Statistical Analysis**

The analyses were conducted in triplicate, and the data were presented as mean  $\pm$  standard deviation (SD). Excel 2021 (XLSTAT) was used for the statistical analysis of the data. One-way analysis of variance (ANOVA), two-way and three-way ANOVA (factorial ANOVA), and Duncan's multiple range tests were carried out.

## **RESULTS AND DISCUSSIONS**

#### **Biometric characteristics of the fruit**

The main biometric characteristics of the blueberry fruit refer to the size, shape, and texture of the pulp, and are expressed through: average weight, size index, and firmness. Significant differences have been observed between genotypes as regards the above-mentioned biometric indicators. The average fruit weight is a genetically determined characteristic, influenced by technical and cultural conditions and it has recorded different values in the current study.

Figure 2 shows that the values for average berry weight oscillated between 1.73 g for 'Northblue' to 2.94 g for '6/38'.

Regarding the size index, no significant differences were recorded between genotypes. The fruit firmness is a crucial parameter for fresh blueberries because it affects the quality and the post-harvest storage potential.

In horticultural studies, the fruit firmness is also referred to as a textural or mechanical quality that can indicate differences in fruit ripeness or horticultural product quality (Abbot, 1999; Musacchi and Serra, 2018). The firmest fruits were harvested from '4/6' and 'Duke'.

The fruit ripening is influenced by total soluble solids, which also affects the nutrient absorption and economic outcomes in fruit trade (Li et al., 2016). The total soluble solids oscillated between 15.9°Brix for 'Northblue' to 8.83°Brix for '6/38'.

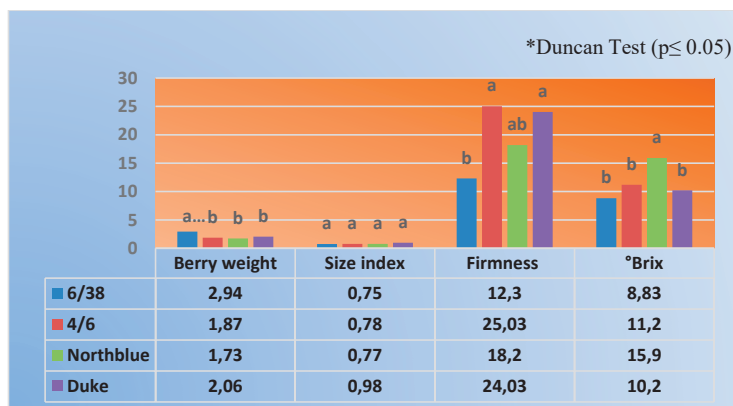


Figure 2. The biometrical parameters of blueberry genotypes  
 Note: \*Different letters between cultivars denote significant differences (Duncan test.  $p < 0.05$ );  
 Different letters between susceptible and resistant cultivars denote significant differences (LSD test.  $P < 0.05$ )

### Juice's Total Tannins Content

The bioactive substances in blueberries, particularly polyphenols and anthocyanins, are subject to various post-harvest factors that influence their content and functionality in different ways (Michalska and Łysiak, 2015). It has been observed that tannins accumulation continues in overripe berries and it may even increase after storage. This is due to the outer layer which by losing firmness, it allows water

to evaporate more quickly (Kalt et al., 2001). The blueberry juice contains multiple phenolic compounds which makes it a potent solution that manages to enhance the nutritional value of this food (Seraglio et al., 2023). In all the studied genotypes, the content of tannins (TTC) in the juice has increased from the first chemical analysis on August 1, 2023, to the last one on February 1, 2024 (Figures 3 and 4).

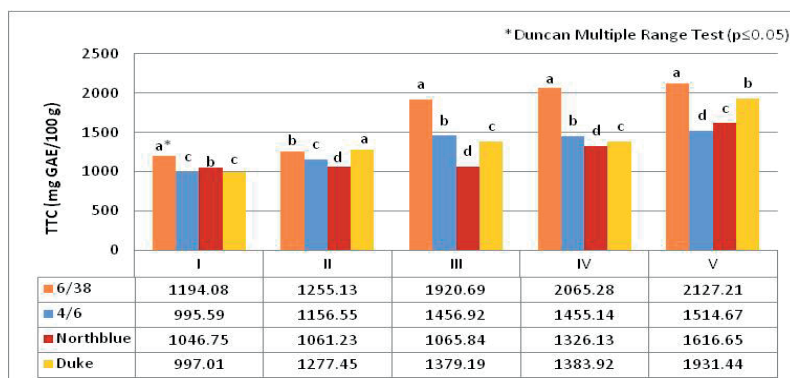


Figure 3. The influence of genotype on TTC depending on the storage period in blueberry juices without honey  
 Note: \*Different letters between cultivars denote significant differences (Duncan test.  $p < 0.05$ );  
 Different letters between susceptible and resistant cultivars denote significant differences (LSD test.  $P < 0.05$ )

