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THE INFLUENCE OF GASEOUS OZONE FUMIGATION ON THE CHEMICAL AND MECHANICAL PROPERTIES OF HIGHBUSH BLUEBERRY (*VACCINIUM CORYMBOSUM* L.) FRUITS

– Research paper –

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Abstract: Ozonation is an effective method of post-harvest preservation and reducing the loss of health-promoting compounds such as phenolic compounds during storage. Fruits of highbush blueberry (*Vaccinium corymbosum* L.) have strong antioxidant properties, they are recognised as one of the most valuable sources of health-promoting bioactive compounds. In the present study, changes in total acidity and pH of highbush blueberry fruit depended on ozonation and storage time and genotype. The total acidity of fresh blueberries of tested genotypes ranged from 0.59 - 0,90 g·100 g^{-1}. The highest total acidity was recorded for clone '21', which was ozonated for 15 min. It was found that the application of ozone gas during storage of *V. corymbosum* fruit had mainly positive effect on the content of analysed bioactive compounds. Results of our study showed that ascorbic acid content varied depending on genotype, storage time and ozonation. The average content of this antioxidant in the non-ozonated fresh fruit was 22.32 mg·100 g^{-1}, and slightly decreased with ozonated 15 min and 30 min - 21.87 mg·100 g^{-1} and 21.95 mg·100 g^{-1}, respectively. Average content of total phenolic in fruits subjected to ozonation for 30 min. and stored for 15 days was higher by 10.3% in a comparison to control sample (fresh fruits). The antioxidant values of DPPH and FRAP in fruits subjected to storage and ozonation for 30 min. increased by 22.6% and 15.5%, respectively. Using ozone had a minor positive effect on the measured mechanical properties of the fruits. Throughout the different storage periods of blueberry fruits, there was a decrease in destructive energy and an increase in force.

Key words: highbush blueberry, ozone fumigation, antioxidant activity, chemical composition, storage

INTRODUCTION

Ozone (O_3) in gaseous form has a strong oxidising and antimicrobial effect, destroying proteins and phospholipid molecules present in the cell membranes of micro-organisms. The use of gaseous ozone after fruit harvest reduced the loss of bioactive compounds during storage by neutralizing ethylene (Pinto et al., 2020, Zardzewiały et al., 2020). However, long-term storage of fruit resulted in a decrease of bioactive compounds in the stored raw material. Ozonation of fruit, due to its disinfectant effect and reduction of the loss of health-promoting compounds, could be used in storage as an opportunity to increase their commercial value (Piechowiak, 2021; Ji et al., 2014). Gaseous ozone can be freely used as a non-thermal method of extending the storage life of soft fruit, fully organic, with no residues in the raw material.

The highbush blueberry (*Vaccinium corymbosum* L.) is a plant naturally native to the North America and belongs to the genus *Vaccinium* L., which includes more than 400 species. It is a shrub with erect or semi-erect shoots that produce blue-black globular fruits, usually reaching 15-20 mm (1.0 - 2.5 g), known for their sweet taste and rich nutritional composition (Macrodi et al., 2020). The fruit of *V. corymbosum* are also recognised as one of the most valuable sources of bioactive compounds, with health-promoting and antioxidant effects. Blueberries are rich in anthocyanins, flavonoids,

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organic acids and proanthocyanidins (Reszka et al., 2017).

The amount of health-promoting compounds in the fruit depends on the growing year, ripeness, environmental conditions and cultivar. The total content of phenolic compounds in the fruit composition ranged from 170.7 - 320.9 mg GAE·100 g^{-1} f.w., the antioxidant value (Ferric Reducing Antioxidant Power - FRAP) reached 18.41 mM Fe^{2+}·kg f.w., 53.49 - 84.69 mg·g^{-1} f.w. (Chiabrando et al., 2016; Skupień et al., 2006). Blueberry fruits showed a protective effect on the cardiovascular system by preventing atherosclerosis caused by excessive cholesterol levels. In addition, they reduced oxidative damage and endothelial inflammation by inhibiting inflammation and protected against ischaemic heart damage (Onuh et al., 2023; Torri et al., 2007). Due to their rich composition and health-promoting properties, blueberries are mainly used for fresh consumption and for drying, juices, jams and tinctures.

Stored under appropriate conditions, *V. corymbosum* fruit retain their freshness on average for only 10 to 18 days, forcing the raw material to be used quickly. The use of gaseous ozone could increase the quality of fresh blueberries during storage (Concha-Meyer et al., 2015), reduced the population of microorganisms on the fruit surface (Piechowiak et al., 2019; Concha-Meyer et al., 2014). Ozonation could also increase the firmness of the fruit while reducing the possibility of mechanical damage that might occur during transport and storage. The aim of the study was to determine the effect of ozone gas fumigation treatment on the chemical composition and selected mechanical properties of the fruit of 4 breeding clones and 2 cultivars of highbush blueberry during storage.

MATERIAL AND METHODS

Materials

The material for analysis and studies included fruits of 6 genotypes: 4 new Polish breeding clones - '17', '19', '20' and '21', and 2 highbush blueberry cultivars - 'Bluecrop' and 'Duke', commonly cultivated commercially in Poland and in other countries in the world. Advanced clones are the result of breeding and selection work as part of the applied breeding program of the highbush blueberry (*V. corymbosum* L.), Northern type, conducted at the Department of Horticultural Crop Breeding of the National Institute of Horticultural Research (InHort) in Skierniewice, central Poland. The fruits were collected manually from bushes of the above-mentioned genotypes grown in a trial located in a field at the Pomological Orchard (InHort) in Skierniewice (51°57'38" N, 20°8'39" E, Łódzkie Voivodeship, Poland) in July 2022. The fruits were harvested at their full ripeness, determined on the basis of color and the strength of the fruit's adhesion to the peduncle. The fruits were stored for 15 days, during which their individual chemical and mechanical properties were examined. The plant material (fruits) analyzed in this manuscript is a continuation of research on the identification of the polyphenol profile and sugar content in the analyzed fruits and clones, conducted by the team and published in Gorzelany et al. (2023).

Ozone treatment of the fruit

Immediately after harvest, the highbush blueberry fruit of all genotypes were randomly divided into three batches. The first batch was a control, left without ozonation. The other two batches were treated with ozonation in plastic containers. Gaseous ozone of 10 ppm for 15 and 30 minutes (flow rate 40 g O_3·h^{-1}) was applied. Ozone was produced by a KORONA A 40 Standard generator (Korona, Piotrków Trybunalski, Poland), the concentration of gaseous ozone was measured using a 106 M UV Ozone Solution Detector (Ozone Solution, Hull, MA, USA).

