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Changes in quality characteristics of fresh blueberries: Combined effect of cultivar and storage conditions

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ABSTRACT

The influences of two storage conditions (regular atmosphere-RA and modified-atmosphere packaging-MAP) and different storage time on fruit textural parameters, chemical composition, and total quality index (TQI) of two blueberry cultivars were investigated. Freshly harvested fruit of mid and late season cultivars ('Bluecrop' and 'Liberty', respectively) were placed in plastic punnets, packed into low-density polyethylene bags of 25 μm thickness with two perforations of 3 mm and stored at 2 °C and 90% relative humidity for 30 days, either in RA or in MAP. Changes in gas composition inside the package and fruit quality characteristics were analyzed at 10-day intervals during storage: 0, 10, 20, and 30 days. 'Liberty' was dominant over 'Bluecrop' in terms of hardness (428 g and 296 g, respectively), as well as individual and total sugars (100 and 76 g·kg^{-1}, respectively), organic acids (19 and 12 g·kg^{-1}, respectively) and most subclasses of phenolic compounds (anthocyanins, flavonols, and hydroxycinnamic acids). In addition, a novel mathematical index of TQI was introduced to compare all evaluated parameters in order to obtain a quantitative single score, as an indicator of overall fruit quality. 'Liberty' had the better TQI score in RA, whereas 'Bluecrop' behaved better in MAP. Accordingly, for longer storage of blueberry fruit MAP should not be assumed to be uniformly helpful, since the effect of storage duration in the specific type of atmosphere substantially depends on the proper cultivar selection.

1. Introduction

Highbush blueberry (*Vaccinium corymbosum* L.) is one of the most popular edible fruits worldwide, which consumption constantly increases due to its high nutritional value and delicious taste (Milivojevic et al., 2012; Okan et al., 2018; Zorenc et al., 2016). Considering that sugars and organic acids significantly affect flavor perception, their optimal ratio can act as a predictor of consumer acceptability. Since fruit taste depends not only on the total sugar and organic acid content but also on the type and quantity of individual components, their composition may reflect changes in internal fruit quality (Milivojevic et al., 2012; Talcott, 2007).

The most abundant sugars in blueberry fruit are glucose, fructose, and galactose, the sum of which is in the range between 99.02% and 99.36% of all sugars detected (Fotirić Akšić et al., 2019b). In their study, the cultivar 'Bluecrop' had a significantly higher sweetness index, which is due to the much higher level of fructose (43.047 mg·g^{-1}). Among organic acids, citric acid is prevalent in blueberry fruit, followed by quinic and malic acids (Mikulic-Petkovsek et al., 2012). As previously reported by Bremer et al. (2008), the combination of citric and malic acid provides a sour taste and affects fruit color development and decay susceptibility.

Blueberries have also gained significant attention by consumers related to a high content of health promoting compounds which are mainly represented by phenolic compounds, such as anthocyanins, flavonols, flavanols, hydroxycinnamic acids, hydrolysable and condensed

Abbreviations: RA, regular atmosphere; MAP, modified-atmosphere packaging; CA, controlled-atmosphere; TQI, total quality index; HPLC, high pressure liquid chromatography.

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tannins (Fotirić Akšić et al., 2019a; Nile and Park, 2014; Veberic et al., 2015). These berry-fruit phenolics are well known for their antioxidant, anti-inflammatory, antihypertensive, antimicrobial, and antidiabetic properties (Castrejón et al., 2008; Del Bó et al., 2013; Giongo et al., 2011; Okan et al., 2018).

Quantitative variations in sugars, organic acids, and phenolic compounds in blueberry fruit are mostly associated with the genetic background; particularly in its interaction with the environmental factors, stage of ripeness, cultivation techniques, and post-harvest manipulations (Fotirić Akšić et al., 2019a; Milivojević et al., 2016; Wang et al., 2008; Zoratti et al., 2015). Peano et al. (2015) reported that blueberries are very perishable and vulnerable to tissue damage during post-harvest handling, whereby firmness losses and the reduction in fruit weight are considered critical for long-term storage. The variable storage life among cultivars is also a result of inherent factors determining fruit quality as well as their interaction with the storage environments (Forney, 2009).

Blueberries stored in the regular atmosphere (RA) at the temperature of 0–0.5 °C and 90–95% of relative humidity can be maintained for only 2–3 weeks (Peano et al., 2015). Although temperature control is considered essential in maintaining the flavor and quality of the fresh product (Tietel et al., 2012), high relative humidity and variations in the gas composition of the storage environment are also important factors to achieve shelf life extension. In general, strategies to extend shelf life became a fundamental requirement in marketing high-quality fresh blueberry fruit. Cold storage (0–1 °C) and controlled-atmosphere (CA) techniques with elevated CO_2 (10–12%) and reduced O_2 (2–4%) are already used commercially for the long-term storability of blueberries (Hancock et al., 2008; Sargent et al., 2006). Moreover, the combined effect of sulfur dioxide fumigation followed by controlled atmosphere storage (3% O_2 + 6 or 12% CO_2) was considered a promising postharvest strategy for fresh blueberries to reduce decay and extend market life (Cantín et al., 2012).

Conditions of high CO_2 and low O_2 slow the physiological breakdown by inhibiting the ripening process or by suppressing the decay organism's activity, especially at sufficiently high CO_2 concentrations. In addition, modified-atmosphere packaging (MAP) has the potential to provide low O_2 and high CO_2 regimes similar to those of CA storage, generating a physiologically adequate O_2 partial pressure inside the package by matching total respiratory O_2 uptake of the packaged product to the total permeation through the film (Beaudry et al., 1992). Rodriguez and Zoffoli (2016) reported that the benefits of MAP were mainly attributed to the humid environment within the packages that allow reducing weight loss and symptoms of dehydration. Nevertheless, little is known about the effect of storage conditions on changes in the content of primary and secondary metabolites in blueberries (Yuan et al., 2011) and their textural fruit properties (Giongo et al., 2013). Moreover, there are contradictory studies about the responses of plant tissues to storage under a modified atmosphere (Khorshidi et al., 2011) in which the results are not conclusive in terms of variation in phenolic composition related to the storage environment.