At the initial time (I), by adding acacia honey to blueberry juice, TTC in juices of '6/38' and 'Northblue' decreased, while in the other two juice samples there was an increase. This result is certainly influenced by TCC of acacia honey (62.73 mg GAE/100 g, Vujan et al., 2023). The highest increase in TTC values was recorded for 'Duke' (93.72%) and '6/38' (78.15%) in

juice samples without honey and for '6/38' (66.05%) in juice samples with acacia honey. The smallest increase in TTC values was recorded for 'Northblue' in the sample of blueberry juice with acacia honey (49.53%), followed by the sample without honey (54.44%). Statistically, very significant differences were recorded in all cases.

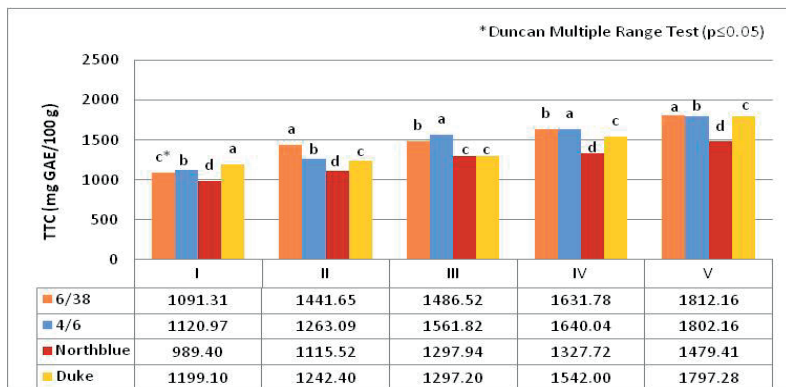


Figure 4. The influence of genotype on TTC depending on the storage period in blueberry juices with acacia honey  
 Note: \*Different letters between cultivars denote significant differences (Duncan test.  $p < 0.05$ );  
 Different letters between susceptible and resistant cultivars denote significant differences (LSD test.  $P < 0.05$ )

Regarding the influence of the storage period on TTC depending on genotype (Figures 5 and 6), a very significant increase was observed

from the beginning of the study to the final storage moment in all blueberry juices.

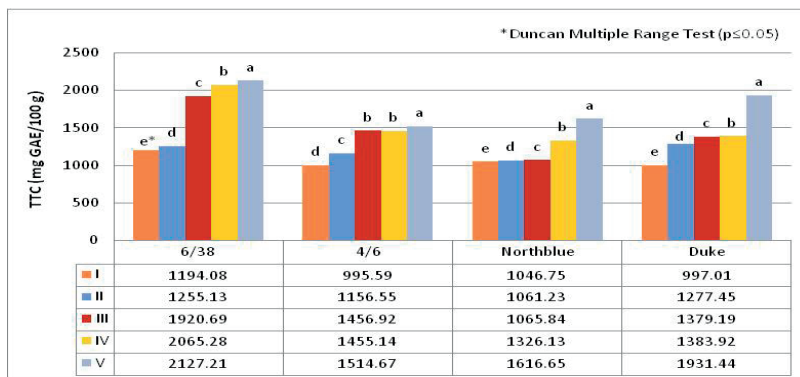


Figure 5. The influence of the storage period on TTC depending on blueberry genotype in juices without honey  
 Note: \*Different letters between cultivars denote significant differences (Duncan test.  $p < 0.05$ );  
 Different letters between susceptible and resistant cultivars denote significant differences (LSD test.  $P < 0.05$ )