Determination of pH and acidity in highbush blueberry fruit

Total acidity per citric acid and pH were determined by potentiometric titration of the test sample with a standard dilution of 0.1 M NaOH to pH = 8.1 using a titrator (Titroline 5000, Mainz, Germany) according to the method in PN-EN 12147:2000. The analysis was performed in triplicate.

Determination of the content of bioactive compounds

The ascorbic acid content of the highbush blueberry fruit was determined according to PN-A-04019:1998. The total polyphenol content of the fruit was determined using the Folin-Ciocalteu method according to the methodology described in Kuźniar et al. (2022). The antioxidant activity of DPPH was determined according to the methodology described by Jurćaga et al. (2021). The result was expressed as % inhibition of the DPPH reagent. FRAP antioxidant activity was determined according to the methodology described by Gawroński et al. (2014) the result was given in μM Fe^{2+}·g^{-1}. ABTS antioxidant activity was determined according to the methodology presented by Rupasinghe et al. (2012); the result was given in mM TE·g^{-1}. All analyses were performed in triplicate.

Determination of the morphological and physical characteristics of blueberry fruits

Morphological and mechanical properties, and water content were determined for fruit non-ozonated (control) and ozonated for 30 minutes and stored for 1, 8 and 15 days. The sample size was 15 fruits for the experimental combinations studied. For each fruit, diameter (d) and length (l) were measured to the nearest 0.01 mm, mass (weight) to the nearest 0.01 g, and density as the ratio of their mass to the volume of a rotating ellipsoid of diameter (d) and length (l). The sphericity factor (φ) was calculated from the formula developed by Szpunar-Krok et. al. (2021):

$$\boldsymbol{\varphi} = \frac{(\boldsymbol{l} \cdot \boldsymbol{d}^2)^{\frac{1}{3}}}{\boldsymbol{l}} 100\% \qquad (1)$$

where: φ - sphericity (%),; l - length of the fruit (mm); d - diameter of the fruit (mm).

The water content of the blueberry fruit was determined using the drying method (105°C), in accordance with PN-90/A-75101-03:1990.

Determination of the mechanical properties of blueberry fruits

A uniaxial compression test between two horizontal planes, perpendicular to the length of the fruit was used, on a Brookfield CT3-1000 texture analyser (AMETEK Brookfield, Middleboro, USA) with TexturePro CT software. The sample size was 15 fruits for the experimental combinations studied. Destructive force (F_D), absolute strain (λ) and destructive energy (E_D) were recorded. The specimen preload was 0.05 N and the compression velocity was 0.2 mm·s^{-1}. The relative deformation (ε) was calculated as the ratio of the absolute deformation (λ) to the fruit diameter (d) described by PN-90/A-75101-03 and Kulig et al. (2015). The value of the apparent modulus of elasticity, E_C, was calculated from the modified formula of Kuźniar et al. (2022) and Gorzelany et al. (2022):

$$E_C = \frac{E_D}{0.26 \cdot d \cdot l \cdot \lambda} \qquad (2)$$

where: E_c – apparent modulus of elasticity (MPa); E_D - destructive energy (mJ); d – diameter of the fruit (mm); l – length of the fruit (mm); λ – absolute deformation of the fruit in the direction of the load (mm).

Statistical analysis

STATISTICA 13.3. software (TIBCO Software Inc., Tulsa, OK, USA) was used to perform statistical analysis of the data. Evaluation included analysis of variance (ANOVA) and LSD significance test at the significance level of α = 0.05.

RESULTS AND DISCUSSION

pH and acidity of the highbush blueberry fruit

The fruit of the highbush blueberry (*V. corymbosum*) contains organic acids (e.g. linolenic acid, α-linolenic acid, palmitic acid). The content of individual organic acids has a significant impact on the taste of the fruit; the saturation of acids may decrease with the stages of ripening. Organic acids can be degraded by various factors, e.g. temperature. Changes in total acidity and pH of highbush blueberry fruit depending on ozonation and storage time and genotype are shown on Figures 1 and 2. The total acidity of fresh highbush blueberries ranged from 0.59 - 0,90 g·100 g^{-1}. The highest total acidity was recorded for clone '21' - 0.90 g·100 g^{-1}, which was ozonated for 15 min; the lowest total acidity of fresh blueberries was noted for clone '19' - 0.59 g·100 g^{-1}, in the control sample. After 15 days of storage, significant differences in acidity were observed between the clones and cultivars tested. The fruit acidity of all tested genotypes, regardless of the treatment, decreased by 11.1%. The greatest differences were observed between the sample ozonated for 30 min and the control sample for clone '21' - 0.2 g·100 g^{-1}. The results of our study showed that the total acidity in the analysed fruits was comparable to the data described by Skupień et al. (2006), in which the acidity of highbush blueberry ranged from 0.27 - 1.12 g·100 g^{-1}, depending on the cultivar and year of cultivation. The literature data showed that the total acidity of highbush blueberry was lower compared to other fruit species cultivated in Poland, e.g.: honeyberry (blue honeysuckle) - 1.22 - 1.31 g·100 g^{-1} (Gorzelany et. al., 2023), red currant - 0.95 - 1.09 g·100 g^{-1} (Kuźniar et al., 2022), cranberry - 1.56 - 1.60 g·100 g^{-1} (Gorzelany et al., 2022), saskatoon berry - 0.3 - 1.5 g·100 g^{-1} (Gorzelany et al., 2022).

Depending on genotype and length of storage, the pH value of *V. corymbosum* fruit ranged from 3.6 to 4.3. The highest value was recorded for the cultivar 'Duke', which was ozonated for 30 min and stored for 15 days. No significant differences were observed depending on storage length and ozonation. Only in the case of fruit stored for 15 days, ozonated for 30 minutes, an increase in pH value of 2.3% was observed, compared to fresh fruit.