Therefore, the purpose of this study was to investigate the effects of two storage conditions (RA and MAP) and different storage time on variation in textural parameters and chemical fruit composition of mid and late season blueberry cultivars 'Bluecrop' and 'Liberty', respectively, which were selected according to their different ability to maintain postharvest fruit quality. In addition, this study employed a novel model for calculating the total quality index (TQI) of two blueberry cultivars stored in two different conditions during the designated storage time.

2. Materials and methods

2.1. Chemicals

HPLC grade carbohydrate standards (glucose, fructose and sucrose), as well as citric and malic acid were procured from Fluka Chemie (Buchs, Switzerland), while quinic and shikimic acid were purchased from Sigma-Aldrich Chemie (Steinheim, Germany). The following standards were used for the quantification of phenolic compounds: quercetin-3-glucoside, kaempferol-3-glucoside, quercetin-3-galactoside, procyanidin B1, quercetin-3-rutinoside, quercetin-3-rhamnoside, chlorogenic acid (5-caffeoylquinic acid), delphinidin-3-glucoside, cyanidin-3-galactoside, cyanidin-3-glucoside and malvidin-3-glucoside from Sigma-Aldrich Chemie; ferulic and caffeic acid, (+)-catechin from Roth (Karlsruhe, Germany), *p*-coumaric acid and (−)-epicatechin from Fluka Chemie; quercetin-3-arabinofuranoside, quercetin-3-xyloside, quercetin-3-arabinopyranoside, and myricetin-3-rhamnoside from Apin Chemicals (Abingdon, UK); isorhamnetin-3-glucoside, petunidin-3-glucoside and peonidin-3-glucoside from Extrasynthese (Genay, France). The chemicals for the sample extractions and for the mobile phases were HPLC-MS grade methanol and acetonitrile and formic acid from Sigma-Aldrich Chemie. Water for the mobile phase was double distilled and purified with the Milli-Q system (Millipore, Bedford, USA).

2.2. Fruit sample collection

The experimental field was situated near Belgrade (44°45′N, 20°35′E, 112 m altitude), the Republic of Serbia. The climate of the region is temperate continental, with a mean annual air temperature of 10.8 °C and a mean annual precipitation of 650 mm.

Nursery plants of 'Bluecrop' and 'Liberty' (mid and late season cultivars, respectively) were imported from Austria and possessed a valid certificate that confirms the identity of both cultivars. Nurseries were planted in the spring of 2016 in 50 L polypropylene pots filled with a mix of pine sawdust (60%), white peat (30%), and perlite (10%). Pots were positioned under the protected environments of the black hail net at a distance of 0.8 m within the row and 3.0 m between the rows (4170 bushes ha^{-1}). During the vegetative season, the plants were irrigated by using four drip emitters in each pot according to sensor measurements (when the substrate water content dropped below 950 mV). Different water quantities were applied according to the weather conditions and water requirements in each stage of plant development. The electrical conductivity of the irrigation water was maintained at 0.8–1.0 dS m^{-1}, and the pH of the growing media was controlled and maintained at a sufficiently low level (4.5) by adding sulfuric acid into the irrigation water.

The plants were fertigated every day with smaller concentrations of different formulations of water soluble NPK fertilizers (0.03%), depending on the phenophase, from mid April to the end of August.

The experiment was set up in a completely randomized block design where each cultivar was represented by four replications of four bushes (a total of 16 bushes in the plot). Fruits were hand-harvested at full maturity (100% of the surface dark blue colored) during late June ('Bluecrop') and mid July ('Liberty').

2.3. Storage conditions

For each cultivar, four fruit samples consisting of 400 berries were harvested per replication and each sample of 100 berries was placed into 250 g plastic punnets. The total weight of 'Bluecrop' berries was 190 g per punnet, whereas for 'Liberty' was 200 g per punnet. Immediately after packaging, one sample was analyzed at the beginning of storage (T0), while the rest of the fruit samples were divided into two groups stored in a cold room at 2 °C and 90% RH for 30 days, either in regular air (RA) or modified atmosphere packages (MAP), using low-density polyethylene bags (DECCO, Italy) of 25 μm thickness with two perforations of 3 mm. For each cultivar, sixteen punnets were placed in both atmospheres; whereby in MAP four punnets per replication were placed into one bag. These bags slowed down the natural process of fruit senescence, eliminating harmful gases and reducing dehydration phenomena. Changes in gas composition inside the package were monitored

with a gas analyzer OxyBaby M+ (WITT-Gasetechnik GmbH & Co KG, Germany) and fruit quality characteristics were analyzed at four storage time points, as follows: T0 - the beginning of storage (0 d), T1 - after 10 days, T2 - after 20 days and T3 - after 30 days.

2.4. Texture profile analysis

Texture profile analysis of homogenous-size blueberries was conducted at harvest (T0) and during postharvest storage (T1, T2, and T3) using a texture analyser (Brookfield Ametek CT3 Texture analyser, Middleboro, USA). The trigger was set at 5 g, deformation at 9 mm with the speed of the probe set at 1.7 mm·s^{-1} during the penetration, as proposed by Giongo et al. (2013), using compression Probe TA4 (38.1 mm diameter). Hardness (as peak loads of the compression cycles), cohesiveness (as a ratio of energies expanded in compression), and springiness (the rate at which a deformed sample returns to its original size and shape) were recorded. Measurements were performed on twenty berries within each sample for 15 min after opening the packaging under the ambient conditions. Data were collected using TexturePro CT software.

2.5. Fruit color changes evaluation

The fruit color of two blueberry cultivars during the period of storage within each type of atmosphere was measured using a color analyser (RGB-1002, Lutron Electronic Enterprise CO., LTD, Taiwan). Measurements were performed on twenty berries within each sample. Data were expressed in CIELAB coordinates (L^*, a^*, and b^*). Total color difference (ΔE) was determined by using Eq. (1) (Hunter and Harold, 1987):

$$\Delta E = \sqrt{(a^* - a_o^*)^2 + (b^* - b_o^*)^2 + (L^* - L_o^*)^2} \quad (1)$$

Average values for a_o, b_o, L_o were obtained from the fresh blueberries (T0) in order to analyse changes within the subset of each cultivar depending on packaging and storage time. Differences (ΔE) were calculated per individual berry and then averaged. L scale is associated with light/dark dimension; a scale for red vs. green and the b scale for yellow vs. blue (Mokrzycki and Tatol, 2011).

