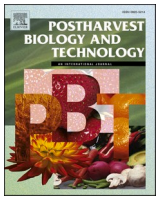
Postharvest Biology and Technology 222 (2025) 113355

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio



Ester content of blueberry fruit can be ruled by tailored controlled atmosphere storage management

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ARTICLE INFO

Keywords:
Vaccinium spp
VOCs
Texture
PTR-ToF-MS
SPME/GC-MS
Germplasm
Postharvest
Aroma

ABSTRACT

This study examines the effects of controlled atmosphere (CA) storage with high CO2 concentration (16 KPa) on the volatile organic compound (VOC) profile of blueberries (*Vaccinium* spp.), considering their genetic variability. The research focuses on the *denovo* production of esters and their association with fermentation related VOCs, employing complementary analytical techniques for comprehensive VOC profiling: direct injection mass spectrometry using Proton Transfer Reaction - Time of Flight - Mass Spectrometry (PTR-ToF-MS) and gas chromatography utilizing Solid-phase microextraction coupled to gas chromatography-mass spectrometry (SPME/GC-MS).

In the first experiment, PTR-ToF-MS and SPME/GC-MS were applied to analyze the volatilome of seven blueberry cultivars under regular (RA) and controlled (CA) atmosphere storage conditions for 42 days. In the second experiment, 39 cultivars were tested to evaluate genetic variability in response to CA storage using PTR-ToF-MS. The third experiment focused on the effect of different oxygen concentrations during storage (1, 7, and 12 kPa O2), studying four cultivars using PTR-ToF-MS.

Results: of the three experiments revealed high variability among *Vaccinium* genotypes for all quality traits, which was amplified during storage, particularly under modified atmosphere conditions. CA storage generally enhanced the positive effects of cold storage by reducing texture decay and water loss and improving VOC profiles. Several ester compounds were synthesized *de novo* under low oxygen conditions, possibly as a response to hypoxic stress.

The study concludes that CA storage offers potential to enhance postharvest fruit quality beyond shelf-life extension. The increase in fruity ester compounds during storage may improve blueberries' organoleptic properties. However, the variability in responses among cultivars needs tailored storage protocols. This research provides valuable insights for market segmentation and breeding programs aimed at enhancing blueberry quality and storability, while also validating PTR-ToF-MS as a rapid phenotyping tool for blueberry assessment.

1. Introduction

Blueberries (*Vaccinium* spp.) are highly valued fruits, prized for their organoleptic qualities and health benefits. Together with texture and taste the aroma of blueberries plays a crucial role in their overall fruit quality, significantly influencing consumer satisfaction and preferences (Beaulieu et al., 2014; Gilbert et al., 2015). Aroma assessment is an indicator of fruit freshness and ripeness. Ripe blueberries typically emit a sweet, fruity, and floral aroma (Du et al., 2011), while an unpleasant smell may suggest decay or overripeness.

The complex aroma profile of blueberries results from the interaction of numerous volatile organic compounds (VOCs) produced during fruit ripening. This VOC composition varies significantly depending on genetic factors, environmental conditions, and fruit ripening stage (Beaulieu et al., 2014; Du and Rouseff, 2014; Qian et al. 2022; Farneti et al., 2020; Ferrão et al. 2020; Sater et al. 2020; Shi et al. 2023). These VOCs include various chemical classes such as aldehydes, alcohols, esters, terpenes, and ketones.

Most aroma compounds, including linalool, several monoterpenes, (*Z*)-2-hexen-1-ol, and hexanal, are synthesized by the fruit in the full ripe

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https://doi.org/10.1016/j.postharvbio.2024.113355
Received 24 October 2024; Received in revised form 3 December 2024; Accepted 4 December 2024
Available online 9 December 2024

stage. In contrast, compounds like (*E*)-2-hexenal are produced earlier, in the pink stage of ripening (Farneti et al., 2017). Esters, although present in lower concentrations, may have a strong influence on blueberry aroma, contributing notably to "sweet" and "fruity" scents. Many esters, such as ethyl acetate, methyl isovalerate, ethyl isovalerate, and methyl 2-methylbutanoate, are produced in small concentration only in the final ripening phase and become more pronounced in overripe fruit (Farneti et al., 2017).

The organoleptic quality of blueberries can be significantly enhanced by ensuring suitable growing conditions (Wang et al., 2008), harvesting at the appropriate ripening stage (Kalt et al., 2003; Retamales and Hancock, 2018), and improving postharvest management practices (Forney et al., 2022; Hancock et al., 2008; Moggia et al., 2017). Proper storage optimization can enhance not only shelf life but also overall fruit quality (Forney et al., 2022; Hancock et al., 2008). Unlike other soft berries such as strawberries, raspberries, or blackberries, which require optimized storage to prevent rapid fruit senescence, blueberries have a comparatively longer shelf life. This extended shelf life provides more flexibility to optimize storage management based on both fruit senescence and qualitative organoleptic traits.

Recent studies have shown that low-temperature storage can alter the VOC profile of blueberries, with effects varying depending on the cultivar. Some cultivars, such as “Duke”, “Bluecrop”, and “Jersey”, produce new esters that are absent in the ripe fruit at harvest (Beaulieu et al., 2014; Farneti et al., 2020). This suggests that postharvest conditions can potentially modulate the aromatic profile of blueberries, indicating new possibilities for quality improvement.

The aromatic profile of blueberries can also be influenced by controlled or modified atmosphere storage (Forney et al., 2022; Harb and Streif, 2004). While these storage conditions can potentially increase off-flavours, primarily from fermentation processes due to excessive hypoxia, they may also induce ester synthesis in some blueberry cultivars, similar to what has been observed in other fruits (Both et al., 2017; Maoz et al. 2023; Maoz et al. 2019). Forney et al. (2022) found that ester synthesis (especially ethyl acetate and ethyl butyrate) was more strongly associated with high CO_2 levels rather than low O_2 levels, highlighting the complex interplay between storage conditions and aroma development.

Controlled atmosphere (CA) storage conditions for highbush blueberries typically range from 1 to 10 kPa O_2 and 5–16 kPa CO_2 at 0°C (Harb and Streif, 2004; Hancock et al., 2008; Alsmairat et al., 2011; Moggia et al., 2017). Unlike other fruits, particularly pome fruits, the primary advantage of CA for blueberries lies in its high CO_2 concentration (>6 kPa), which inhibits fungal proliferation (Alsmairat et al., 2011; Beaudry, 1993; Harb and Streif, 2004; Schotsmans et al., 2007), rather than reducing physiological activities through low O_2 levels (Forney, 2009).

The O_2 level has been found to have little effect on fruit quality, although some evidence suggests it can modify fruit CO_2 tolerance (Beaudry, 1993; Hancock et al., 2008). This unique response of blueberries to CA storage conditions sets them apart from many other fruits and necessitates specialized storage protocols.

However, the CO_2 concentration required for decay prevention is often near the tolerance level of the product (Alsmairat et al., 2011). For some blueberry cultivars, such as “Elliott”, “Aurora”, and “Ozarkblue”, high CO_2 levels ($\geq$18 kPa CO_2) may lead to a rapid and continuous decline in berry firmness (Harb and Streif, 2004; Hancock et al., 2008; Duarte et al., 2009; Alsmairat et al., 2011; Cantin et al., 2012; Rodriguez and Zoffoli, 2016; Catuneanu et al., 2017) and flesh discoloration throughout the storage period, regardless of the O_2 level (Harb and Streif, 2004). This highlights the importance of tailoring CA storage conditions to specific cultivars to maximize quality retention while preventing decay.

Since CA storage is the most effective postharvest technology for extending the market life of fresh blueberries, especially during long sea shipments (Moggia et al., 2017; Retamales and Hancock, 2018), developing tailored storage strategies for each blueberry genotype is essential. Linking postharvest research with breeding programs can significantly enhance the natural aroma of blueberries and facilitate the development of new cultivars with improved aroma and flavor profiles (Gilbert et al., 2015; Farneti et al., 2020; Ferrão et al. 2020).

Currently cultivated blueberry cultivars exhibit high genetic variability, with many derived from inter-and intraspecific crosses (Lobos and Hancock, 2015). This genetic diversity contributes to the wide range of responses observed in different cultivars to postharvest treatments, including CA storage. The published information on the effect of storage is often inconsistent, likely due to the use of different cultivars and varying storage conditions across studies (Farneti et al. 2020; Farneti et al. 2022; Moggia et al., 2017). Therefore, it is crucial to clarify how storage management impacts the organoleptic quality of blueberries, taking into account the genetic variability present in most cultivars.