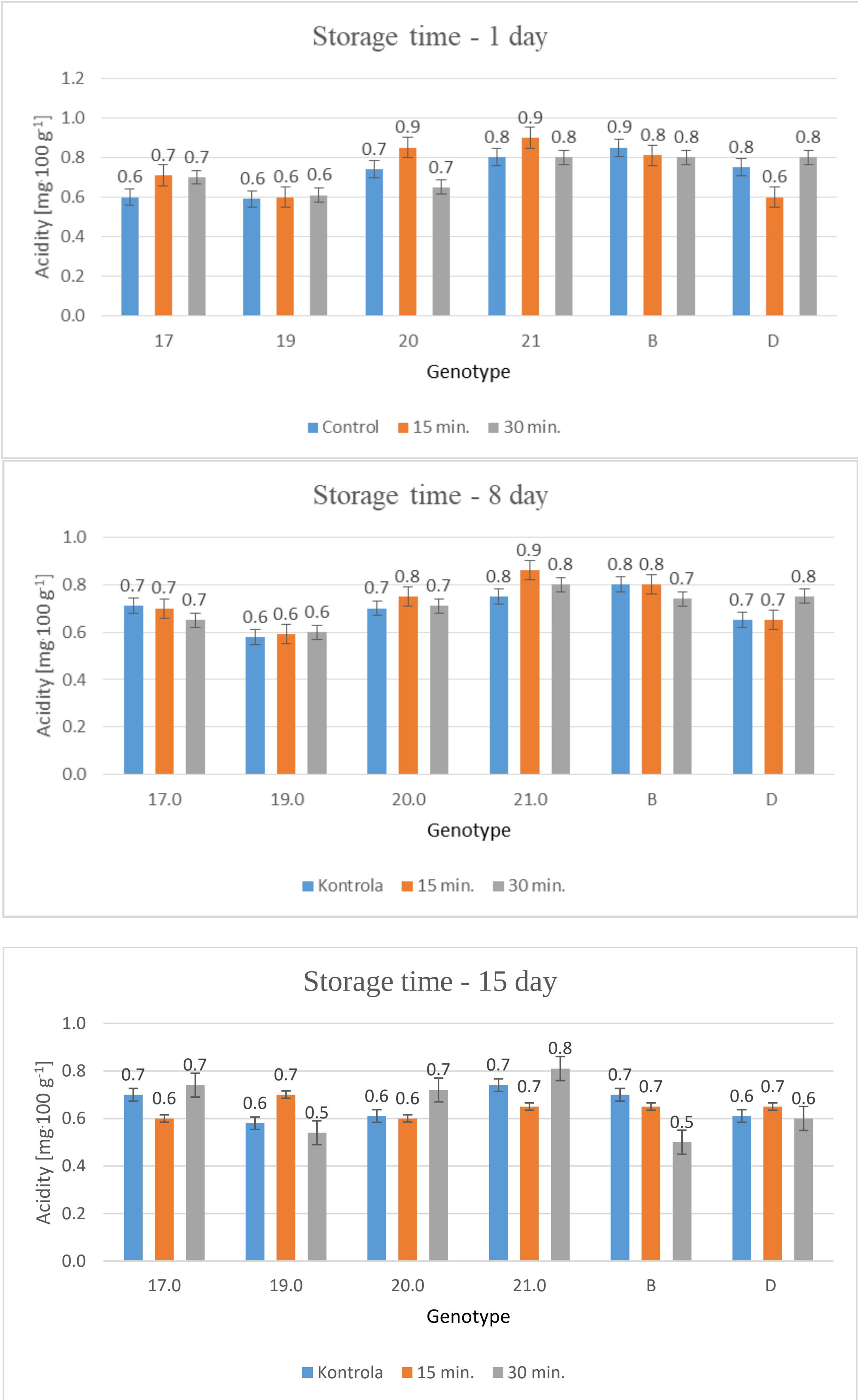

Figure 1. Changes in total acidity of the highbush blueberry fruit depending on genotype and ozone concentration; B – 'Bluecrop', D – 'Duke'

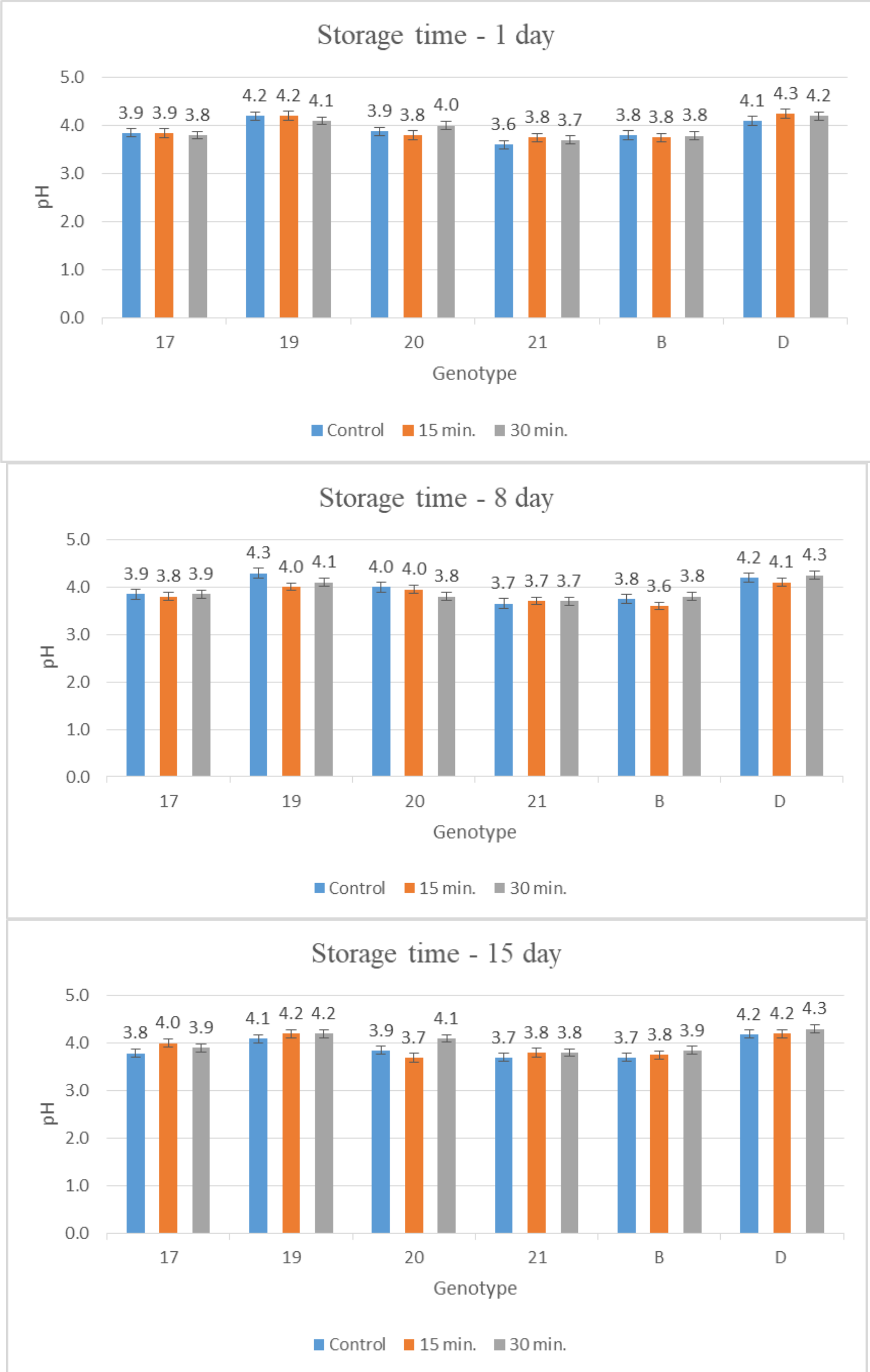

Figure. 2. Changes in pH of the highbush blueberry fruit in relation to genotype and ozone concentration; B – 'Bluecrop', D – 'Duke'