2.6. Extraction and determination of sugars and organic acids

Primary metabolites (sugars and organic acids) were analyzed in whole berry fruit. Four replicates were performed for each blueberry cultivar; for both storage conditions and each storage time treatment, each replicate included 20 fruits. For extraction of primary metabolites, 4 g of fruit was ground to a fine paste in a mortar and homogenized with 18 mL of double-distilled water. The extraction of sugars and acids was carried out for half an hour at room temperature with constant stirring. After the extraction, the methods followed procedures reported by Mikulic-Petkovsek et al. (2012). Sugars and organic acids were analyzed by a HPLC system (Thermo Scientific, Finnigan Spectra System, USA). Sugars analysis was carried out with Rezex RCM-monosaccharide Ca+ 2% column (Phenomenex) heated at 65 °C and by isocratic elution for 30 min using the bidistilled water at a flow rate of 0.6 mL min^{-1} and measured with refractive index (RI) detector. For organic acid separation, we used a UV detector set at 210 nm, Rezex ROA column (Phenomenex) operated at 65 °C and the mobile phase was 4 mM sulfuric acid with a flow rate of 0.6 mL min^{-1}. Sugars and organic acids content levels were expressed in mg g^{-1} on a fresh weight basis of blueberry fruit.

2.7. Extraction and determination of phenolic compounds using HPLC–DAD–MSn analysis

For the determination of phenolic compounds, four replications (each including 20 fruits) were carried out for both cultivars from each storage condition and storage time treatment. Berries were homogenized with liquid nitrogen and 6 g of homogenate was extracted with 12 mL of methanol containing 3% (v/v) formic acid in a cooled ultrasonic bath for 1 h. The fruit extracts were centrifuged at 9700 × g for 7 min at 4 °C, and the supernatant was filtered through a 0.20 μm Chromafil AO-20/25 polyamide filter (Macherey-Nagel) into a vial pending analysis (Mikulic-Petkovsek et al., 2015). Phenolic compounds were analyzed on a Thermo Scientific Accela HPLC system (Thermo Fisher Scientific) with a diode array detector (DAD) at 280 nm (flavanols, hydroxycinnamic and hydroxybenzoic acid derivatives), 350 nm (flavonols), and 530 nm (anthocyanins) according to the procedures previously described by Mikulic-Petkovsek et al. (2015).

Phenolic components were identified by comparing their UV–vis spectra and retention time with standards and were also confirmed using a mass spectrometer (Thermo Scientific, LCQ Deca XP MAX) with an electrospray interface (ESI) operating in positive (for anthocyanins) and negative (for all other phenolics) modes. Data-dependent MSn scanning with a full scan from m/z 115–1700 was performed. All conditions on the mass spectrometer were the same as reported by Mikulic-Petkovsek et al. (2015). Phenolic contents were expressed in mg kg^{-1} on a fresh weight basis of blueberry fruit.

2.8. Total quality index (TQI)

The novel method of calculating a unique TQI is capable of assessing the effects of different storage conditions on the quality of fresh blueberries in a quantitative way. The main advantage of such an approach is that all quality parameters are evaluated regardless of the unit they are expressed and presented as a single score (Djekic et al., 2017).

The set of quality parameters was separated into two distinct groups in line with the study of Finotti et al. (2007) and Djekic et al. (2018), depending on the two rules applied (Table 1). Quality indexes (QI) were considered as vectors in a newly developed Euclidean space (QI_1, QI_2, … QI_N) ⊂ R^N (N - number of quality characteristics). Upon calculation of all QIs, the total quality index (TQI) was calculated using the Eq. (2) (Finotti et al., 2007):

$$TQI = \sqrt{\sum_{j=1}^{N} (QI_j)^2} \quad (2)$$

Interpretation of the calculated TQI is as follows: 'the lower the TQI value, the better the quality', Measuring the distance of TQI from the origin of the vector, means that 'the nearer from the origin, the better the TQI', and vice versa.

2.9. Statistical analysis

All data were subjected to analysis of variance (ANOVA). Tukey's HSD post-hoc test was used to compare treatments when ANOVA showed significant differences among means. The level of statistical significance was set at 0.05. Statistical processing was performed using Microsoft Excel 2010 (Microsoft Corporation, USA) and SPSS Statistics

Table 1
Rules for calculating quality indices of specific quality characteristics.

Rule	Formula	Quality characteristics
The lower the value, the better the quality	$QI = \frac{x_i}{x_{max}}$	Total acids content; total color difference
The higher the value, the better the quality	$QI = \frac{x_{max} - x_i}{x_{max} - x_{min}}$	Total sugars content; total anthocyanins; total flavonols; total hydroxycinnamic acids; total flavanols; hardness; cohesiveness, springiness

QI – quality index for a parameter; xi – measured value in the subset of values; xmax – maximal value in the subset of values; xmin – minimal value in the subset of values.

17.0 (IBM, Armonk, NY, USA).

3. Results and discussion

3.1. Effects of storage period at different atmospheres on the fruit texture and color changes

Since the texture is a significant sensory attribute in whole fruit, which changes during storage could have a profound effect on consumer acceptability, it can be used as an indicator of fruit postharvest quality. Testing of fruit texture for scientific purposes provides information on the storability and resistance to injury of fruit that could be extremely useful for their storage management and marketing.

In our texture experiment, three mechanical textural attributes were investigated: (1) hardness - the force required to compress the sample (2) cohesiveness - the degree to which fruit can be deformed before it breaks, and (3) springiness - the rapidity and degree of recovery from a deforming force (Di Monaco et al., 2008). In Table 2 it is shown that fruit hardness was lower in cv. 'Bluecrop' meaning that this cultivar is softer than 'Liberty'. The hardness of both cultivars gradually decreased during storage, while cohesiveness and springiness did not significantly differ between cultivars, packaging types, and during the storage time, with the exception of springiness which increased during storage.