The aim of this study was to assess how controlled atmosphere storage, with high CO_2 concentration, affects the VOC profile of blueberries, with a specific focus on exploring whether these modifications are genetically dependent, thereby necessitating an investigation of the highest genetic variability to create a dataset for future detailed studies. We investigated the *de novo* production of esters and their association with fermentation related VOCs, such as ethanol or acetaldehyde. These compounds are of particular interest as they can significantly impact fruit flavour and may be indicators of fruit stress or quality changes during storage (Forney et al., 2022).

To obtain a comprehensive, untargeted measurement of the VOC profile we employed two complementary analytical techniques: direct injection mass spectrometry using Proton Transfer Reaction - Time of Flight - Mass Spectrometry (PTR-ToF-MS) and gas chromatography utilizing Solid-phase microextraction coupled to gas chromatography-mass spectrometry (SPME/GC-MS). The use of these complementary techniques allows for both rapid screening of overall aroma profiles and detailed identification of specific compounds, providing a more complete picture of effects of CA storage on blueberry aroma.

Additionally, we aimed to validate the PTR-ToF-MS methodology as a fast and untargeted phenotyping tool for blueberry breeding and postharvest management. The development of rapid, high-throughput methods for assessing fruit quality is crucial for both breeding programs and postharvest management strategies. By demonstrating the effectiveness of PTR-ToF-MS for blueberry aroma analysis, we hope to provide a valuable tool for researchers and industry professionals working to improve blueberry quality.

2. Material and methods

2.1. Plant material and fruit sampling

Thirty-nine *Vaccinium* cultivars (Supplementary Table 1) were chosen from the experimental field of Fondazione Edmund Mach (FEM) Research and Innovation Centre in Pergine (Trento), located in the north of Italy (Trentino-Alto Adige region: 46.0744 °N, 11,2334 °E, 525 m a.s. l.). This set of blueberry cultivars was selected based on the genetic variability information available within our germplasm collection, which was characterized using a set of microsatellite (SSR) markers (Farneti et al. 2020 and unpublished data). At the time of the analyses, which were conducted across three distinct production seasons for each of the three experiments, plants were in the full production phase. Bushes were maintained following standard pruning and surface bark mulching renewal. The experimental plot contained a minimum of five plants for each cultivar.

Fruit were harvested at the commercial harvest maturity stage based on berry color. Homogeneous fruit of each cultivar, free from external damages or irregularities, were sorted immediately at harvest and divided into batches of around 80 fruit, for each storage condition tested in the three independent experiments conducted across three distinct production seasons.

For each CA condition, 34 L air-tight polypropylene boxes were used to store fruit. Each box was connected to a "Multiplex Analyzer" (Isolcell S.p.A., Laives, BZ, Italy) via PVC tubes. The Isolcell Multiplex Analyzer included an automated sample sequencing system to measure and control every 60 minutes the gas concentration introduced in CA environments.

2.2. Experimental plan

2.2.1. Experiment 1: untargeted characterization of the blueberry volatilome

Seven blueberry cultivars, namely "Biloxi", "Brigitta Blue", "Centurion", "Chandler", "Northland", "Ozark Blue", and "Star" (Supplementary table 1), were tested. Two storage conditions of 42 days were considered: i) regular atmosphere (RA), 2 °C and RH of 90 % with regular atmosphere gas concentration (20.9 kPa O2 and 0.03 kPa CO2) and ii) controlled atmosphere (CA), 2 °C and RH of 90 % with modified atmosphere gas concentration (7 kPa O2 and 16 kPa CO2). Fruit VOC composition was analysed in triplicate by SPME/GC-MS and PTR-ToF-MS at harvest and after 42 days of storage.

2.2.2. Experiment 2: estimation of genetic variability

39 blueberry cultivars (Supplementary table 1) were tested. Fruit were stored for 42 days at the same storage condition of the first experiment: i) regular atmosphere ("RA", 20.9 kPa O2 and 0.03 kPa CO2) and ii) controlled atmosphere ("CA", 7 kPa O2 and 16 kPa CO2). Fruit VOC composition was analysed in triplicate by PTR-ToF-MS at harvest and after 42 days of storage.

2.2.3. Experiment 3: evaluation of the role of hypoxia level

Four cultivars chosen based on the second experiment results ("Brigitta Blue", "Centurion", "Northland", and "Star"; Supplementary table 1) were tested. Fruit were stored for 42 days of storage at 2 °C and RH of 90 %. Four storage conditions were considered: i) regular atmosphere ("RA"), with regular atmosphere gas concentration (20.9 kPa O2 and 0.03 kPa CO2), ii) controlled atmosphere with 1 kPa O2 and 16 kPa CO2 ("O2_1"), iii) controlled atmosphere with 7 kPa O2 and 16 kPa CO2 ("O2_7"), and iv) controlled atmosphere with 12 kPa O2 and 16 kPa CO2 ("O2_12"). Fruit VOC composition was assessed in triplicate by PTR-ToF-MS at harvest and after 42 days of storage.

2.3. Texture analysis

Texture was assessed after harvest and after storage on 10 homogenous fruit from each accession, following a 2-hour equilibration period at room temperature (22°C). We used a texture analyzer (Zwick Roell, Ulm, Germany) equipped with a 5 kg loading cell and a cylindrical flat head probe with a diameter of 4 mm entering the berry flesh from the sagittal side (Farneti et al., 2020; Giongo et al., 2022). The mechanical profile was defined by two variables: force (N) and distance (strain, %). The force was measured using the following instrumental settings: test speed of 100 mm min^{-1}, post test speed of 300 mm min^{-1}, auto force trigger of 2 g, and stop plot at target position. Each berry was compressed until reaching 90 % deformation. From the force displacement profile, we considered three parameters: maximum force (FM), deformation at maximum force (DFM) and gradient (or Apparent Young's module). All data were processed by TaxtExpertII software (Zwick Roell, Ulm, Germany).

2.4. Sample preparation for VOC analysis

After 2 hours of equilibration period at room temperature (22 °C) fruit, free from external damages or irregularities, were frozen with liquid nitrogen and grinded. Replicates of 0.5 g of blueberry powdered frozen samples, conserved at −80°C, were weighed into 20 ml glass vials equipped with PTFE/silicone septa (Agilent, Cernusco sul Naviglio, Italy) and mixed with 0.5 ml of deionized water, 200 mg of sodium chloride, 2.5 mg of ascorbic acid, and 2.5 mg of citric acid (Farneti et al., 2020).

2.5. VOC Analysis by SPME/GC-MS

Samples were equilibrated (40 °C, 10 min) under constant stirring prior to analysis. A DVB/CAR/PDMS fiber (2 cm coating, Supelco, Bellefonte, PA, USA) was employed for headspace solid-phase microextraction (HS-SPME), with a 30-min exposure period and then desorbed at 250 °C in the injector port of a GC (GC Clarus 500, PerkinElmer, Norwalk CT, USA)coupled with mass spectrometry (MS). The MS operated in electron ionization mode (70 eV) with a scan mass range of m/z 33–350.Separation was carried out in an HP-INNOWax fused silica capillary column (30 m, 0.32-mm ID, 0.5-μm film thickness; Agilent Technologies, Palo Alto, CA, USA). The initial GC oven temperature was 40 °C rising to 220 °C at 4 °C min^{-1}, the temperature of 220 °C was maintained for 1 min, then increased at 10 °C min^{-1} until it reached 250 °C, which was maintained for 1 min. The carrier gas, helium, was kept at a constant column flow rate of 1.5 ml min^{-1}.

Analytes were identified through mass spectral comparison (NIST 14 and Wiley 7th libraries) and linear retention indices (LRI). LRI were calculated using a C7-C30 n-alkane standard (Supelco). Quantification was performed in triplicate, with results expressed as μg/L 2-octanol equivalents.

2.6. VOC Analysis by PTR-ToF–MS

Analysis was conducted using a PTR-ToF-MS 8000 (Ionicon Analytik GmbH, Innsbruck, Austria) with parameters as described by Farneti et al. (2020).

The sample headspace was withdrawn through PTR-MS inlet with 40 sccm flow. Pure nitrogen was flushed continuously through the vial to prevent pressure drop. Each measurement was conducted automatically after 20 min of sample incubation at 40°C and 2 min between each measurement was applied to prevent any instrumental memory effect. Samples were analysed in triplicates. An adapted GC autosampler (MPS Multipurpose Sampler, GERSTEL) was integrated with the PTR-ToF-MS for automated sample handling and measurement. Spectral analysis was performed following the methodology outlined in Farneti et al. (2017)

2.7. Data and statistical analysis

The array of masses detected with PTR-ToF-MS was refined by applying noise and correlation coefficient thresholds. Initially, peaks not significantly different from blank samples were removed. Peaks with over 99 % correlation were excluded, as these mostly corresponded to isotopes of monoisotopic masses (Farneti et al., 2017).