The results of our study are comparable to those published by Angeletti et al. (2010), where the pH of fruit of the same species ranged from 3.31 - 4.41, depending on the cultivar and length of storage; in the study of Lee et al. (2015), the pH of these fruits was 3.26. The pH value of blueberries is comparable to other berry species, e.g. raspberry – 2.86 (Souza et al., 2014), red currant - 3.05 - 4.15 (Kuźniar et al., 2022), sea buckthorn - 3.02 - 3.19 (Gorzelany et al., 2022), haskap - 3.13 - 3.52 (Gorzelany et al., 2023).

Ascorbic acid content in the highbush blueberry fruit

Ascorbic acid is a powerful antioxidant with anti-cancer effects. This compound is essential for the functioning of the human body and the activation of many enzymes, affects the circulatory and immune systems, slows down skin ageing and supports cell and tissue function (Du et al., 2012). Analysis of the results of our study showed that ascorbic acid content varied depending on genotype, storage time and ozonation (Table 1). The average ascorbic acid content of non-ozonated fresh fruit was 22.32 $mg\cdot 100\ g^{-1}$, ozonated 15 min and 30 min - 21.87 $mg\cdot 100\ g^{-1}$ and 21.95 $mg\cdot 100\ g^{-1}$, respectively. The results obtained in our study were comparable with the study of Skupień (2006), in which the ascorbic acid content ranged from 19.9 to 22.7 $mg\cdot 100\ g^{-1}$. After 15 days of storage, a decrease in the average ascorbic acid content of 13.0% was found, regardless of genotype and ozonation time. The lowest degree (7.8%) of ascorbic acid degradation after 15 days of storage was determined in fruit of the analysed clones and cultivars ozonated for 15 min, while fruit non-ozonated had the highest loss of ascorbic acid - 15.1%. In the study by Kuźniar et al. (2022), a 24.2% decrease in ascorbic acid content was observed after 14 days of storage of red currant fruit ozonated for 30 min in one of the tested cultivars; this result was comparable to that obtained in our study on highbush blueberries. The ozonation process had a favourable effect on enzymatic activity and organic acid metabolism, including ascorbic acid content (Piechowiak et al., 2021). The content of ascorbic acid in the blueberry fruit was comparable with other berry species, e.g. haskap - 36.80 - 51.30 $mg\cdot 100\ g^{-1}$ (Gorzelany et al., 2023), red currant - 35.10 - 69.70 $mg\cdot 100\ g^{-1}$ (Kuźniar et al., 2022), blackberry - 33.85 $mg\cdot 100\ g^{-1}$, blackthorn - 21.94 $mg^{-}100\ g^{-1}$ (Jabłońska-Ryś et al., 2009). As stated, the use of gaseous ozone fumigation in strawberries reduced the rate of superoxide radical anion formation, increased the activity of superoxidase (SOD), catalase (CAT), ascorbate peroxidase (APX) and monodehydroascorbate reductase (MDHAR), which contributed to the accumulation of ascorbic acid in these fruits (Zhang et al., 2020).

Phenolic compounds in the highbush blueberry fruit

Highbush blueberries contain polyphenolic compounds in their composition, especially anthocyanins, which show health-promoting effects. Consumption of these fruits improves health, the cardiovascular system (Erlund et al., 2008), cognitive abilities and memory (Williams et al., 2008), can inhibit inflammation and reduce oxidative stress (Youdim et al., 2002). The total content of health-promoting compounds depended on soil and climatic conditions, as well as cultivar and growing season (Aires et al., 2017). In our study, the average total polyphenol content of fresh, non-ozonated fruit was 202.17 $mg\ GAE^{-}100\ g^{-1}$, ozonated 15 min and 30 min - 204.67 and 215.33 $mg\ GAE^{-}100\ g^{-1}$, respectively (Table 1). After 15 days of storage, an increase in average total polyphenol content of 6.6% was observed, irrespective of genotype and length of ozonation period. The greatest increase (10.3%) in average phenolic compound content was found for fruit ozonated for 30 min. Fruits ozonated for 30 min and stored for 15 days were characterised by the highest content of polyphenols - 218.67 $mg\ GAE\cdot 100\ g^{-1}$. In the research of Gorzelany et al. (2023) regarding the highbush blueberry genotypes analyzed in this study, the highest average content of polyphenolic compounds had malvidin-3-O-galactoside (M3Ga), malvidin-3-O-glucoside (M3Gl), delphinidin-3-O-glucoside (D3G) and cyanidin-3-O-glucoside (C3G) throughout the storage period, regardless of the ozone dose used. The results obtained in our study were comparable with the data of Skupień (2006), where the total content of polyphenols ranged from 170.7 $mg\ GAE\cdot 100\ g^{-1}$ to 320.9 $mg\ GAE\cdot 100\ g^{-1}$, depending on the cultivar and year of cultivation. However, these results were lower with that obtained in the study by Jabłońska-Ryś et. al. (2009), in which highbush blueberry fruit contained 424.72 $mg\ GAE\cdot 100\ g^{-1}$. The results of our study indicated that the total content of polyphenols in the fruit of the evaluated genotypes was significantly higher than for redcurrant fruit at an average of 73.27 $mg\ GAE\cdot 100\ g^{-1}$ (Kuźniar et al., 2022), comparable to blackberry - 247.25 $mg\ GAE\cdot 100\ g^{-1}$, but much lower than for elderberry - 535.98 $mg\ GAE\cdot 100\ g^{-1}$ (Zhang et al., 2020), honeyberry - 522.06 - 747.85 $mg\ GAE\cdot 100\ g^{-1}$, haskap (416.94 - 597.29 $mg\ GAE\cdot 100\ g^{-1}$; Gorzelany et al., (2023). Literature data indicated that appropriate ozonation of fruit could significantly increase peroxidase (POD) activity, inhibit polyphenol oxidase (PPO) activity, while maintaining high total levels of phenolic compounds (Youdim et al., 2002).