In cv. 'Liberty' no statistical differences were observed between the two packaging types for hardness. In the same cultivar, after 10 days cohesiveness and springiness were statistically different between the two packaging atmospheres. Cell walls provide support to plant cells, contributing to the cohesiveness and springiness of the plant tissue (Guiné et al., 2011). The changes associated with cell wall disassembly could be related to the hemicellulosic polymers depolymerization accompanying specific fruit ripening and softening phases, previously reported for the cell wall of the "soft fruit" (Vicente et al., 2007). The turgor pressure of mesocarp cells could also be an important texture factor in small fruits such as blueberry. The linear relationship between elasticity and turgor pressure during grape berry development suggested that the latter may be the primary component that determines fruit softening (Thomas et al., 2008). Physiological changes at the cell or membrane level could lead to morphological modifications at the tissue level. This indicates that MAP conditions may delay fruit softening of specific blueberry cultivars, thus remaining the overall fruit quality and improving the resistance to postharvest storage.

The color of blueberry fruit depends on anthocyanin profile and content, having both aesthetic and nutritional values (Spinardi et al., 2019). However, the low stability of these pigments could cause serious difficulties during storage: they could easily undergo condensation reactions with procyanidins, be degraded as a result of endogenous enzyme activities (peroxidase and polyphenol oxidase) or be bonded to macromolecules, such as proteins and cell-wall polysaccharides forming a precipitate (Brownmiller et al., 2009). This could cause a shift of skin color from blue-purple toward a reddish hue, thereby increasing the risk of rejection by consumers. Therefore, the investigation of the color properties of blueberry fruit during storage in different atmospheres could be useful for the development of an effective preservation strategy with high retention of anthocyanins. Besides anthocyanins, the light blue color of fresh blueberries is also determined by the amount of waxy 'bloom' (quantity and structure) on the skin. This whitish material or 'bloom' on the surface of the fruit is a rather thin and fragile wax deposit which makes it sensitive to even gentle brushing and bouncing of the fruit. Hence, preservation of the waxy bloom during handling and storage is an important goal for shelf life extension (Retamales and Hancock, 2018).

Total color difference (ΔE) is a colorimetric parameter extensively used to characterize the variation of colors in fruit. If the difference between the samples is less than 1.0, it is assumed that the difference in color would not be sensitively perceptible. When this value is below 2.0, trained observers would notice the difference, while when this value is over 3.5, a clear color difference could be noticed even by average observers (Mokrzycki and Tatol, 2011). In this study, cv. 'Bluecrop' displayed slightly higher color changes than 'Liberty' (Table 3). On the other side, atmosphere packaging and storage time did not affect the color differences of fruit. It was previously reported that the ΔE of table grape fruit increased after 10 days of storage, indicating that metabolic activity such as enzymatic browning occurs during storage (Watanabe et al., 2018). In addition, chlorophyll degradation is one of the factors affecting the color changes in fruit flesh during storage (Montefiori et al., 2005; Park et al., 2018). Based on the results, color changes in both blueberry cultivars were not statistically different and not noticeable by an average consumer (max ΔE<2.5).

3.2. Effects of storage period at different atmospheres on biochemical constituents of fruit

It is well known that sugars and acids are principal biochemical drivers of human sensory experiences (Yarmolinsky et al., 2009). Perceived sweetness is best elucidated by measures of sugars, primarily glucose and fructose, but much less sucrose (Gilbert et al., 2015). As previously reported, sucrose was presented in very low amounts in the blueberry fruit, suggesting the action of invertase, a cell wall-bound

Table 2
The effects of different atmospheres and storage periods on the fruit textural properties of the two selected blueberry cultivars.

Treatments	Hardness (g)	Cohesiveness	Springiness (mm)
'Bluecrop'	296 ± 15.3^{b}	0.14 ± 0.01	6.71 ± 0.08
'Liberty'	428 ± 10.2^{a}	0.11 ± 0.01	6.68 ± 0.18
F cultivar (C)	* **	*ns*	*ns*
RA	349 ± 22.2	0.14 ± 0.01	6.63 ± 0.14
MAP	375 ± 14.6	0.12 ± 0.01	6.76 ± 0.14
F atmosphere (A)	*ns*	*ns*	*ns*
0 d	410 ± 22.3^{a}	0.12 ± 0.01	6.27 ± 0.31^{b}
10 d	376 ± 25.1^{ab}	0.12 ± 0.01	6.83 ± 0.09^{ab}
20 d	344 ± 27.1^{b}	0.12 ± 0.01	6.67 ± 0.15^{ab}
30 d	319 ± 26.7^{b}	0.15 ± 0.02	7.02 ± 0.10^{a}
F storage period (SP)	* *	*ns*	*
F C × A	*ns*	*ns*	*ns*
F C × SP	*ns*	*ns*	* *
F A × SP	*ns*	*ns*	*ns*
F C × A × SP	*ns*	*ns*	*ns*

RA (regular atmosphere); MAP (modified atmosphere packaging); Blueberry cultivars: 'Bluecrop'; 'Liberty'. Values are the arithmetic mean ± standard error. Different letters in columns denote significant differences among the treatments (Tukey's HSD test, P ≤ 0.05). Statistically significant differences at * P < 0.05. ** P < 0.01. *** P < 0.001; ns - not significant.

Table 3
The effects of different atmospheres and storage periods on the fruit color properties of the two selected blueberry cultivars.

Treatments	Total color difference (ΔE)
'Bluecrop'	1.84 ± 0.09
'Liberty'	1.64 ± 0.15
F cultivar (C)	*ns*
RA	1.80 ± 0.10
MAP	1.68 ± 0.15
F atmosphere (A)	*ns*
10 d	1.86 ± 0.21
20 d	1.57 ± 0.12
30 d	1.80 ± 0.12
F storage period (SP)	*ns*
F C × A	*ns*
F C × SP	*ns*
F A × SP	*ns*
F C × A × SP	*ns*

RA (regular atmosphere); MAP (modified atmosphere packaging); Blueberry cultivars: 'Bluecrop'; 'Liberty'. Values are the arithmetic mean ± standard error. ns - not significant (Tukey's HSD test, P ≤ 0.05).

enzyme responsible for sucrose hydrolysis to glucose and fructose, which was found to be inversely proportional to sucrose concentration (Kader et al., 1993). Correspondingly, Table 4 showed that in both cultivars concentrations of reducing sugars (glucose and fructose) were presented in almost equal proportion, while sucrose was detected in far smaller concentrations.