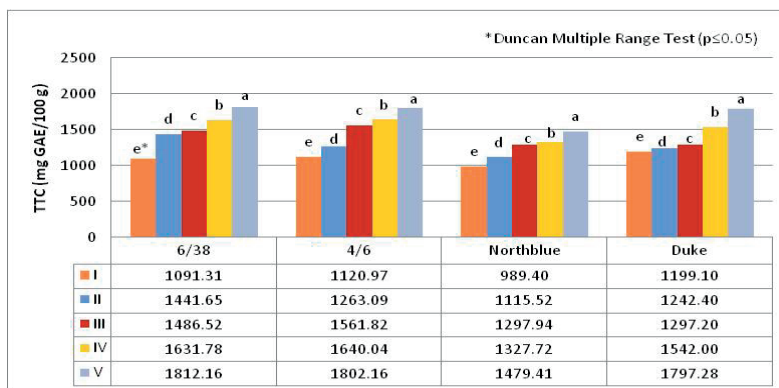


Figure 6. The influence of the storage period on TTC depending on blueberry genotype in juices with acacia honey  
 Note: \*Different letters between cultivars denote significant differences (Duncan test,  $p < 0.05$ );  
 Different letters between susceptible and resistant cultivars denote significant differences (LSD test,  $P < 0.05$ )

In the case of blueberry juices without honey (Figure 5), TTC was 995.59-1194.08 mg GAE/100 g at time point I, and 1514.67-2127.21 mg GAE/100 g at time point V. The same increase was observed in the juices to which acacia honey was added, but the percentage increase for the entire storage period was lower compared to juices without honey. Similar results were found in the blueberry juice (Bett-Garber et al., 2015; Li et al., 2021), ginkgo kernel juice (Wang et al., 2019), sea buckthorn and sea buckthorn-apple

juices (Tkacz et al., 2020), and mulberry juice (Kwaw et al., 2018).

Regarding the influence of sweetener on TTC depending on genotype and the storage period (Figure 7), a very significant effects between the juices without honey and the juices with acacia honey was observed at each time point for all blueberry genotypes. However, of the three factors (genotype, storage period and sweetener) the sweetener showed the lowest effect size on TTC values (data not presented).

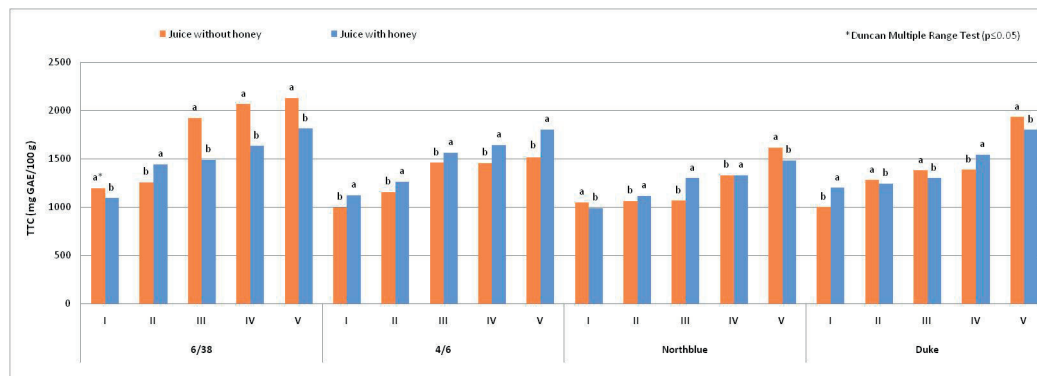


Figure 7. The influence of sweetener on TTC depending on genotype and storage period in blueberry juices  
 Note: \*Different letters between cultivars denote significant differences (Duncan test,  $p < 0.05$ );  
 Different letters between susceptible and resistant cultivars denote significant differences (LSD test,  $P < 0.05$ )

### Total flavonoid content in juice

Flavonoids in blueberry are well-known for being synthesized in specific locations. They contribute to the colour and fragrance of flowers, as well as to an effective fruit dispersion that attracts pollinators since they support the spore germination, as well as the

growth and development of seedlings (Panche et al., 2016).

Regarding the influence of the genotype on TFC depending on the storage period in juices obtained without adding honey and the juices to which acacia honey was added, a decrease is observed continuously over the entire storage



period for each genotype with very significant differences between genotypes (Figures 8 and 9).

At the initial time (I), by adding acacia honey to blueberry juices, the TFC values decreased in all juices regardless of blueberry genotype, this decrease being explained by the small intake of the acacia honey, which presented a TFC value of 11.83 mg CE/100 g (Vijan et al., 2023).