Antioxidant value of the highbush blueberry fruit

The fruit of *V. corymbosum* is recognised as a valuable source of phenolic compounds with antioxidant properties. The blueberry fruit show antioxidant and anti-cancer effects. The antioxidant value was determined by three methods: DPPH, FRAP and ABTS (Table 1). The average degree of inhibition determined by the DPPH method of fresh fruit non-ozonated was 63.75%, ozonated 15 min and 30 min - 65.83% and 52.78%, respectively. After 15 days of storage, the average degree of inhibition as determined by the DPPH method of fruit ozonated for 30 min was 5.2% higher, compared to the non-ozonated sample. Only fruit ozonated for 30 min and stored for 15 days showed an increase in the average antioxidant value of 18.5%. However, the results of our study under Polish conditions did not coincide with those obtained by Aires et al. (2007), where the inhibition value of the analysed highbush blueberry fruits ranged from 80% - 92% under Portuguese soil and climatic conditions.

The average antioxidant value determined by the FRAP method of fresh blueberry fruit non-ozonated was 19.02 µM $Fe^{2+} \cdot g^{-1}$, ozonated 15 min and 30 min - 20.20 and 18.62 µM $Fe^{2+} \cdot g^{-1}$, respectively. After 15 days of storage, the fruit had a 4.0% decrease in average FRAP value, regardless of genotype and ozonation time. Fruits ozonated for 30 min, after 15 days of storage, were characterised by a 2.4% increase in antioxidant value. After the storage period, the average antioxidant value determined by FRAP of blueberry fruit ozonated for 30 min was the highest at 19.07 µM $Fe^{2+} \cdot g^{-1}$. The results of our study were 17.1% higher to those obtained by Rupasinghe et al. (2012), in which the average FRAP value of fresh highbush blueberry fruit was 16.24 µM $Fe^{2+} \cdot g^{-1}$.

The average antioxidant value determined by the ABTS method of fresh, non-ozonated fruit was 0.437 mM·TE g^{-1}, ozonated 15 min and 30 min - 0.375 and 0.305 mM·TE g^{-1}, respectively. After 15 days of storage, there was a 10.0% decrease in the mean ABTS value regardless of genotype and ozonation time. After the storage period, the greatest decrease (21.2%) in the average antioxidant value using the ABTS method was determined in the fresh fruit (control sample). The values obtained in our study were consistent with the data obtained by Nikolić et al. (2019), where the antioxidant value determined by the ABTS method in the three highbush blueberry cultivars ranged from 0.313 - 0.509 mM·TE g^{-1} under the growing conditions prevailing in Serbia.

Table. 1 Total content of the ascorbic acid, phenolic compounds and antioxidant value of the highbush blueberry fruits (*an average for all tested genotypes*)

Ozone exposure time [min]	Storage time [days]	Ascorbic acid (mg·100 g^{-1})	Total phenolics content (mg GAE·100 g^{-1})	DPPH (inhibition %)	FRAP ($Fe^{2+} \cdot g^{-1}$)	ABTS (mmol· TE g^{-1})
0	1	22.32a±3.21	202.17a±12.95	63.75a±14.68	19.02a±2.89	0.437a±0.197
	8	21.33a±3.61	204.67ab±13.03	66.11a±7.04	19.25a±1.05	0.357a±0.190
	15	18.97a±4.88	215.33b±9.91	61.53a±11.67	17.98a±2.47	0.342a±0.187
	Average	20.87A±3.99	207.39A±16.08	63.80A±11.03	18.75A±2.21	0.378A±0.185
15	1	21.87a±3.11	200.17a±20.65	65.83a±18.45	20.20a±2.16	0.375a±0.217
	8	23.32a±3.84	204.50a±14.38	60.83a±11.74	19.00a±1.92	0.297a±0.162
	15	20.17a±2.42	208.33a±18.24	57.78a±8.85	18.50a±2.72	0.333a±0.194
	Average	21.78A±3.27	204.33A±16.88	61.48A±13.24	19.23A±2.27	0.335A±0.184
30	1	21.95a±2.36	198.16a±14.85	52.78a±2.72	18.62a±2.05	0.305a±0.183
	8	20.62a±3.14	203.17ab±15.24	59.31a±7.27	19.90a±1.57	0.295a±0.163
	15	18.82a±3.54	218.67b±14.00	64.72a±8.72	19.07a±0.43	0.325a±0.170
	Average	20.46A±3.16	206.67A±16.47	58.94A±8.08	19.19A±1.52	0.308A±0.162
Duration of storage [days]	1	22.04b±2.75	200.17a±17.64	60.79a±14.16	19.28a±2.36	0.372a±0.195
	8	21.76b±3.53	204.11ab±13.58	62.08a±8.93	19.38a±1.52	0.316a±0.164
	15	19.32a±3.57	214.11b±14.38	61.34a±9.69	18.52a±2.06	0.333a±0.173
Average		21.04±3.47	206.13±16.30	61.40±10.97	19.06±2.01	0.341±0.176

Statistical data are expressed as a mean value (n = 15) ± SD. Means in a column followed by different lowercase and uppercase letters show significant differences ($p<0.05$) according to the LSD test

The morphological and physical characteristics of the highbush blueberry fruits

Analysis of variance showed a significant effect of genotype (with the exception of density) and storage time (with the exception of sphericity coefficient) on selected morphological characteristics and water content in the fruit (Table 2). Fruits of clone '17' were characterised by the largest diameter, length, weight and moisture content, while fruits of cultivar 'Bluecrop' had significantly the smallest diameter, length, weight and moisture content. Significantly the least spherical fruits had clone '19', while the most spherical fruits were those of the cultivars 'Duke' and 'Bluecrop' and clones '17' and '21'. The fruit of the tested clones and cultivars of highbush blueberry did not differ significantly in density. Ozone-treated fruit had smaller diameter, length, weight and moisture content and higher density, but these differences were not statistically significant.