The content of all sugar compounds was significantly higher in 'Liberty' cultivar, including fruit stored in a regular atmosphere. Glucose varied most on the basis of cultivar and atmosphere, but with no significant variability for the storage period. The content of fructose increased during storage reaching the highest point after 30 d, with an opposite pattern for sucrose, indicating enhancement of invertase activity. Kader et al. (1993) also noticed a highly positive correlation (r^2 =0.97) between invertase activity and the degree of ripeness of blueberry fruit during cold storage designated by lowering of sucrose content. This is due to the breathing processes that use sugars as organic substrate, which is less pronounced in MAP-treated fruit due to the use of gas (Tinebra et al., 2021). So, in MAP fruits gasses decrease the respiration rate, thus decreasing the consumption of substrates, resulting in higher sugar content. Furthermore, fruits treated with MAP showed an increased sugar/acidity ratio during the storage period, thus suggesting good quality characteristics for consumption, even after storage (Medlicott and Thompson, 1985). These findings may have useful implications for producers and stakeholders, since that the recommendation of specific cultivars for MAP storage could contribute to better fruit behavior on the market shelf.

The earliest reference dealing with the organic acid composition of highbush blueberries is that of Nelson (1927) who reported citric acid as predominant in blueberry fruit, followed by malic acid to a far less extent. Back in the early 60 s, 16 organic acids were identified in the fruit of two highbush blueberry cultivars ('Rubel' and 'Jersey'), but predominant organic acids were citric, malic, and quinic averaging more than 80% (Markakis et al., 1963). The same authors reported quantitative changes in non-volatile organic acids during the ripening of the fruit. A few decades later, the profile of organic acids in 6 highbush blueberry cultivars reported the contribution of citric acid at about 75%, succinic acid at 17%, while malic and quinic acid were presented at < 5% each (Ehlenfeldt et al., 1994). As opposed to that, our cultivars revealed a higher level of quinic acid that was equally represented with citric acid, making more than 90% of total acids, while malic acid contributed with 6–8%, but shikimic acid < 0.2%, regardless of cultivar, atmosphere or storage period (Table 5).

In the study of Ehlenfeldt et al. (1994), it was also observed that the total quantity of acids decreased during ripening in highbush blueberry fruit due to a decrease in citric acid content, because it was superior in the composition of the total acids. Alike, citric acid content decreased during the storage period in our study, while quinic acid didn't change significantly, thus less affecting the downtrend of total acids content during storage.

The determination of the phenolic composition of the blueberries allows one to estimate the content of all compounds belonging to the subclasses of phenolic compounds (Table 6) among which anthocyanins have received the most attention. Up to 60% of the total phenolic content in blueberries is accounted for anthocyanins, which are responsible for the blue color in fruit and are dependent on environmental pH values (Milivojević et al., 2016; Okan et al., 2018; You et al., 2011). Okan et al. (2018) have reported that 16 different types of anthocyanins are responsible for the coloring of blueberries ranging from bright red to purple/blue depending on their composition, concentration, and structure - which electron donor groups (methoxy or hydroxyl) are bonded to the aglycones. Since color is a parameter of fruit quality, its determination is useful to correlate with the concentration of the pigments present in the fruit. In the present study, anthocyanins were the dominant among four investigated phenolic subgroups, ranging from 48% to 59% ('Bluecrop' and 'Liberty', respectively), which content was affected by cultivar, atmosphere, and storage period. When comparing cultivars, a significantly higher average content of total anthocyanins was registered in cv. 'Liberty', whereas RA expressed a more prominent effect on

Table 4
Content of sugars (g·kg^{-1}) of the two selected blueberry cultivars determined at different atmospheres and storage periods.

Treatments	Glucose	Fructose	Sucrose	Total sugars
'Bluecrop'	35.9 ± 0.49^{b}	39.5 ± 0.67^{b}	0.22 ± 0.02^{b}	75.6 ± 1.01^{b}
'Liberty'	49.1 ± 1.21^{a}	49.7 ± 1.33^{a}	0.70 ± 0.10^{a}	99.5 ± 2.41^{a}
F cultivar (C)	* **	* **	* **	* **
RA	45.3 ± 1.81^{a}	47.4 ± 1.67^{a}	0.38 ± 0.07^{b}	93.1 ± 3.46^{a}
MAP	39.7 ± 1.22^{b}	41.8 ± 1.01^{b}	0.54 ± 0.10^{a}	82.0 ± 2.15^{b}
F atmosphere (A)	* **	* **	* *	* **
0 d	42.0 ± 1.93	43.0 ± 1.75^{b}	0.76 ± 0.15^{a}	85.9 ± 3.81
10 d	41.8 ± 2.01	43.2 ± 1.42^{b}	0.54 ± 0.13^{b}	86.3 ± 3.23
20 d	43.3 ± 2.62	45.4 ± 2.53^{ab}	0.35 ± 0.08^{bc}	88.2 ± 5.12
30 d	42.9 ± 2.84	46.7 ± 2.53^{a}	0.20 ± 0.04^{c}	89.8 ± 5.31
F storage period (SP)	*ns*	*	***	*ns*
F C × A	***	**	***	***
F C × SP	*ns*	**	***	*ns*
F A × SP	***	**	*ns*	***
F C × A × SP	**	*ns*	*ns*	*

RA (regular atmosphere); MAP (modified atmosphere packaging); Blueberry cultivars: 'Bluecrop'; 'Liberty'. Values are the arithmetic mean ± standard error. Different letters in columns denote significant differences among the treatments (Tukey's HSD test, P ≤ 0.05). Statistically significant differences at * P < 0.05, ** P < 0.01, *** P < 0.001; ns - not significant.

Table 5
Content of organic acids (g·kg^{-1}) of the two selected blueberry cultivars determined at different atmospheres and storage periods.