For all quality parameters, both texture and volatiles, a storage index (SI) was computed using the formula $SI = \log_{10}(Q_{iPH}/Q_{iH})$, where Qi_H is the value of the i- th quality parameter measured at harvest, and Qi_{PH} is the value of the same parameter measured after storage (Giongo et al. 2013). Positive SI values indicate a quality trait enhancement, while negative values highlight a loss of the quality trait during storage.

Multivariate statistical methods were applied using internal statistical functions from R (version 3.4.1, R Foundation for Statistical Computing, Vienna, Austria) and the external packages "mixOmics" and "ggplot2".

3. Results

3.1. Untargeted VOCs analysis combining SPME/GC-MS and PTR-ToF-MS

Gas chromatography-mass spectrometry (GC-MS) analysis was conducted to evaluate VOC profiles of seven blueberry cultivars ("Biloxi",

"Brigitta Blue", "Centurion", "Chandler", "Northland", "Ozark Blue", and "Star") under three distinct conditions: i) at harvest (H), ii) after regular atmosphere storage (RA), and iii) following controlled atmosphere storage (CA). The results, presented in Fig. 1 and detailed in Supplementary Table 2, illustrate the complex dynamics of VOC composition across cultivars and storage conditions.

Substantial inter-cultivar variation was observed in both total VOC concentrations and individual compound profiles. Postharvest storage conditions markedly influenced VOC profiles. CA storage led to increased total VOC concentrations compared to harvest levels, with some exceptions. This trend was particularly pronounced in "Biloxi", "Brigitta Blue", and "Chandler", while "Centurion" demonstrated relatively stable VOC levels across all conditions, suggesting a cultivar-specific response to storage.

The blueberry VOC profiles were dominated by several key compound classes. Aldehydes constituted a significant portion of the VOC profile in most cultivars, especially at harvest. "Northland" exhibited the highest aldehyde concentration at harvest (1974 μg/kg), with (*E*)-2-hexenal being the major contributor. "Biloxi" showed the lowest total aldehyde content. Only "Centurion" maintained relatively high aldehyde levels across all conditions, including after CA storage (70 % of the content at harvest). In the other six cultivars, aldehyde levels decreased significantly after CA storage ranging from 2 % (cv. "Star") to 31 % (cv. "Northland") of their initial harvest content. In contrast, regular atmosphere (RA) storage resulted in a more moderate reduction, with aldehydes maintaining approximately 70 % of their initial levels.

Esters were present in trace concentration in blueberries at harvest. However, esters concentration increased significantly during storage, emerging as a major contributor to the fruit VOC profile under CA conditions. "Chandler" showed the most pronounced increase, from 12 μg/kg at harvest to 3120 μg/kg after CA storage, while "Centurion" increased the least, from 13 μg/kg to 483 μg/kg. In total, 29 esters were identified, most of which were absent or present in minimal traces in freshly harvested fruits. Ethyl acetate was the dominant ester in most cultivars after CA storage (from 91 μg/kg of "Centurion" to 1146 μg/kg of "Biloxi"). Other esters present at higher concentrations, particularly after CA storage, included ethyl isovalerate (from 180 μg/kg of "Centurion" to 1135 μg/kg of "Chandler"), ethyl crotonate (from 2 μg/kg of "Centurion" to 574 μg/kg of "Ozark Blue"), ethyl 2-methylbutanoate (from 15 μg/kg of "Centurion" to 361 μg/kg of "Chandler"), (*E*, *E*)-ethyl 2,4-hexadienoate (from 0 μg/kg of "Centurion" to 113 μg/kg of "Brigitta Blue"), and ethyl butanoate (from 1 μg/kg of "Centurion" to 75 μg/kg of "Ozark Blue").

Monoterpenes were significant contributors to the blueberry VOC profile at harvest. Linalool was the predominant monoterpene in all cultivars at harvest, with the highest concentration observed in "Northland" (249 μg/kg) and the lowest in "Centurion" (61 μg/kg). Another relevant monoterpene is 1,8-cineole ranging at harvest from 5 μg/kg of "Biloxi" to 81 μg/kg of "Brigitta Blue". In general, the total monoterpene content decreased during storage in both RA and CA conditions. However, for some compounds, such as 4-terpineol, we found different trends depending on the cultivar. For example, the highest 4-terpineol content was found in the fruit of "Centurion" after CA storage (129 μg/kg), while the contents in the fruit at harvest and after RA storage were both 0 μg/kg.

Alcohol concentrations varied widely among cultivars and storage conditions. "Northland" showed the highest alcohol levels, particularly after RA storage (382.33 μg/kg). Hexanol and (*Z*)-2-hexen-1-ol were the predominant alcohols in most cultivars.

Acids were generally present in low concentrations (4.54–13.82 μg/kg) at harvest and after RA storage. However, "Biloxi" exhibited a remarkable increase in acid content after CA storage (321.99 μg/kg), primarily due to acetic acid (317.67 μg/kg).

Ketone levels varied among cultivars and storage conditions. "Biloxi" showed a notable increase in ketones after CA storage (155.92 μg/kg), primarily due to acetoin formation (140.86 μg/kg). Acetoin was present at harvest and RA storage and was produced only during CA storage. Only Centurion did not contain acetoin even after CA.

To provide a more comprehensive analysis of the VOC profile, all samples were also analyzed using Proton Transfer Reaction-Time of Flight-Mass Spectrometry (PTR-ToF-MS) in addition to SPME/GC-MS. The whole VOC spectra, assessed in triplicate for each cultivar, were reduced to 117 VOC mass peaks (Supplementary Table 3), applying noise and correlation coefficient thresholds. Tentative identification (t. i.) of each mass peak detected by PTR-ToF-MS relied on an in-house

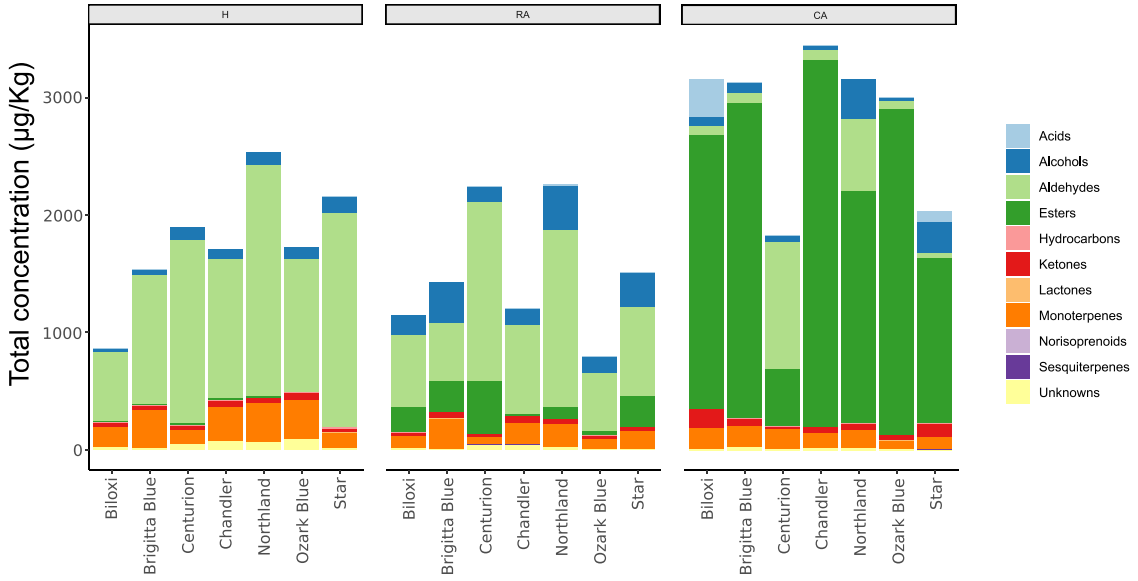

Fig. 1. Stacked bar plot depicting changes in VOCs concentration, expressed as μg/L of 2-octanol, assessed by SPME/GC-MS, for each blueberry cultivars ("Biloxi", "Brigitta Blue", "Centurion", "Chandler", "Northland", "Ozark Blue", and "Star") at harvest time ("H") and after 42 days of storage under Regular Atmosphere ("RA": 2°C, 90 % RH, 20.9 kPa O_2, and 0.03 kPa CO_2) and Controlled Atmosphere ("CA": 2°C, 90 % RH, 7 kPa O_2, and 16 kPa CO_2) conditions. Each data point represents the arithmetic mean of three biological replicates. VOC classes (acids, alcohols, aldehydes, esters, hydrocarbons, ketones, lactones, monoterpenes, norisoprenoids, sesquiterpenes, and unknowns) are represented by different colours.

library of chemical standards, on the list of compounds detected by SPME/GC-MS analysis, and on the list of VOCs previously detected in blueberry fruit with different analytical techniques (Sater et al. 2020). The headspace analyses carried out with PTR-ToF-MS allowed for the evaluation of VOCs that are omitted from the gas chromatographic methodology used in this study, despite their importance for the characterization of fruit quality and freshness, such as t.i. methanol (*m/z* 33.033), ethanol (*m/z* 47.049), acetaldehyde (*m/z* 45.033), and dimethyl sulfide (*m/z* 63.026).