The highest decrease in TFC values registered between de time I and the last storage moment was recorded for 'Northblue' (73.96%) and '6/38' (74.46%) in juice samples without honey and for '4/6' (84.30%) and 'Northblue' (74.85%) in juice samples with acacia honey. The smallest decrease in TFC values was recorded for 'Duke' in the sample of blueberry juice

without acacia honey (57.16%), followed by the sample with honey (63.05%), this genotype proved a superior stability of TFC both in the unsweetened juice and in the juice with honey.

As regards the influence of the storage period on TFC depending on genotype (Figures 10 and 11), very significant differences were noticed for all juices. For blueberry juices without honey, the highest TFC was recorded at Time point I: 588.99-685.68 mg CE/100 g, and it decreased to 161.14-252.31 mg CE/100 g at Time point V. The same decrease was observed in the juices to which acacia honey was added, the percentage decrease being comparable between the two types of juices: 57.16-74.46% in the juices without honey and 63.05-84.30% in the juices with acacia honey.

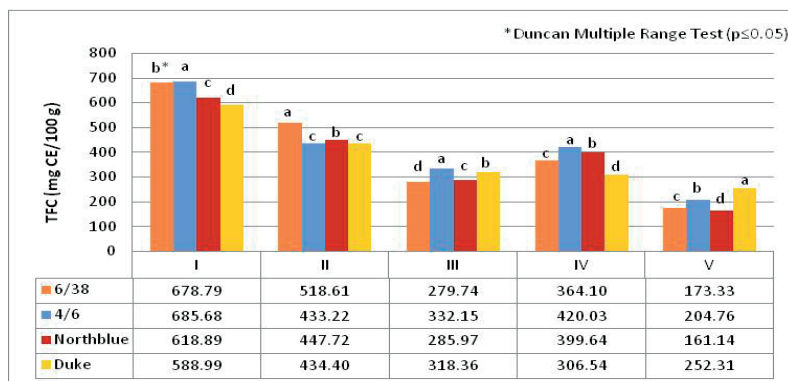


Figure 8. The influence of genotype on TFC depending on the storage period in blueberry juices without honey

Note: \*Different letters between cultivars denote significant differences (Duncan test.  $p < 0.05$ );

Different letters between susceptible and resistant cultivars denote significant differences (LSD test.  $P < 0.05$ )

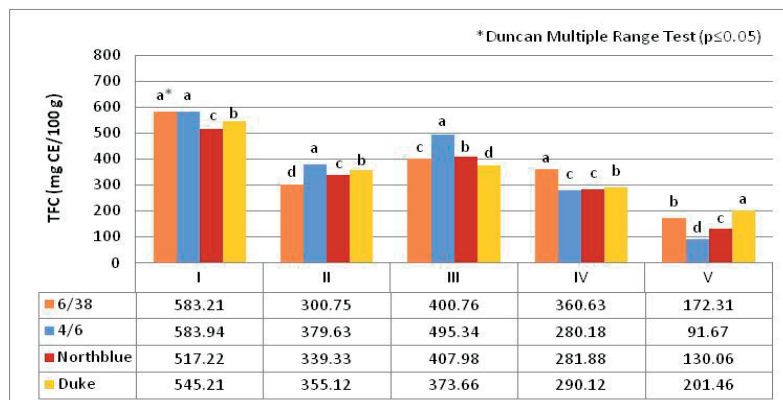
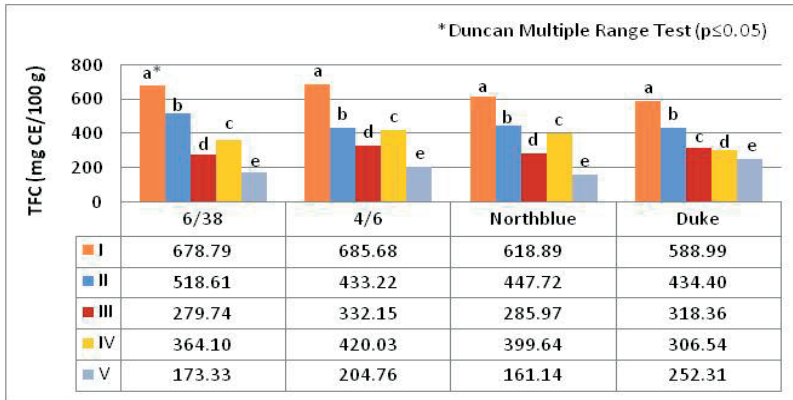


Figure 9. The influence of genotype on TFC depending on the storage period in blueberry juices with honey

Note: \*Different letters between cultivars denote significant differences (Duncan test.  $P < 0.05$ );

Different letters between susceptible and resistant cultivars denote significant differences (LSD test.  $P < 0.05$ )



Note: \*Different letters between cultivars denote significant differences (Duncan test,  $p < 0.05$ ).