Treatments	Citric acid	Malic acid	Quinic acid	Shikimic acid	Total acids
'Bluecrop'	5.01 ± 0.24^{b}	1.04 ± 0.02^{b}	5.92 ± 0.12^{b}	0.02 ± 0.00^{b}	12.0 ± 0.34^{b}
'Liberty'	8.52 ± 0.27^{a}	1.13 ± 0.06^{a}	8.87 ± 0.28^{a}	0.04 ± 0.00^{a}	18.7 ± 0.34^{a}
F cultivar (C)	* **	* *	* **	* **	* **
RA	6.40 ± 0.43^{b}	1.20 ± 0.05^{a}	7.78 ± 0.46^{a}	0.03 ± 0.00	15.5 ± 0.88
MAP	7.14 ± 0.42^{a}	0.97 ± 0.02^{b}	7.01 ± 0.24^{b}	0.03 ± 0.00	15.2 ± 0.65
F atmosphere (A)	* **	* **	* **	*ns*	*ns*
0 d	8.51 ± 0.59^{a}	0.99 ± 0.02^{b}	7.75 ± 0.35	0.05 ± 0.01^{a}	17.4 ± 0.94^{a}
10 d	6.72 ± 0.52^{b}	1.06 ± 0.04^{ab}	7.13 ± 0.40	0.03 ± 0.01^{b}	14.9 ± 0.90^{b}
20 d	6.10 ± 0.52^{c}	1.13 ± 0.07^{a}	7.38 ± 0.64	0.03 ± 0.00^{b}	14.7 ± 1.12^{b}
30 d	5.74 ± 0.60^{c}	1.16 ± 0.09^{a}	7.32 ± 0.69	0.03 ± 0.00^{b}	14.3 ± 1.24^{b}
F storage period (SP)	***	**	*ns*	***	***
F C × A	*ns*	***	***	*ns*	***
F C × SP	*ns*	***	**	*ns*	*
F A × SP	*ns*	***	**	*ns*	*ns*
F C × A × SP	*ns*	**	***	*ns*	**

RA (regular atmosphere); MAP (modified atmosphere packaging); Blueberry cultivars: 'Bluecrop'; 'Liberty'. Values are the arithmetic mean ± standard error. Different letters in columns denote significant differences among the treatments (Tukey's HSD test, P ≤ 0.05). Statistically significant differences at * P < 0.05, ** P < 0.01, *** P < 0.001; ns - not significant.

Table 6
Content (mg·kg^{-1}) of total anthocyanins, flavonols, flavanols and hydroxycinnamic acids of the two selected blueberry cultivars determined at different atmospheres and storage periods.

Treatments	Total anthocyanins	Total flavonols	Total flavanols	Total hydroxycinnamic acids
'Bluecrop'	718 ± 20.9^{b}	107 ± 3.76^{b}	236 ± 4.17^{a}	423 ± 17.8^{b}
'Liberty'	1213 ± 38.8^{a}	216 ± 5.43^{a}	107 ± 1.92^{b}	532 ± 39.7^{a}
F cultivar (C)	* **	* **	* **	* **
RA	999 ± 64.9^{a}	168 ± 13.2^{a}	177 ± 14.0^{a}	491 ± 38.9^{a}
MAP	933 ± 54.2^{b}	155 ± 11.2^{b}	166 ± 13.7^{b}	465 ± 24.9^{b}
F atmosphere (A)	* *	* **	* **	* **
0 d	814 ± 66.7^{d}	133 ± 16.1^{d}	158 ± 14.6^{c}	283 ± 11.9^{d}
10 d	905 ± 70.7^{c}	155 ± 15.4^{c}	169 ± 20.7^{b}	465 ± 25.9^{c}
20 d	1004 ± 79.1^{b}	170 ± 17.1^{b}	179 ± 22.0^{a}	536 ± 35.9^{b}
30 d	1141 ± 96.0^{a}	188 ± 18.5^{a}	180 ± 21.4^{a}	627 ± 32.5^{a}
F storage period (SP)	***	***	**	***
F C × A	*	***	*ns*	***
F C × SP	*	***	**	***
F A × SP	*ns*	***	**	***
F C × A × SP	*ns*	***	*ns*	***

RA (regular atmosphere); MAP (modified atmosphere packaging); Blueberry cultivars: 'Bluecrop'; 'Liberty'. Values are the arithmetic mean ± standard error. Different letters in columns denote significant differences among the treatments (Tukey's HSD test, $P \leq 0.05$). Statistically significant differences at * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; ns - not significant.

anthocyanin concentration. Evaluating the phytochemical content in different parts of blueberry fruit, Pertuzatti et al. (2016) found a total content of anthocyanins in the range of 700–2000 mg kg^{-1} in the whole fruit, similar to the values found in the present study. In the examination of the total flavonols and flavanols of the blueberries (Table 6), higher values of both subclasses were determined in the regular atmosphere. However, an abundance of flavonols was detected in cv. 'Liberty', while cv. 'Bluecrop' was rich in flavanols, and the values were in line with other studies (Justesen et al., 1998; Ochmian et al., 2015).

Flavonoids represent the important subgroup of polyphenols that are believed to be the most relevant constituents contributing to the blueberry's health benefits. Due to the health implication of blueberry consumption, there has been scientific interest in flavonoids characterization and profiling, including the effect of plant growth conditions and fruit maturity level on the concentration of blueberry flavonoids (Castrejón et al., 2008; Wang et al., 2008; Zheng et al., 2003). However, comprehensive studies of external and internal fruit quality attributes of blueberry cultivars, including different storage conditions, are still limited.

In the course of our study, an increase in the content of all detected phenolic compounds in fruit during storage was found statistically significant (Table 6). During postharvest storage, phenolics could be synthesized from nonphenolic constituents resulting in their accumulation in fruit after removing from the plant (Kalt et al., 2003). After harvest, respiratory metabolism and antioxidant synthesis continue in fruit. Thus, carbon skeletons for phenolic synthesis could be obtained from carbohydrates or organic acids, consequently increasing their content. In this respect, prolonged synthesis of anthocyanins during storage has been previously documented in blueberries (Kalt and McDonald, 1996; Mallik and Hamilton, 2017).