The results obtained with the two analytical techniques were compared and combined to verify both the complementarity of the two methodologies and the possibility of using PTR-ToF-MS as a fast, comprehensive, and reliable VOC phenotyping tool for blueberry fruit, despite its known analytical limitations in separating and identifying isomers. To analyse the relationships between VOC profiles, cultivars, and storage conditions, we employed a multi-block statistical approach using the DIABLO mixOmics framework [Data Integration Analysis for Biomarker discovery using a Latent cOmponents (Singh et al., 2019)], Fig. 2. The core DIABLO method extends Generalised Canonical Correlation Analysis (GCCA), which generalizes partial least squares regression (PLS) for multiple matching datasets, and the sparse sGCCA method. This method allowed us to integrate data from both SPME/GC-MS and PTR-ToF-MS analyses, providing a comprehensive view of the VOC signatures across different sample groups.

The correlation structure between the two analytical techniques was visualized using a component-based plot, as shown in Supplementary Figure 1. This function allows for the plotting of the components across the different data sets for a given dimension. The results revealed a high correlation between the SPME/GC-MS and PTR-ToF-MS datasets, resulting in a correlation of 0.98 and 0.95 for the first and second components, respectively (Supplementary Figure 1).

Our analysis indicated that the first component primarily captured variations in VOC profiles associated with storage conditions, while the second component predominantly reflected differences among cultivars (Fig. 2A). The high degree of similarity between the SPME/GC-MS and PTR-ToF-MS datasets can be attributed to the consistent detection of individual volatile compounds by both techniques.

The high similarity between the two matrices is explainable by the strong correlation between the content of individual molecules acquired by the two analytical techniques (Fig. 2B). This result confirms the possibility of using a direct injection mass spectrometry (DI-MS) technique, like PTR-ToF-MS, for blueberry flavour profile analysis as an alternative to gas chromatographic analysis performed after headspace accumulation, like SPME/GC-MS.

To further illustrate the relationships between the two analytical techniques and the fruit VOC composition, we created a Clustered Image Map (Fig. 2C). This visualization represents the multi-omics molecular signatures for each sample, with color-coded cells reflecting the similarity between VOC datasets. The associated dendrograms illustrate the hierarchical clustering of cultivars, storage conditions, and volatile compounds. Blocks of homogeneous colour depict subsets of features from each dataset which are correlated and suggest a potential causative

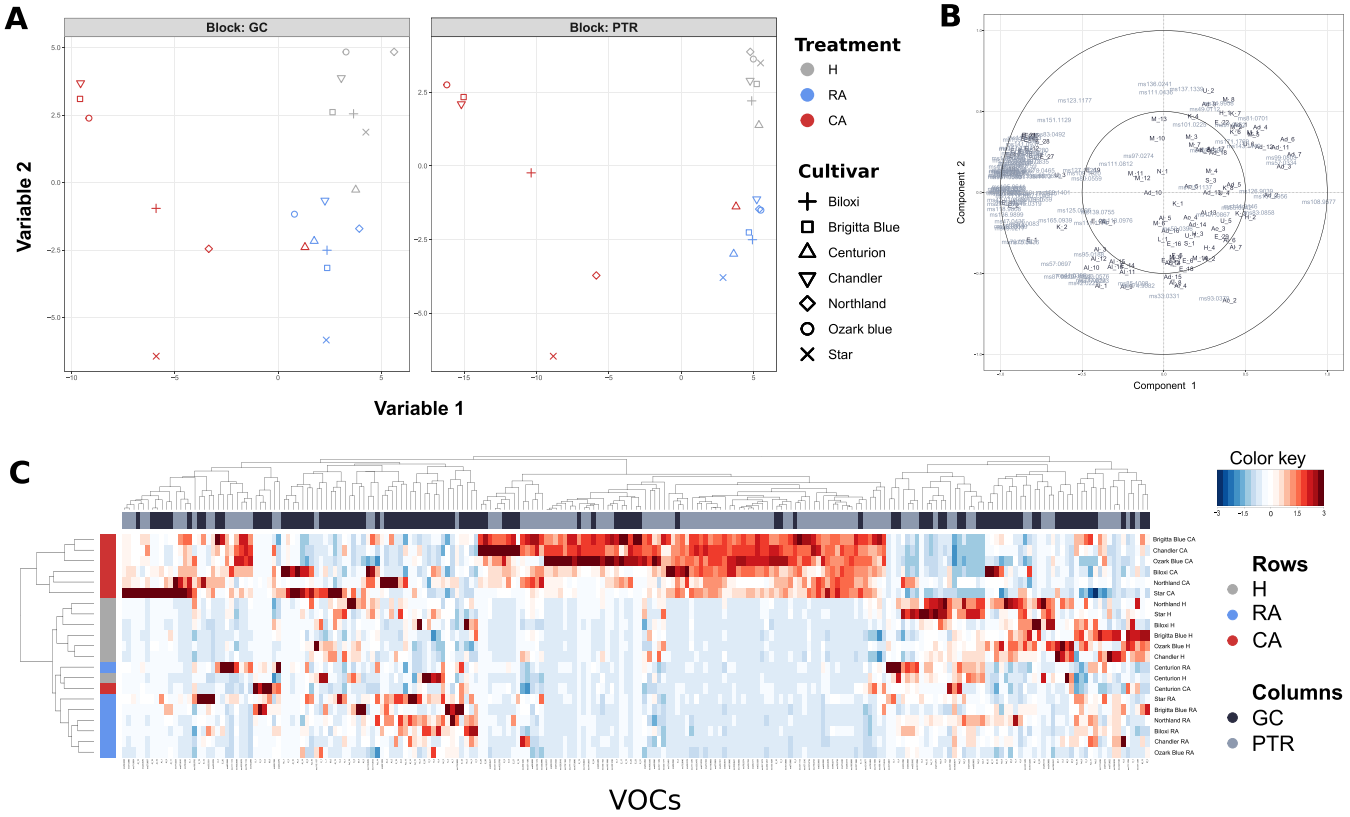

Fig. 2. Complementarity between SPME/GC-MS and PTR-ToF-MS headspace VOC assessments defined by applying the Data Integration Analysis for Biomarker discovery using Latent cOmponents (DIABLO) multi-block discriminant analysis. VOC analyses were performed for seven blueberry cultivars ("Biloxi", "Brigitta Blue", "Centurion", "Chandler", "Northland", "Ozark Blue", and "Star") at harvest time ("H") and after 42 days of storage under Regular Atmosphere ("RA": 2°C, 90 % RH, 20.9 kPa O_2, and 0.03 kPa CO_2) and Controlled Atmosphere ("CA": 2°C, 90 % RH, 7 kPa O_2, and 16 kPa CO_2) conditions. Each data point represents the arithmetic mean of three biological replicates. **Plot A)** Sample plot from multiblock sparse Partial Least Squares Discriminant Analysis (sPLS-DA) performed on data obtained with SPME/GC-MS and PTR-ToF-MS. **Plot B)** Correlation circle plot from multiblock sPLS-DA reported in plot a. **Plot C)** Clustered Image Map for the variables selected by multiblock sPLS-DA, representing the multi-omics molecular signature expression for each sample. Each cell's color is based on the values of the similarity matrix performed on the two VOC datasets. Euclidean distance and Complete linkage methods are used. The correlation structure at the component level is reported in supplementary figure #.

relationship.

This integrated analysis approach proveshow cultivar differences and storage conditions influence the complex VOC profiles in blueberries, while demonstrating the complementarity of SPME/GC-MS and PTR-ToF-MS techniques in VOC analysis. The results primarily highlight a clear effect of storage on the composition of the aromatic profile, followed by a cultivar effect. In particular, the cultivar "Centurion" differentiates itself from the others, especially in its postharvest behaviour, maintaining an almost unchanged VOC profile of the fruit. Regarding the other six cultivars, a significant cluster of VOCs increases after CA storage. In this cluster, as previously observed, we mainly find mass peaks tentatively associated with esters (i.e. *m/z* 75.044, 89.060, 103.076, 115.076, 117.092, 131.108, 141.09, 145.124), higher alcohols (i.e. 57.069,71.085, 91.068), and with fundamental compounds of the fermentation process such as ethanol (*m/z* 47.043) and acetaldehyde (*m/z* 45.031).