Different letters between susceptible and resistant cultivars denote significant differences (LSD test,  $P < 0.05$ )

Figure 10. The influence of the storage period on TFC depending on blueberry genotype in juices without honey

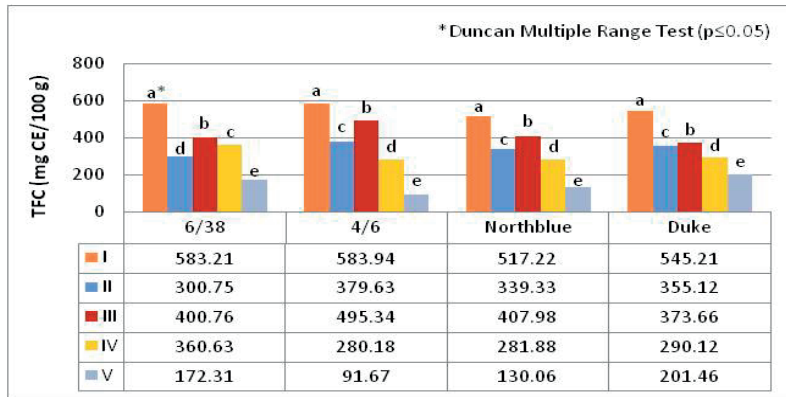


Figure 11. The influence of the storage period on TFC depending on blueberry genotype in juices with honey

Note: \*Different letters between cultivars denote significant differences (Duncan test,  $p < 0.05$ );

Different letters between susceptible and resistant cultivars denote significant differences (LSD test,  $P < 0.05$ )

Regarding the influence of sweetener on TFC depending on genotype and the storage period (Figure 12), a very significant effects between

the juices without honey and the juices with acacia honey was observed at each time point for all blueberry genotypes.

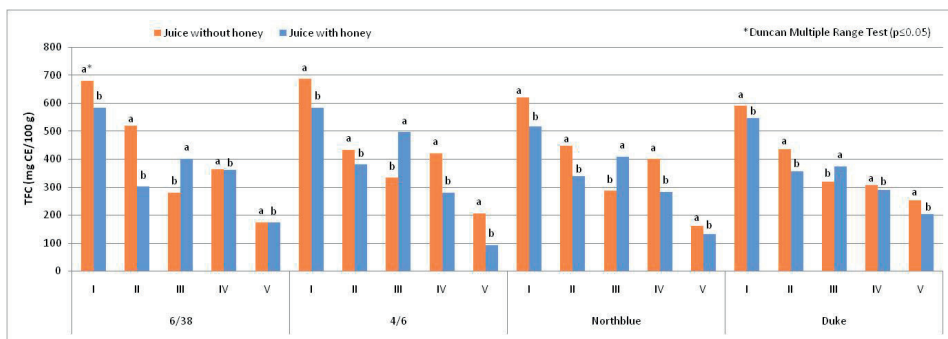


Figure 12. The influence of sweetener on TFC depending on genotype and storage period in blueberry juices

Note: \*Different letters between cultivars denote significant differences (Duncan test,  $p < 0.05$ );

Different letters between susceptible and resistant cultivars denote significant differences (LSD test,  $P < 0.05$ )



## CONCLUSIONS

The results revealed very significant effects of the three analysed factors (genotype, storage period and sweetener) on the total tannin content and the total flavonoid content, with the sweetener (acacia honey) showing the lowest effect size.

Extending the storage time led to opposite trends on TTC and TFC as from the first analysis to the fifth, the tannins content increased, while the flavonoids content decreased.

Among the genotypes, 'Duke' stood out, from which juices were obtained (unsweetened, but also with honey) that presented at the end of the study the highest levels of flavonoids. 'Northblue' was also noted, which at the end of the storage period accumulated the lowest level of tannin. Top-quality blueberry juices can be obtained only by taking into account the fruit compositional variability and its preservation along the processing chain.

## ACKNOWLEDGEMENTS

This research study was supported by a “Henri Coandă” programme granted by the Romanian Ministry of Research, Innovation and Digitalization, contract number 4/16.01.2024. The authors would like to thank the Romanian beekeeper Florin Voicu for providing the acacia honey.

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