Four flavonoid groups, anthocyanins, flavonols, flavan-3-ols, and hydroxycinnamic acids, which commonly exist as plant secondary metabolites, are covered by our study. Individual compounds of two selected blueberry cultivars exposed to different atmospheres and storage periods were separated, identified and quantified by HPLC. Obtained anthocyanin profile indicated different glycone moiety: glucosides, galactosides and arabinosides, as well as acetylglucosides, of the five common blueberry anthocyanidins (delphinidin, cyanidin, petunidin, peonidin and malvidin) in both cultivars. The glycosylation pattern of 'Liberty' showed that 43% of anthocyanins were presented as galactosides, 22% as glucosides, 34% as arabinosides and less than 1% as acetylglucosides. In 'Bluecrop' glycosylation pattern was more uniform, where 29% of anthocyanins were presented as galactosides, 31% as glucosides, 33% as arabinosides and up to 7% as acetylglucosides. Among 15 detected anthocyanin compounds (Table S1), delphinidin-3-galactoside was predominant in both cultivars, 'Bluecrop' and 'Liberty': 25% and 38% of total anthocyanin content, respectively (Table 7). Similarly, in the study of Bunea et al. (2011), delphinidin-3-galactoside was the major anthocyanin contributor in 'Bluecrop' together with peonidin-3-glucoside, each represented by 17%. In the present study, the latter was represented to a far lesser extent (around 4%) in 'Bluecrop', while the joint contribution of these two compounds was around 30%.

A total of 19 flavonols were detected, of which 9 compounds were derived from quercetin, 3 from isorhamnetin, 2 from myricetin, laricitrin, and kaempferol, and 1 from syringetin (Table S2). As dominant flavonols (Table 7), quercetin-3-rhamnoside and quercetin-3-rutinoside accounted for 64% of quercetin derivatives, which concentrations were followed by laricitrin-, myricetin-, isorhamnetin- and kaempferol-derivatives (Table S2). Our HPLC profile corresponds to those reported in the literature (Becker Pertuzatti et al., 2021; Vrhovsek et al., 2012). The content of quercetin-3-rutinoside is in line with previously reported data for 'Bluecrop' (19 mg·kg^{-1}), while quercetin-3-rhamnoside content was 4-fold higher than the value of 12.6 mg·kg^{-1} reported by the same authors (Cho et al., 2005).

The term "flavanols" is commonly used to refer to the related subgroup of flavonoids "flavan-3-ols". These strong polyphenolic antioxidants found in berries are more abundant in the external tissues of fruit (Nile and Park, 2014). The condensation of the monomeric flavonoids, such as catechins and epicatechins, leads to the formation of procyanidins dimers (ProCy: Cat-epiCat and epiCat-epiCat). ProCy composition was found to be the strongest driver in genotypic profiles (Günther et al., 2020), thus the concentration of measured ProCy, as well as monomers, differed among cultivars – it was higher in 'Bluecrop' compared to 'Liberty' (Table S3). It was interesting that ProCy concentrations declined during storage despite the increase of monomers concentration, but without affecting total flavanols content (Table 6) which indicates the favorisation of monomeric forms during storage.

Among phenolic compounds produced in fruit flesh, hydroxycinnamic acid derivatives play an important role because of both their abundance and diversity. Derived from cinnamic acid, they are essentially present in fruit as combined forms of the four basic molecules: caffeic, ferulic, *p*-coumaric, and sinapic acids, but rare as free forms (Murkovic, 2003). In the group of 8 detected hydroxycinnamic acids (Table S4), derivatives of caffeic acids were dominant in both tested cultivars (Table 7). 5-caffeoylquinic acid or chlorogenic acid was found to be the dominant compound in blueberries, with the amount of 254 mg·kg^{-1} (Zheng et al., 2003) and 400 mg·kg^{-1} for the 'Bluecrop' cultivar (Rodriguez-Mateos et al., 2012), which was in line with its content in this study. Widely distributed in blueberry fruit, chlorogenic acid is a major compound among the phenolic compounds with the highest radicals scavenge effect (Sawa et al., 1999) which, together with other bioactive compounds presented, makes blueberry a rich source of natural antioxidants.

Most phenolic compounds had higher concentrations in the regular atmosphere, while the content of all quantified compounds was gradually increased during storage. Besides prolonged secondary metabolism during storage, the increase of the content of various classes of phenolic

Table 7
Content (mg·kg^{-1}) of representative compounds in each subclass of anthocyanins, flavonols, flavanols and hydroxycinnamic acids of the two selected blueberry cultivars determined at different atmospheres and storage periods.

	Anthocyanins	Flavonols		Flavanols	Hydroxycinnamic acids	
	delphinidin-3-galactoside	quercetin-3-rhamnoside	quercetin-3-rutinoside	catechin	caffeic acid hexoside 2	5-caffeoylquinic acid (chlorogenic acid)
'Bluecrop'	180 ± 7.01^{b}	58.8 ± 1.44^{a}	20.9 ± 0.55^{b}	141 ± 4.55^{a}	54.3 ± 0.86^{b}	321 ± 16.1^{b}
'Liberty'	459 ± 8.10^{a}	10.8 ± 0.41^{b}	40.7 ± 0.47^{a}	31.4 ± 1.12^{b}	136 ± 16.8^{a}	340 ± 21.9^{a}
F cultivar (C)	* **	* **	* **	* *	* **	* **
RA	325 ± 30.9	36.6 ± 5.42^{a}	31.7 ± 2.18^{a}	89.9 ± 12.3^{a}	110 ± 17.3^{a}	326 ± 21.2^{b}
MAP	313 ± 29.1	33.0 ± 4.75^{b}	29.9 ± 2.05^{b}	82.2 ± 11.4^{b}	80.1 ± 10.5^{b}	335 ± 17.2^{a}
F atmosphere (A)	*ns*	* **	* **	* *	* **	* *
0 d	290 ± 43.1^{b}	29.7 ± 6.52^{d}	27.7 ± 3.21^{d}	66.4 ± 12.4^{c}	36.6 ± 3.80^{d}	202 ± 9.33^{d}
10 d	299 ± 41.9^{b}	32.9 ± 6.88^{c}	30.3 ± 2.93^{c}	85.1 ± 16.9^{b}	89.0 ± 13.3^{c}	327 ± 15.6^{c}
20 d	330 ± 42.6^{a}	37.0 ± 7.76^{b}	31.9 ± 2.92^{b}	95.0 ± 18.7^{a}	114 ± 19.3^{b}	368 ± 16.5^{b}
30 d	358 ± 43.2^{a}	39.5 ± 7.97^{a}	33.3 ± 2.98^{a}	97.5 ± 18.3^{a}	141 ± 26.2^{a}	424 ± 9.7^{a}
F storage period (SP)	* **	* **	* **	* *	* **	* **
F C × A	*ns*	* **	*	* *	* **	* **
F C × SP	*ns*	* **	* **	* *	* **	* **
F A × SP	*ns*	* **	* **	* *	* **	* **
F C × A × SP	*ns*	* **	*ns*	* *	* **	* **