3.2. Role of genetic variability on the fruit response to controlled atmosphere storage

3.2.1. Fruit texture and fresh weight loss

In this study, fresh weight loss and texture differences among blueberry accessions (totally 39 cultivars) were heightened during storage under both normal and controlled atmosphere conditions. CA storage significantly reduced the average weight loss after 42 days compared to standard storage (2.1 % vs. 9.0 %, respectively). Despite high variability among the 39 blueberry accessions under both storage conditions (Supplementary Figure 2), no significant correlation was observed between storage conditions, highlighting the need for tailored storage management strategies for each accession.

The average weight loss of blueberry cultivars during standard storage was approximately 9.0 %. Three cultivars with the lowest weight loss were "Brigitta Blue" (6.2 %), "Legacy" (6.4 %), and "Ozark Blue" (6.7 %), while the three cultivars with the highest weight losses were "Cosmopolitan" (14.9 %), "Jersey" (13.3 %), and "Star" (12.6 %). For blueberries stored under CA conditions, the average weight loss was around 2.1 %. Cultivars with the lowest weight loss were "Nui" (0.6 %), "Centurion" (0.7 %), and "Bluecorp" (1.0 %), while "Cosmopolitan" (3.7 %), "Azur" (3.2 %), and "Roxy Blue" (3.2 %) exhibited the highest weight losses (Supplementary Figure 2).

The effect of different atmospheric compositions (RA vs CA) on the texture profile of blueberry fruit is summarized in the Principal Component Analysis (PCA) presented in Fig. 3. Based on previously published results (Farneti et al. 2020, Giongo et al. 2022), we considered only three texture parameters extracted from the destructive analysis of fruit texture: maximum force value ("FM"), deformation at maximum force ("DFM"), and gradient (or Young's modulus).

The PCA, conducted on the average value of 10 fruit for each variety, demonstrates that storage under CA conditions significantly reduces the degradation of blueberry texture, particularly by reducing the deformation at maximum force (Fig. 3). However, this positive effect of CA storage is closely linked to the genotype. A more detailed analysis of each of the three texture characteristics reveals that the fold changes (storage index) during the two types of storage are highly cultivar-dependent (Supplementary Figure 3).

For deformation at maximum force (Supplementary Figure 3), some

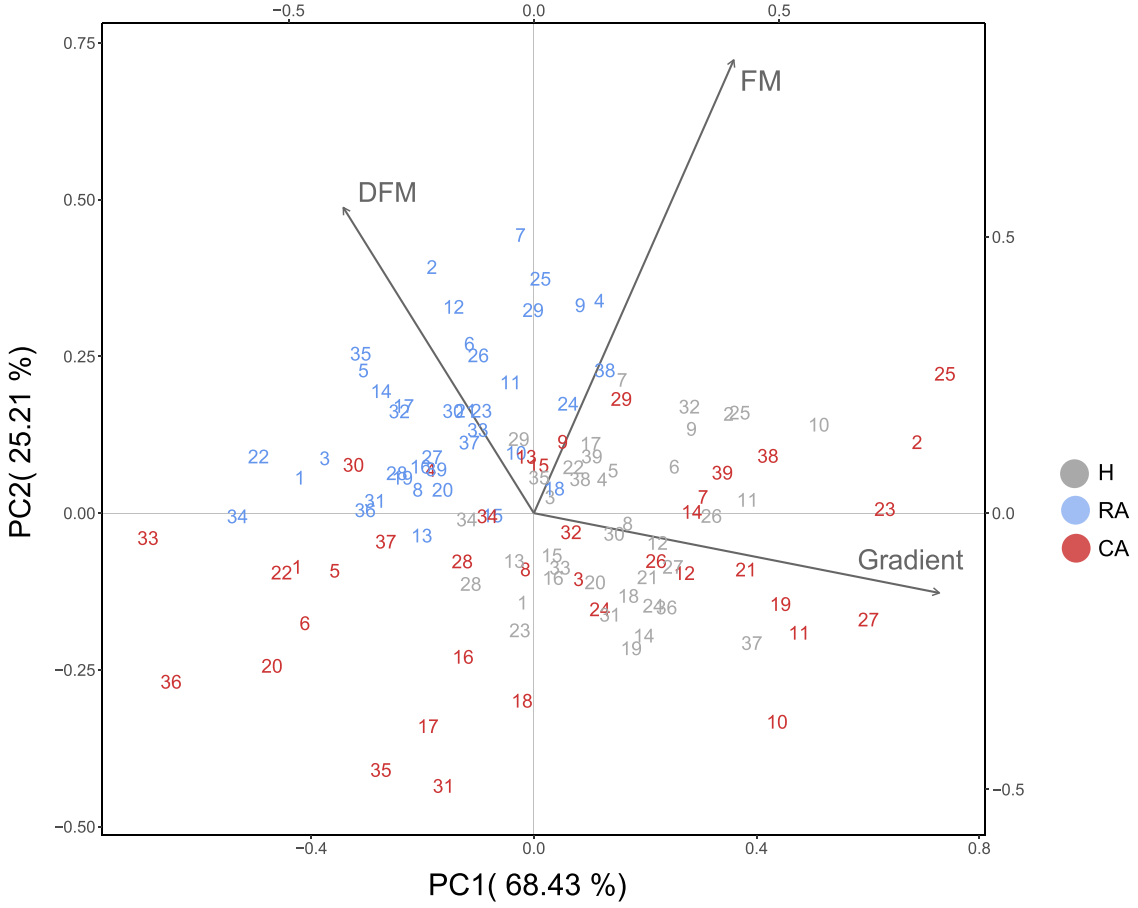

Fig. 3. Effect of storage conditions on blueberry texture. PCA biplot based on log-transformed texture values of 39 blueberry cultivars (listed in Supplementary Table 1) at harvest and after 42 days of regular atmosphere ("RA": 2°C, 90 % RH, 20.9 kPa O_2, and 0.03 kPa CO_2) and controlled atmosphere ("CA": 2°C, 90 % RH, 7 kPa O_2, and 16 kPa CO_2) storage. Each data point represents the mean of 10 biological replicates. Each of the 39 cultivars is represented in the graph by a number (corresponding to Supplementary Table 1).

cultivars like “Jersey”, “Blueray”, “Southern Belle”, and “Star” showed increased values after CA storage, while most cultivars maintained values similar to those at harvest (e.g., “Biloxi”, “Darrow”, “Mistyo”, “Northland”) or even lower values (e.g., “Sky Blue”, “Centra Blue”, “Top Hat”, “Aurora”).

Regarding the maximum force value (Supplementary Figure 3), on average, the value observed in the 39 blueberry cultivars was around 15 % lower in fruits stored under CA compared to regular atmosphere (RA) storage. As previously observed for deformation values, the variation in maximum force is closely linked to the genotype. Some cultivars, like “Compact”, “Jubilee”, “Elliott”, and “Liberty”, exhibited increased maximum force values, while most of the remaining accessions showed a decrease, particularly in “Southern Belle”, “Early Blue”, “Blue Moon”, and “Sky Blue”.

The gradient associated with the perception of gumminess/elasticity of the fruit, is another important parameter. RA storage caused a decrease in the gradient across all analysed cultivars, while CA storage resulted in higher variability (Supplementary Figure 3). Some cultivars, such as “Southern Belle”, “Roxy Blue”, and “Emerald”, exhibited a significant decrease in gradient values, while others, like “Biloxi”, “Star”, and “Brigitta Blue”, showed similar values between the two storage conditions. Additionally, certain cultivars, such as “Jubilee”, “Aurora”, “Mondo”, and “Liberty”, exhibited increased gradient values compared to those at harvest.

Neither texture parameters nor fresh weight loss changes correlated significantly with fermentation and senescence VOC changes, including t.i. methanol (*m/z* 33.033), acetaldehyde (*m/z* 45.031), and ethanol (*m/z* 47.043) (Fig. 4). Notably, cultivars such as “Toro”, “Mondo”, “Legacy”, and “Misty” demonstrated significant fold increases in both ethanol and acetaldehyde concentrations during CA storage, while exhibiting comparatively modest fold changes in textural parameters. Conversely, cultivars like “Star”, “Roxy Blue”, “Centra Blue”, and “Sky Blue”, which displayed more pronounced fold changes in textural parameters, were characterized by comparatively smaller alterations in the concentrations of these volatile compounds.

3.2.2. *Fruit VOC profile*

The VOC profile of the 39 blueberry cultivars was analyzed using PTR-ToF-MS in an untargeted mode, both at harvest and after RA and CA storage. Prior to statistical analyses, the raw spectrum obtained with PTR-ToF-MS was refined by excluding masses not significantly different from the blank and redundant ones such as isotopes and various chemical clusters (Supplementary table 3).