Table 2. Morphological features and moisture content of the highbush blueberry fruits depending on the genotype, duration of storage and time of gaseous ozonation

Variables		Diameter (mm)	Length (mm)	Weight (g)	Sphericity (%)	Density (10^{-3} kg·m^{-3})	Moisture content (%)
Genotype	‘17’	12.9c±1.7	17,7d± 2,5	3.4e±1.0	81.0c±3.8	2.22a±0.43	88.7e±1.9
	‘19’	10.8b±1.3	16,8c±2,1	2.5d±0.8	74.5a±2.8	2.38a±0.41	88.1de±2.2
	‘20’	10.6b±1.2	15,4b±1,9	2.1c±0.6	78.1b±3.4	2.29a±0.38	87.3cd±2.5
	‘21’	10.6b±1.0	14,8b±1,6	1.9bc±0.4	80.1c±4.1	2.23a±0.42	86.9c±2.2
	‘Bluecrop’	9.7a±1.1	13,4a±1,6	1.6a±0.6	80.9c±4.0	2.33a±0.46	84.6a±4.3
	‘Duke’	10.1a±1.3	13,9a±1,8	1.7ab±0.4	81.0c±3.3	2.30a±0.51	85.7b±3.0
Time of ozonation [min]	0	10.9a±1.7	15,5a± ,4	2.3a±1.0	79.3a±4.4	2.28a±0.42	87.0a±2.9
	30	10.7a±1.5	15,2a±2,5	2.1a±0.9	79.2a±4.1	2.30a±0.45	86.8a±3.3
Duration of storage [days]	1	11.5c±1.5	16,2c±2,2	2.5b±0.8	79.7a±4.1	2.15b±0.37	87.8b±2.1
	8	10.0a±1.3	14,2a±2,2	2.1a±0.9	79.4a±4.7	2.67c±0.39	86.5a±2.9
	15	10.8b±1.7	15,6b±2,5	2.1a±1.0	78.8a±4.1	2.05a±0.24	86.5a±3.9
	Mean	10.8 ±1.6	15,3±2,4	2.2±0.9	79.3± 4.3	2.29±0.44	86.9±3.1

Statistical data are expressed as a mean value (n = 15) ± SD. Means in a column followed by different letters show significant differences ($p<0.05$) according to the LSD test

The weight and moisture content of the highbush blueberry fruit of the tested genotypes decreased during storage. However, this decrease was significant only after 8 days of fruit storage. The diameter, length and density of highbush blueberry fruit after 15 days of storage were significantly lower than in fresh fruit. The fruit diameter and length were significantly smallest after 8 days of storage, and their density was highest after 8 days of storage (Table 2). In the study by Kuźniar et al. (2022), the ozonation process, regardless of its time, resulted in a reduction in the weight, diameter and moisture of currant fruits. The opposite relationship, i.e. a smaller decrease in humidity after ozonation, was observed by Zapałowska et al. (2021) for sea buckthorn fruit and Zrdzewiały et al. (2020) for rhubarb petioles.

The mechanical properties of the highbush blueberry fruits

Analysis of variance showed the effect of genotype on the analysed mechanical parameters of the highbush blueberry fruit and storage time on relative strain and conventional modulus of elasticity (Table 3). Indeed, the fruits of the new Polish clones '20', '21' and '19' were the most resistant to mechanical damage, as they required the greatest strength and energy. Fruits of clone '19' were also characterised by the highest apparent modulus of elasticity, while fruits of clones '17' and '19' significantly deformed the least. Fruits of the cultivars 'Bluecrop' and 'Duke' and genotype ‘17’ were the most susceptible to damage. They were damaged significantly with the least force and energy, and had the lowest apparent modulus of elasticity (Table 3). Ozonation slightly increased the magnitude of the determined mechanical parameters of the tested fruits. During storage, destructive energy decreased and force increased. In contrast, strain and modulus of elasticity increased significantly after 8 days of storage and decreased after 15 days, but modulus was still slightly higher than after one day of storage. In the study by Kuźniar et al. (2022), the use of ozonation reduced the determined mechanical parameters of fruit, significantly only for energy, regardless of whether the process lasted 15 or 30 minutes, while for all storage times used, energy and modulus of elasticity decreased significantly, and strength decreased significantly after 8 days of storage.

Table 3. Mechanical properties of highbush blueberry fruits depending on the genotype, duration of storage and time gaseous ozonation

Variables		Destructive force (N)	Relative deformation (%)	Destructive energy (mJ)	Apparent modulus of elasticity (10^{-3} MPa)
Genotype	'17'	11.8a±3.2	56,71a±7,39	40.4a±11.6	99.4a±37.3
	'19'	22.0c±5.3	56,36a±10,45	84.5d±20.0	323.5d±122.5
	'20'	25.3d±7.1	62,61b±7,62	76.3c±19.4	285.4c±103.6
	'21'	24.2d±7.4	64,48b±7,39	67.9b±21.3	253.5b±102.8
	'Bluecrop'	12.9ab±3.9	64,47b±8,53	41.7a±10.3	209.4a±74.1
	'Duke'	14.7b±4.5	63,96b±9,48	43.8a±12.8	193.5a±67.9
Time of ozonation [min]	0	18.3a±8.0	60,79a±9,29	58.2a±25.3	219.5a±114.6
	30	18.7a±7.5	62,08a±9,08	60.0a±23.2	235.4a±113.7
Duration of storage [days]	1	18.2a±7.4	58,88a±7,66	61.8a±23.0	200.1a±86.8
	8	18.0a±7.5	66,70b±9,58	60.2a±24.3	263.8b±124.3
	15	19.2a±8.2	58,72a±7,93	55.3a±25.1	218.4a±119.4
Mean		18.5±7.7	61,43±9,20	59.1±24.2	227.4±114.3

Statistical data are expressed as a mean value (n = 15) ± SD. Means in a column followed by different letters show significant differences ($p<0.05$) according to the LSD test

CONCLUSIONS

The application of ozone gas during storage of the highbush blueberry (*V. corymbosum L.*) fruit had a positive effect on pH and acidity level, as well as on the average content of ascorbic acid, phenolic compounds and antioxidant activities. After 15 days in storage, fruit of these genotypes treated with ozone for 15 min. showed vitamin C loss at 7.8%. In contrast, fruits that did not undergo ozonation showed the highest ascorbic acid loss at 15.1%. After storage, fruits of tested genotypes treated with ozone for 30 minutes showed a 18.5% and 2.4% increase in DPPH and FRAP antioxidant values, respectively. The utilization of ozonation slightly enhanced the measured mechanical characteristics of the fruits. Across the applied storage durations, the destructive energy decreased while the force increased. Generally, the new tested breeding clones show similar fruit content in the range of morphological and chemical properties compared to the old highbush blueberry cvs. 'Bluecrop' and 'Duke'. The breeding clone marked 17 deserves special mention, which is characterized by better mechanical properties. The development of an effective storage method using ozone gas may contribute to the development of the highbush blueberry fruit production technology and thus an increase the commercial value of the final product.