RA (regular atmosphere); MAP (modified atmosphere packaging); Blueberry cultivars: 'Bluecrop', 'Liberty'. Values are the arithmetic mean ± standard error. Different letters in columns denote significant differences among the treatments (Tukey's HSD test, $P \leq 0.05$). Statistically significant differences at * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; ns - not significant.

compounds could be explained by changes in the molecular structure of some phenolic compounds that could lead to a switch from some classes to others, as well as tissue changes and degradation that could influence the extractability of pigments (Cocetta et al., 2015). The storability and preservation of antioxidant quality attributes in a modified atmosphere during 2–4 weeks of storage had been reported to vary among different blueberry cultivars (Connor et al., 2002). In the present study, a statistically significant influence of two factors - cultivar and atmosphere was inhomogeneous (Table 7, S1-S4), so no clear positive influence can be linked to the use of a specific type of storage for fresh blueberry fruit. Likewise, in the study of Hancock et al. (2008) a significant enhancement in the storage life of 'Liberty' and 'Bluecrop' fruit held under controlled atmospheric conditions was not observed.

3.3. Changes in total quality index (TQI) during storage period at different atmospheres

Total quality index (TQI) is a mathematical model proposed to compare individual parameters evaluated using different units in order to obtain a single score for an overall fruit quality (Djekic et al., 2018; Finotti et al., 2007) and it was found as a reliable tool in monitoring during shelf life (Djekic et al., 2017). The method of calculating unique TQI enables the comparison and quantitative evaluation of blueberry fruit of different cultivars packed in different atmospheric conditions. Thus, Fig. 1 shows the final TQI scores of the two cultivars 'Bluecrop' and 'Liberty' stored in two different conditions (RA and MAP). At the beginning of the study, cv. 'Bluecrop' had a better TQI compared to cv. 'Liberty'. After 10 days, TQI scores changed places and kept the same

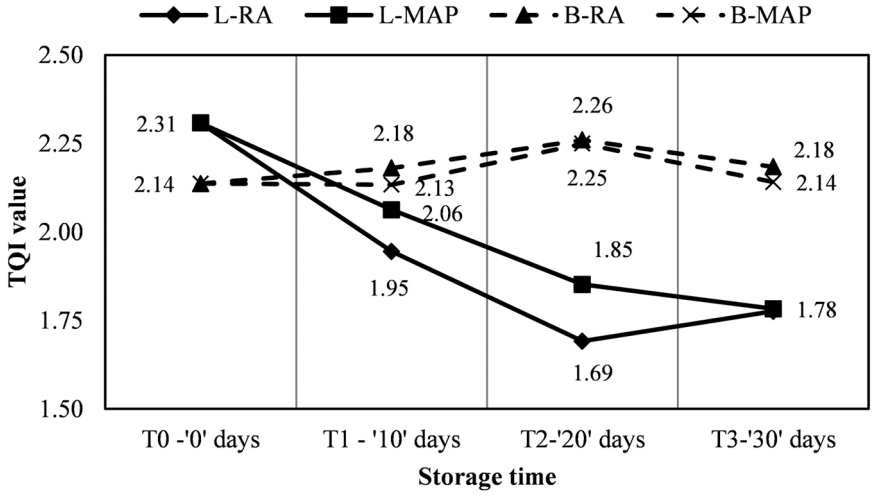

Fig. 1. Total quality index (TQI) of the two blueberry cultivars stored in two different conditions during shelf life. L – Liberty; B – Bluecrop; RA - regular atmosphere; MAP - modified-atmosphere packaging; T0 - 0 days, T1 - 10 days, T2 –20 days; T3 30 days.

trend until the end of the observed shelf life. Regarding the two storage conditions, RA better scored for cv. 'Liberty', opposed to MAP with better TQI scores associated with the cv. 'Bluecrop'. TQI showed that intensive changes occurred after 10 days of storage when differences in the overall fruit quality of blueberry cultivars stored in diverse atmospheric conditions were clearly distinguished.

4. Conclusions

The research of mid and late season blueberry cultivars 'Bluecrop' and 'Liberty', respectively, indicated that fruit textural properties, color, chemical composition and total quality index were dependent mainly upon the cultivar, while storage conditions (RA and MAP) are less effective on variation in tested parameters. In parallel, a novel mathematical index of TQI was introduced to compare all analyzed parameters in order to acquire a single quantitative score. TQI can be employed as a simple tool for monitoring overall fruit quality during storage by transferring obtained quality attributes to measurable features. Regarding TQI for different storage conditions, cv. 'Liberty' had the better score in RA, whereas cv. 'Bluecrop' behaved better in MAP. In conclusion, MAP for longer storage of blueberry fruit should not be assumed to be uniformly helpful; instead, we reckon that the effect of storage duration in a specific type of atmosphere depends on the proper cultivar selection. Changes in the content of primary and secondary metabolites of blueberries during storage at different atmospheres, concerning their textural fruit properties, provide new knowledge in the field of postharvest manipulation of blueberry fruit in order to select promising cultivars with higher fruit quality traits at the specific storage conditions.

Data availability statement

Data sharing is not applicable to this article as all new created data is confidential.

CRediT authorship contribution statement

Jelena Dragišić Maksimović: Investigation, Visualization, Writing – original draft, Writing – review & editing. **Jasminka Milivojević:** Conceptualization, Investigation, Resources, Writing – review & editing, Visualization, Supervision. **Ilija Djekić:** Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing – review & editing. **Dragan Radivojević:** Data curation, Investigation, Writing – review & editing, Visualization. **Robert Veberič:** Formal analysis, Methodology. **Maja Mikulič-Petkovšek:** Formal analysis, Methodology, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper in any way.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jfca.2022.104597.

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