The Principal Component Analysis (PCA) (Fig. 5A) confirms the results previously obtained on a reduced number of genotypes (Fig. 2). The first two components of the PCA in Fig. 5A explain nearly 90 % of the total measured variability. Most of this variability (80 %) is expressed by the first component, which enables a clear separation between the two storage conditions (RA and CA) and harvest. The concentration of most VOCs is higher in stored fruits, except for a few mass peaks that, on average, are present in higher concentrations in fruit measured at harvest, such as *m/z* 57.034, 97.065, 99.08, 115.113, 137.133, and 171.176. These mass peaks are tentatively associated respectively with propenal, 2,4-hexadienal, 2-hexenal, heptanal, monotepenes, and linalool oxide (Supplementary table 3).

To focus more closely on the effect of the different storage atmospheres, a heatmap (Fig. 5B and Supplementary figure 4) was generated considering only the fold change values (storage index) obtained by

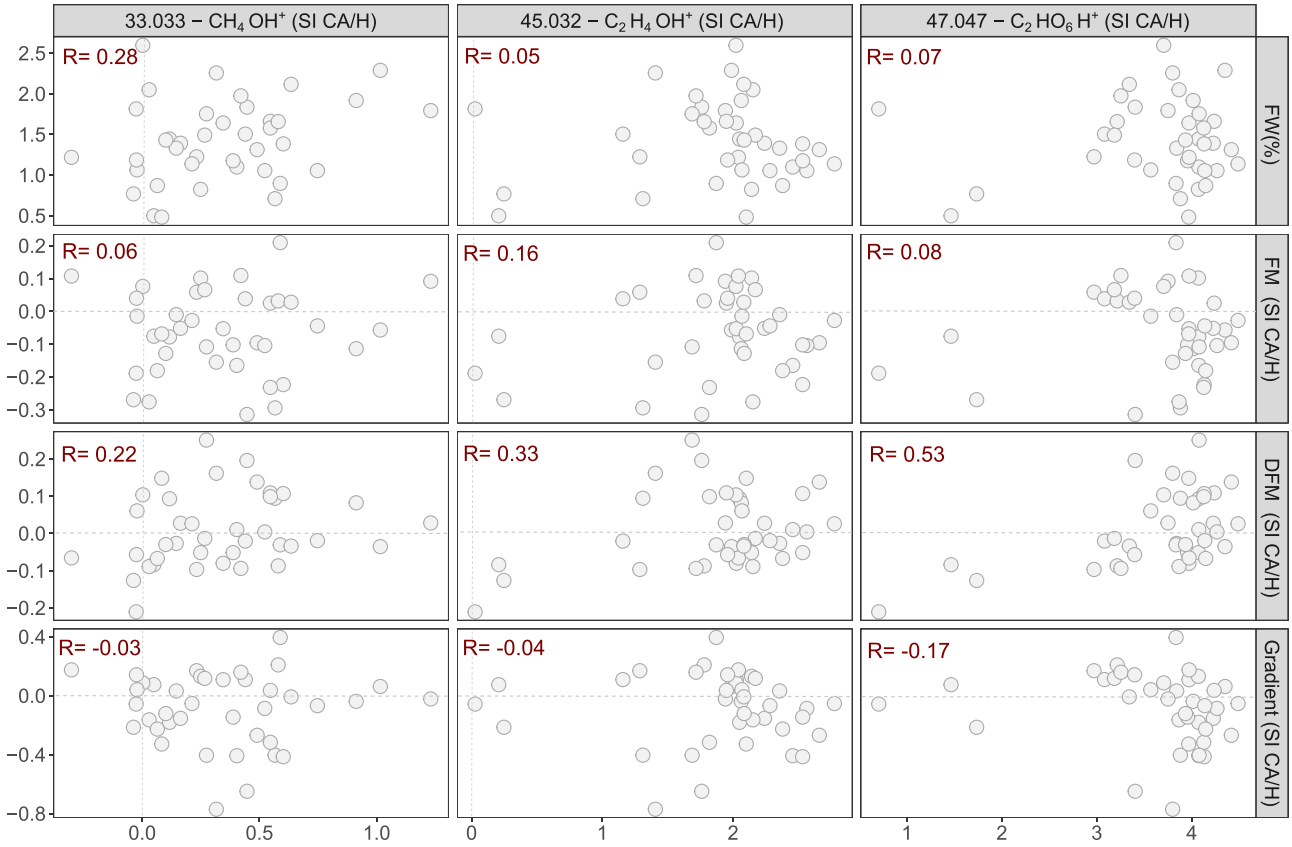

Fig. 4. Correlation plot between changes in three key texture parameters (maximum force, deformation at maximum force, and gradient) during controlled atmosphere (CA) storage (42 days, 2°C, 90 % RH, 7 kPa O_2, and 16 kPa CO_2), fresh weight loss (FW%), and variations in concentration of VOC associated with fruit fermentation and senescence as measured by PTR-ToF-MS (methanol, *m/z* 33.0333; acetaldehyde, *m/z* 45.032; ethanol, *m/z* 47.047). Changes in volatile compounds and texture parameters are reported as Storage Index (SI) values, calculated as the base-10 logarithm of the ratio between measurements taken after CA storage and those at harvest. For each plot the Pearson correlation value (R) is reported.

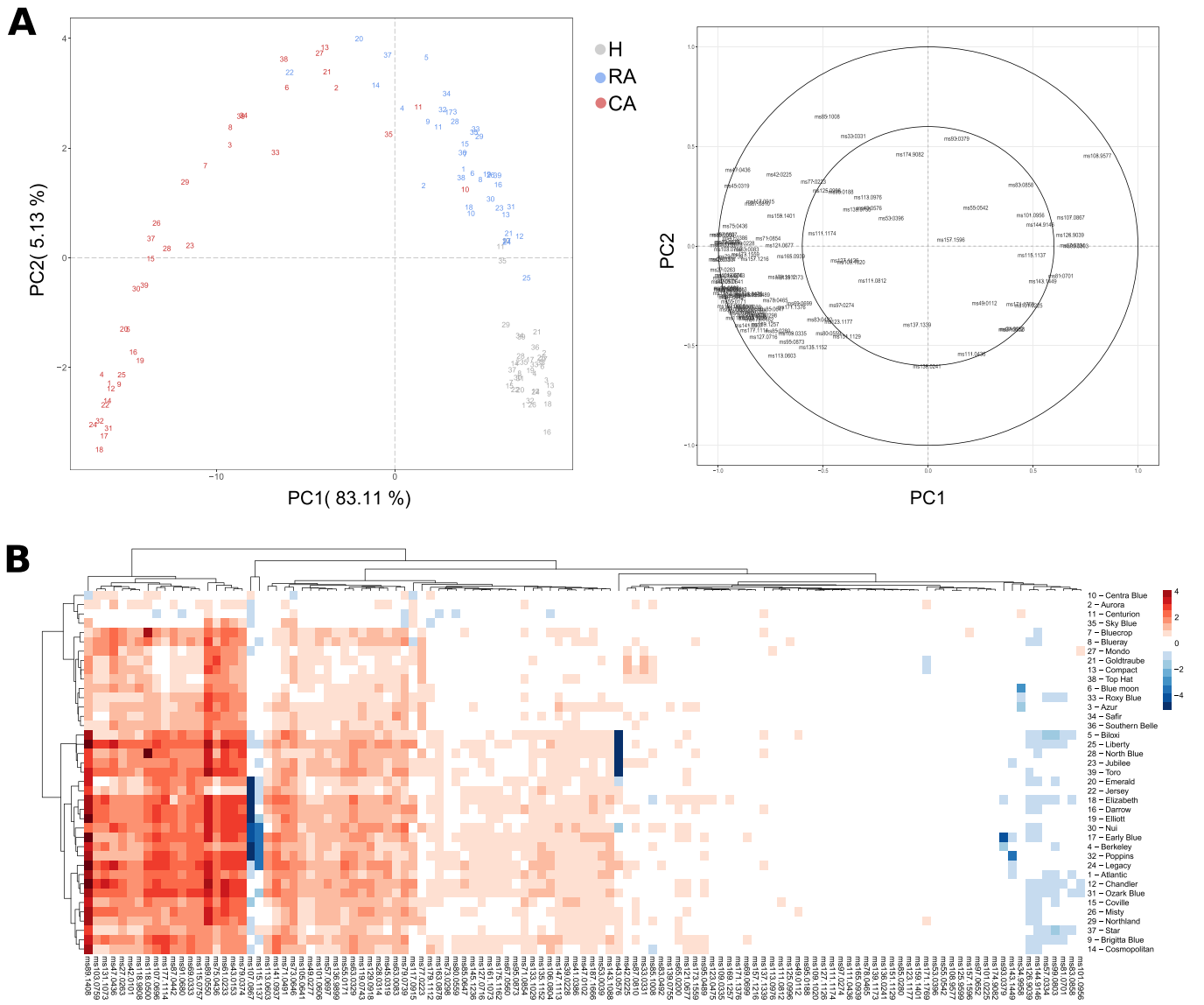

Fig. 5. Effect of storage conditions on blueberry germplasm volatilome assessed by PTR-ToF-MS. Fruit from 39 blueberry cultivars (listed in Supplementary Table 1) were analysed at harvest time ("H") and after 42 days of storage under Regular Atmosphere ("RA": 2°C, 90 % RH, 20.9 kPa O_2, and 0.03 kPa CO_2) and Controlled Atmosphere ("CA": 2°C, 90 % RH, 7 kPa O_2, and 16 kPa CO_2) conditions. Each data point represents the arithmetic mean of three biological replicates. **Plot A**) Principal Component Analysis (PCA) and loading plot performed on the log-transformed concentration of each mass. Each of the 39 cultivars is represented in the graph by a number (corresponding to Supplementary Table 1). **Plot B**) Heatmap and hierarchical clustering based on the Storage Index (SI) values of each VOC, calculated as the fold change between values measured after CA storage and RA storage. Supplementary Figure 4 also shows heatmaps obtained using SI values calculated from the fold change between H and RA, and between H and CA, respectively.