REFERENCES

1. Aires A., Carvalho R., Matos M., Carnide V., Silva A.P., Goncalves B. 2017. Variation of chemical constituents, antioxidant activity, and endogenous plant hormones through different ripening stages of highbush blueberry (*Vaccinium corymbosum* L.) cultivars produced in central of Portugal. *Journal of Food Biochemistry*, 41: 12414. DOI: 10.1111/jfbc.12414
2. Angeletti P., Castagnasso H., Miceli E., Terminiello L., Concellon A., Chaves A., Vicente A.R. 2010. Effect of preharvest calcium applications on postharvest quality, softening and cell wall degradation of two blueberry (*Vaccinium corymbosum*) cariaties. *Postharvest Biology and Technology,* 58: 98-103 doi:10.1016/j.postharvbio.2010.05.015
3. Concha-Meyer A., Eifert J.D., Williams R.C., Marcy J.E., Welbaum G.E. 2015. Shelf life determination of fresh blueberries (*Vaccinium corymbosum*) stored under controlled atmosphere and ozone. *International Journal of Food Science,* 9. https://doi.org/10.1155/2015/164143

4. Concha-Meyer A., Eifert J.D., Williams R.C., Marcy J.E., Welbaum G.E. 2014. Survival of *Listeria monocytogenes* on Fresh Blueberries (*Vaccinium corymbosum*) Stored under cotrolled atmosphere and ozone. *Journal of Food Protection,* 77(5): 832-836. doi.org/10.4315/0362-028X.JFP-13-441
5. Chiabrando V., Giacalone G. 2016. Anthocyanins, phenolics and antioxidant capacity after fresh storage of blueberry treated with edible coatings. *International Journal of Food Sciences and Nutrition,* 33: 248-253 DOI:10.3109/09637486.2014.986075
6. Du J., Cullen J.J., Buettner G.R. 2012. Ascorbic acid: Chemistry, biology and the treatment of cancer. *Biochimica Biophysica Acta,* 1826: 443–457.
7. Erlund I., Koli R., Alfthan G., Marniemi J., Puukka P., Mustonen P., Matilla P., Jula A. 2008. Favorable effects of berry consumption on platelet function, blood pressure, and HDL cholesterol. *American Journal of Clinical Nutrtition*, 87(2): 323–331. doi: 10.1093/ajcn/87.2.323
8. Gawroński J., Hortyński J., Kaczmarska E., Dyduch-Siemińska M., Marecki W., Witorozec A. 2014. Evaluation of phenotypic and genotypic diversity of some Polish and Russian blue honeysuckle (*Lonicera caerulea* L.) cultivars and clones. *Acta Sci. Polonorum. Hortorum Cultus*, 13: 157–169.
9. Gorzelany J., Basara O., Kapusta I., Korfanty P., Belcar J. 2023. Evaluation of the chemical composition of selected varieties of *L. caerulea* var. *kamtschatica* and *L. caerulea* var. *emphyyllocalyx. Molecules*, 28: 2525 https://doi.org/10.3390/molecules28062525
10. Gorzelany J., Kapusta I., Pluta S., Belcar J., Pentoś K., Basara O. 2023. Effect of Gaseous Ozone and Storage Time on Polyphenolic Profile and Sugar Content in Fruits of Selected *Vaccinium corymbosum* L. Genotypes. *Molecules*, 28, 8106. https://doi.org/10.3390/ molecules28248106
11. Gorzelany J., Belcar J., Kuźniar P., Niedbała G., Pentoś K. 2022. Modelling of Mechanical Properties of Fresh and Stored Fruit of Large Cranberry Using Multiple Linear Regression and Machine Learning. *Agriculture*, 12: 200. https://doi.org/10.3390/agriculture12020200
12. Gorzelany J., Kapusta I., Zardzewiały M., Belcar J. 2022. Effects of ozone application on microbiological stability and content of sugars and bioactive compounds in the fruit of the saskatoon berry (*Amelanchier alnifolia* Nutt.). *Molecules*, 27: 6446. https://doi.org/10.3390/molecules27196446
13. Gorzelany J., Basara O., Kuźniar P., Pawłoska A.M., Belcar J. 2022. Effect of ozone treatment on mechanical and chemical properties of sea-buckthorn (*Hippophae rhammonides* L.) fruit. *Acta Universitatis Cibinensis Series E: Food Technology,* 2: 183- 194. 10.2478/aucft-2022-0015
14. Jabłońska-Ryś E., Zalewska-Korona M., Kalbarczyk J. 2009. Antioxidant capacity, ascorbic acid and phenolics content in wild edible fruits. *Journal of Fruit and Ornamental Plant Research,* 17: 115–120.
15. Ji L., Pang J., Li S. 2014. Application of new physical storage technology in fruit and vegetable industry. *African Journal of Biotechnology*, 11(25): 6718–6722. doi.org/10.5897/AJB11.1878
16. Jurćaga L., Bobko M., Kolesárová A., Bobková A., Demianová A., Hašcík P., Belej L. Mendelová A., Bučko O., Kročko M., Cech M. 2021. Blackcurrant (*Ribes nigrum* L.) and Kamchatka Honeysuckle (*Lonicera caerulea* var. *Kamtschatica*) Extract Effects on Technological Properties, Sensory Quality, and Lipid Oxidation of Raw-Cooked Meat Product (Frankfurters). *Foods*, 10: 2957. DOI: 10.3390/foods10122957
17. Kulig R., Łysiak G., Skonecki S. 2015. Prediction of pelleting outcomes based on moisture versus strain hysteresis during the loading of individual pea seeds. *Biosystems Engegry,* 129: 226–236.
18. Kuźniar P., Belcar J., Zardzewiały M., Basara O., Gorzelany J. 2022. Effect of Ozonation on the Mechanical, Chemical, and Microbiological Properties of Organically Grown Red Currant (*Ribes rubrum* L.) Fruit. *Molecules,* 27: 8231. doi.org/10.3390/molecules27238231
19. Lee Y., Lee J.-H., Kim S.-D., Chang M.-S., Jo I.-S., Kim S.-J., Hwang K.T., Jo H.-B., Kim J.-H. 2015. Chemical composition, functional constituents, and antioxidant activities of berry fruits produced in Korea. *Journal of the Korean Society of Food Science and Nutrition,* 44: 1295–1303.
20. Macrodi O.V., Hou J., Blackmon C., Babiker E.M., Mavrodi D.V. 2020. Comparative Analysis of Rhizosphere Microbiomes of Southern Highbush Bluberry (*Vaccinium corymbosum* L.), Darrow's