comparing the mass concentrations after storage in RA and CA. The mass peaks associated with compounds that decreased more significantly in CA compared to RA storage are *m/z* 57.034 (t.i. propenal), 81.07 (t.i. common fragment of hexenal and monoterpenes), 107.086 (t.i. ethyl benezene), and 115.113 (t.i. heptanal). For all other mass peaks, the average values are similar or significantly higher to those found at harvest (leftmost cluster of masses in the heatmap in Fig. 5B).

After CA storage, only few cultivars (i.e. "Centra Blue", "Aurora", "Sky Blue" and "Centurion") exhibited a similar aroma profile to fruit stored under regular atmosphere, while the VOC profile of the other cultivars was significantly altered by CA storage. On average, CA storage more severely decreased the content of t.i. C6 aldehydes and alcohols (e.g., *m/z* 81.07, 99.08, 83.085), while the terpene content (*m/z* 137.134) remained, on average, at the same concentration measured at harvest. Most VOC modifications were associated with increased synthesis of compounds linked to anaerobic fermentation, such as t.i. acetaldehyde (*m/z* 45.032), ethanol (*m/z* 47.043), and ester compounds (Fig. 6), including methyl acetate (*m/z* 75.043), ethyl acetate (*m/z* 89.055), ethyl propanoate (*m/z* 103.075), ethyl (2E)-2-butenoate (*m/z* 115.075), methyl isovalerate (*m/z* 117.091), ethyl isovalerate (*m/z* 131.107), ethyl 2,4-hexadienoate (*m/z* 141.093), and ethyl hexanoate (*m/z* 145.123) which may be by-products of fermentation processes. For instance, *m/z* 89.055 (t.i. ethyl acetate) and *m/z* 131.107 (t.i. ethyl isovalerate) were positively correlated with ethanol (R^2=0.46 and R^2=0.41, respectively) and acetaldehyde (R^2=0.38 and R^2=0.42, respectively). The fold change values vary considerably among different esters, especially after storage in controlled atmosphere (CA). Additionally, there is high variability among cultivars, likely due to the considerable genetic diversity of the varieties examined in this study.

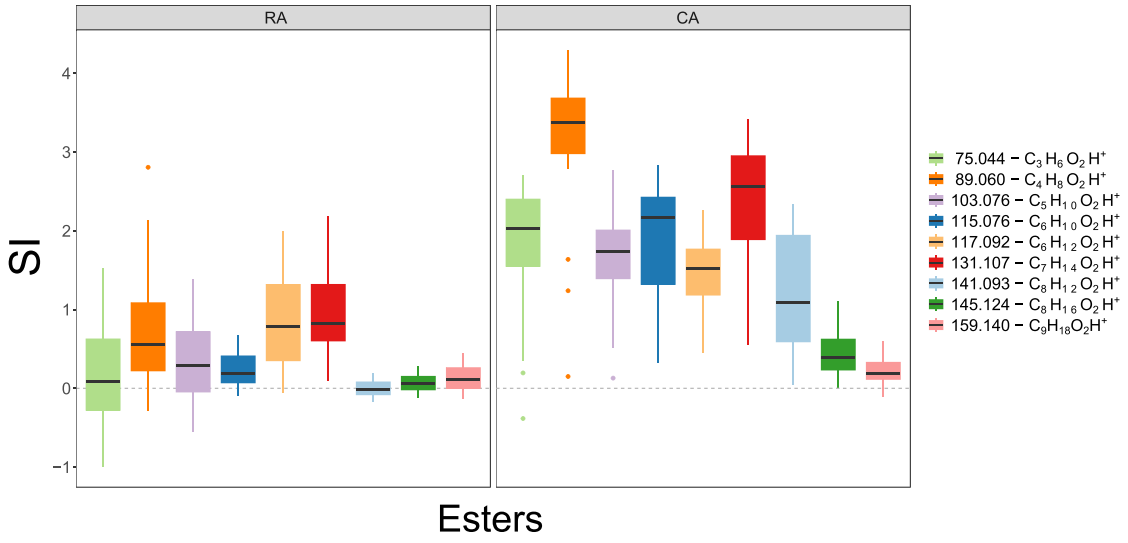

Fig. 6. Effect of storage on the content of main esters. Box plots display fold changes in major esters measured across 39 blueberry cultivars after 42 days of RA (2°C, 90 % RH, 20.9 kPa O_2, and 0.03 kPa CO_2) and CA (2°C, 90 % RH, 7 kPa O_2, and 16 kPa CO_2) storage using PTR-ToF-MS. The Storage Index (SI) values represent the ratio between measurements taken after CA or regular atmosphere (RA) storage and those at harvest.

3.3. Effect of the oxygen partial pressure on the blueberry VOC composition

Based on VOCs and texture results obtained from the germplasm collection, we selected four cultivars with distinct postharvest behaviours to test the effect of varying oxygen concentrations (1,7, and 12 KPa) on their aromatic profiles while maintaining the CO_2 content constant (16 kPa). The four cultivars ("Brigitta Blue", "Centurion", "Northland", "Star") exhibited different responses, showing similar trends but with varying magnitudes (Fig. 7). The PCA in Fig. 7A shows the differences in the VOC profile of the fruits of the four varieties after refrigerated storage at different oxygen partial pressures. The variability due to the first component (PC1: 72 %) explains for the most the effect of oxygen levels in the air, while the second component (PC2: 10 %) explains the variability between cultivars. As the oxygen concentration decreases, a general increase in several compounds associated with the first component (loading plot Fig. 7A) is observed. This effect is cultivar-dependent. For example, for "Centurion", the differences due to the different oxygen concentrations are much lower compared to "Star". However, the PCA indicates that the genetic effect (different cultivars) is also more visible for the fruit stored at the lowest oxygen concentration (1 Kpa), suggesting a different level of reaction to hypoxic stress.

The heatmap in Fig. 7B presents the fold change values of VOC concentrations among the three controlled atmosphere conditions compared to storage in regular atmosphere (RA). For all four cultivars, storage at 12 kPa oxygen does not significantly modify the fruit VOC profile, except for an increase in the concentration of certain esters, particularly for "Brigitta Blue" and "Northland".

Generally, increasing hypoxic stress by reducing oxygen levels led to a significant increase in t.i. ethanol (*m/z* 47.043) and acetaldehyde (*m/z* 45.031) concentrations (Fig. 7B). Most esters, corresponding to mass peak *m/z* 75.044 (t.i methyl acetate), *m/z* 89.06 (t.i. ethyl acetate), *m/z* 103.076 (t.i. ethyl propanoate), *m/z* 115.07 (t.i. ethyl (2E)-2-butenoate), *m/z* 131.108 (t.i. ethyl 2-methylbutanoate), *m/z* 141.093 (t.i. ethyl 2,4-hexadienoate), *m/z* 145.12 (t.i. ethyl hexanoate), and *m/z* 159.142 (t.i. isobutyl pentanoate) tended to increase with decreasing oxygen levels (Fig. 8).

Only the concentration of esters associated with mass peaks *m/z* 117.092 (t.i. methyl Isovalerate) and *m/z* 119.074 (t.i. 2-methoxyethyl acetate) is higher at higher O_2 concentrations and decreases with decreasing oxygen concentration (Fig. 8). However, it remains significantly higher compared to storage in RA. Among the four cultivars, "Centurion", as confirmed by the previous test, appears to be less susceptible to CA storage and thus to different oxygen concentrations.

Other important compounds, such as aldehydes (i.e *m/z* 99.085, 101.095) and monoterpenes (i.e *m/z* 137.133), were less affected by the different oxygen levels and show concentrations similar to storage in RA.