Bluberry (*V. darrowii Camp*), and Rabbiteye Blueberry (*V. virgatum Aiton*). *Frontiers in Microbiology*, 11: 1-14. https://doi.org/10.3389/fmicb.2020.00370
21. Nikolić M.D., Pavlović A.N., Mitić S.S., Tosić S.B., Kalicanin B.M., Manojlović D.D., Stanković D.M. 2019. Use of cyclic voltammetry to determine the antioxidant capacity of berry fruits: correlation with spectrophotometric assays. *European Journal of Horticultural Science*, 84(3): 152-160 DOI: 10.17660/eJHS.2019/84.3.
22. Onuh J.O., Dawkins N.L., Aluko R.E. 2023. Cardiovascular disease protective properties of blueberry polyphenols (*Vaccinium corymbosum*): a concise review. *Food Production, Processing and Nutrition*. 5: 27 doi.org/10.1186/s43014-023-00139-y
23. Pinto L., Palma A., Cefola M., Pace B., D'Aquino S., Carboni C., Baruzzi F. 2020. Effect of modified atmosphere packaging (MAP) and gaseous ozone pre-packaging treatment on the physico-chemical, microbiological and sensory quality of small berry fruit. *Food Packaging and Shelf Life*, 26:100573. doi.org/10.1016/j.fpsl.2020.100573.
24. Piechowiak T. 2021. Effect of ozone treatment on glutathione (GSH) status in selected berry fruit. *Phytochemistry*, 187: 112767. doi.org/10.1016/j.phytochem.2021.112767
25. Piechowiak T., Sowa P., Balawejder M. 2021. Effect of ozonation process on the energy metabolism in raspberry fruit during storage at room temperature. *Food and Bioprocess Technology,* 14: 483-491.
26. Piechowiak T., Antos P., Józefczyk R., Kosowski P., Skrobacz K., Balawejder M. 2019. Impact of Ozonation Process on the Microbiological Contamination and Antioxidant Capacity of Highbush Blueberry (*Vaccinium corymbosum* L.) Fruit during Cold Storage. *Ozone: Science & Engineering*, 41(4): 376-385. DOI: 10.1080/01919512.2018.1540922
27. PN-EN 12147:2000. 2000. Fruit and Vegetable Juices—Determination of Titrable Acidity. *Polish Committee for Standardization*: Warsaw, Poland.
28. PN-A-04019:1998. 1998. Food Products—Determination of Vitamin C Content. *Polish Committee for Standardization*: Warsaw, Poland.
29. PN-90/A-75101-03. 1990. Fruit and Vegetable Preserves—Sample Preparation and Physicochemical Test Methods—Determination of Dry Matter Content by Gravimetry. *Polish Committee for Standardization*: Warsaw, Poland.
30. Reszka A., Lesiów T., Mońka J. 2017. Economic conditions of cultivation and processing of highbush blueberry in Poland. *Engineering Sciences and Technologies*, 2: 25. DOI: 10.15611/nit.2017.2.04
31. Rupasinghe H.P.V., Yu L.J., Bhullar K.S., Bors B. 2012. Short Communication: Haskap (*Lonicera caeruela*): A new berry crop with high antioxidant capacity. *Can. Journal of Plant Science*, 21: 4334.
32. Skupień K. 2006. Chemical composition of selected cultivars of highbush blueberry fruit (*Vaccinium corymbosum* L.). *Folia Horticulturae*, 18: 47 – 45.
33. Skupień K. 2006. Evaluation of chemical composition of fresh and frozen blueberry fruit (*Vaccinium corymbosum* L.). *Acta Scientarium Polonorum,* 5: 19 – 25.
34. Souza V.R., Pereira P.A.P.P., Silva T.L.R., Lima L.C.O., Pio R., Queiroz F. 2014. Determination of the bioactive compounds, antioxidant activity and chemical composition of Brazilian blackberry, red raspberry, strawberry, blueberry and sweet cherry fruits. *Food Chemistry,* 156: 352-368 https://doi.org/10.1016/j.foodchem.2014.01.125
35. Szpunar-Krok E., Kuźniar P., Pawlak R., Migut D. 2021. The effect of foliar fertilization on the resistance of pea (*Pisum sativum* L.) seeds to mechanical damage. *Agronomy-Basel,* 11: 189. https://doi.org/10.3390/agronomy11010189
36. Torri E., Melos M., Caliari V., Kassuya C.A.L., Bastos J.K., Andrade S.F. 2007. Anti-inflammatory and antiociceptive properties of blueberry extract (*Vaccinium corymbosum*). *Journal of Pharmacy and Pharmacology*, 59: 591-596 DOI 10.1211/jpp.59.4.0015
37. Williams C.M., El Mohsen M.A., Vauzour D., Rendeiro C., Butler L.T., Ellis J.A., Whiteman M., Spencer J.P.E. 2008. Blueberry-induced changes in spatial working memory correlate with changes in

hippocampal CREB phosphorylation and brain-derived neurotrophic factor (BDNF) levels. *Free Radical Biology and Medicine*, 45(3): 295–305. doi: 10.1016/j.freeradbiomed.2008.04.008

38. Youdim K.A., McDonald J., Kalt W., Joseph J.A. 2002. Potential role of dietary flavonoids in reducing microvascular endothelium vulnerability to oxidative and inflammatory insults. *The Journal of Nutritional Biochemistry,* 13(5): 282–288. doi: 10.1016/S0955-2863(01)00221-2
39. Zapałowska A., Matłok N., Zardzewiały M., Piechowiak T., Balawejder M. 2021. Effect of Ozone Treatment on the Quality of Sea Buckthorn (*Hippophae rhamnoides* L.). Plants, 10, 847. https://doi.org/10.3390/plants10050847
40. Zardzewiały M., Matlok N., Piechowiak T., Gorzelany J., Balawejder M. 2020. Ozone Treatment as a Process of Quality Improvement Method of Rhubarb (*Rheum rhaponticum* L.) Petioles during Storage. *Applied Sciences,* 10: 8282. https://doi.org/10.3390/app10228282
41. Zhang H., Li K., Zhang X., Dong C., Ji H., Ke R., Ban Z., Hu Y., Lin S., Chen C. 2020. Effects of ozone treatment on the antioxidant capacity of postharvest strawberry. *RSC Advances*, 10: 38142–38157