4. Discussion

4.1. Controlled atmosphere storage with high CO_2 triggers ester formation in blueberries

The present study highlights the complex interplay between controlled atmosphere storage conditions (7 kPa O_2 and 16 kPa CO_2) and VOC profile of blueberries, emphasizing the importance of cultivar-specific responses and the potential for enhancing fruit quality through optimized storage practices.

The comprehensive analysis of the VOC profile in blueberries, employing both gas chromatography (SPME/GC-MS) and direct injection mass spectrometry (PTR-ToF-MS) techniques, provides valuable insights into the uneven nature of aroma compounds during storage. This approach allows for a more qualified understanding of fruit quality beyond traditional parameters such as firmness and colour. As demonstrated by Farneti et al. (2017), the untargeted VOC profiling method based on PTR-ToF-MS allows to measure simultaneously a wide range of VOCs offering a powerful tool for breeders and postharvest researchers to assess and potentially enhance the organoleptic properties of blueberries.

The comparison between the two analytical techniques (Fig. 2) confirmed that most compounds measured and quantified with SPME/GC-MS in blueberry fruit are also detectable using PTR-ToF-MS. This is corroborated by the data merging analysis, which shows a 98 % correlation between the profiling obtained with the two analytical techniques. The analysis carried out using the DIABLO tool not only revealed this high collinearity, but also highlighted the ability of PTR-ToF-MS to quantify molecules not detected by the GC-MS methodology applied in this study, such as methanol, acetaldehyde, ethanol and sulfur compounds like methanethiol and dimethyl sulfide (Supplementary table 3).

The comprehensive detection of these molecules is crucial in studies like this, where different atmospheric storage conditions can induce oxidative stress and fermentative processes in the fruit. This finding is consistent with previous research demonstrating the advantages and complementarities of DI-MS over traditional GC-MS techniques in the

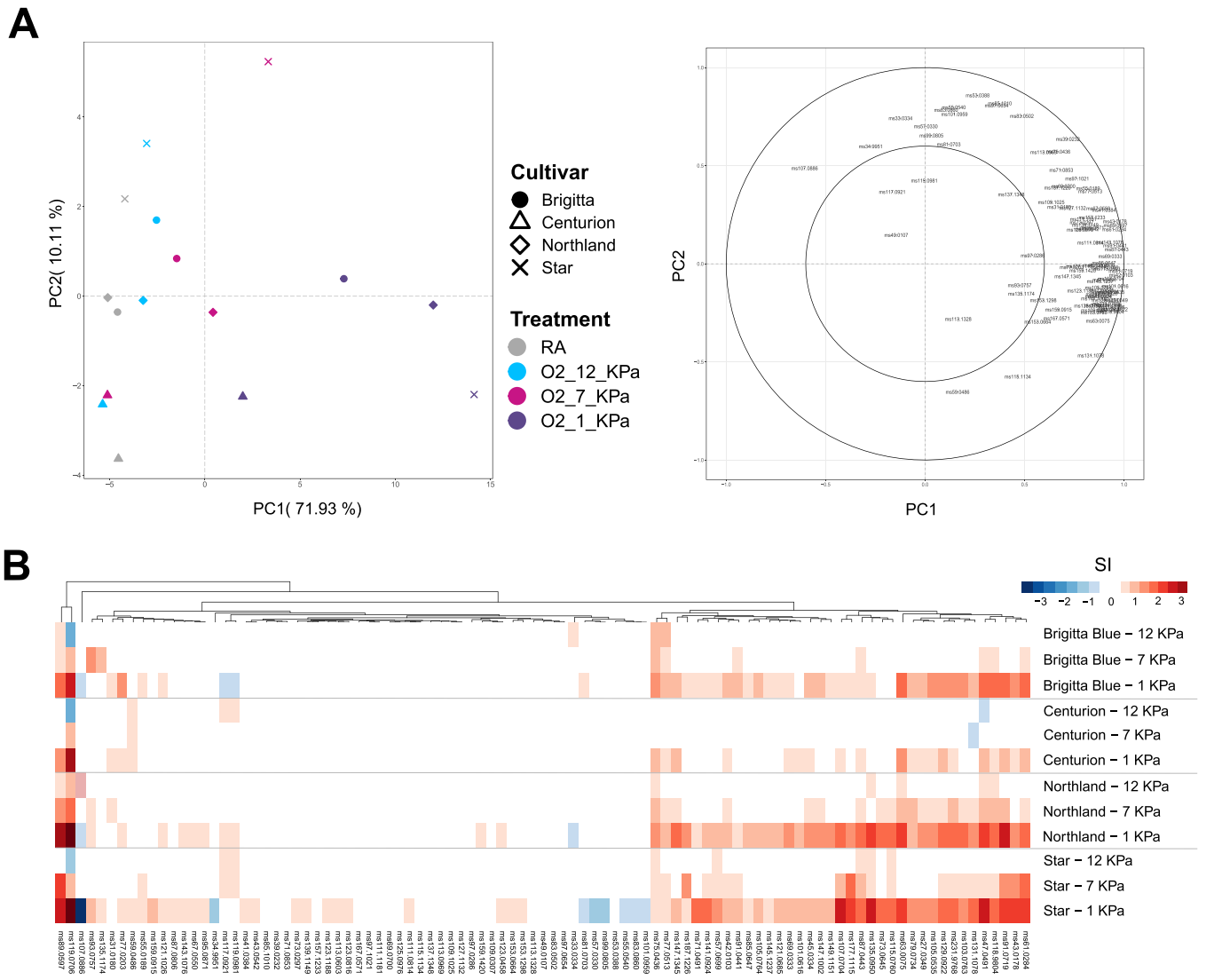

Fig. 7. Effect of varying oxygen levels on the Volatile Organic Compound (VOC) profile of blueberry cultivars "Brigitta", "Centurion", "Northland", and "Star" measured after 42 days of storage using PTR-ToF-MS. Four storage conditions were examined: "RA"(20.9 kPa O_2 and 0.03 kPa CO_2), "O2_1"(1 kPa O_2 and 16 kPa CO_2), "O2_7" (7 kPa O_2 and 16 kPa CO_2), "O2_12" (12 kPa O_2 and 16 kPa CO_2). Each data point represents the arithmetic mean of three biological replicates. **Plot A)** Principal Component Analysis (PCA) and loading plot performed on the log-transformed concentration of each mass. **Plot B)** Heatmap and hierarchical clustering based on the Storage Index (SI) values of each VOC, calculated as the fold change between values measured after Controlled Atmosphere (CA) storage ("O2_1", "O2_7", "O2_12") and Regular Atmosphere (RA) storage.

analysis of VOCs in fruits and vegetables especially during storage (Mazzucotelli et al. 2022). The high sensitivity and real-time monitoring capabilities of PTR-ToF-MS allow for the detection of VOCs that may be missed or underestimated by GC-MS due to their high volatility or low concentration.

The VOC analysis in this study identified only molecules previously reported in other studies of blueberry (Sater et al. 2020), confirming that the aromatic bouquet of blueberries is largely composed of aldehydes (i.e. (*E*)-2-hexenal, hexanal, (*Z*)-3-hexenal, hexadienal, and heptenal), alcohols (i.e. ethanol, (*Z*)-3-hexenol and (*Z*)-2-hexenol), terpenes (i.e. 1,8-cineole, linalool, limonene, α-terpinolene), and ketones (mostly 2-heptanone and 6-methyl-5-hepten-2-one).

The concentration of esters, in particular ethyl acetate, ethyl isovalerate, ethyl crotonate, ethyl 2-methyl butanoate, (*E*,*E*)-ethyl 2,4-hexadienoate, and ethyl butyrate, was significantly higher only in the fruit measured after CA storage, showing values higher than those reported in previous studies (Farneti et al. 2017, Farneti et al. 2020, Gilbert et al. 2015, Forney et al. 2022). Most of previous studies on blueberry aroma, given the relatively low ester contents of fruit at harvest, did not consider esters as key compounds in the aromatic bouquet of blueberries (Gilbert et al. 2015; Sater et al. 2020; Ferrao et al., 2020).

Our results demonstrate that many VOC modifications that are not observed in regular atmosphere (RA) storage occur under CA storage conditions. This finding is particularly significant as it suggests that CA storage not only extends shelf life but can also modulate the organoleptic properties of blueberries. The observed increases in ester compounds, such as ethyl acetate and ethyl isovalerate, under CA conditions are especially noteworthy. These compounds are often associated with fruity and sweet aromas and are typically not present in high concentrations in mature blueberries at harvest (Farneti et al. 2017).

4.2. *Regulatory impact of hypoxic conditions on ester synthesis*

The production of these esters during CA storage presents an opportunity to enhance blueberry aroma profiles by applying the proper postharvest storage condition. This finding is consistent with the literature, which has extensively documented the changes in volatile profiles of various fruits during storage under different atmospheric conditions.

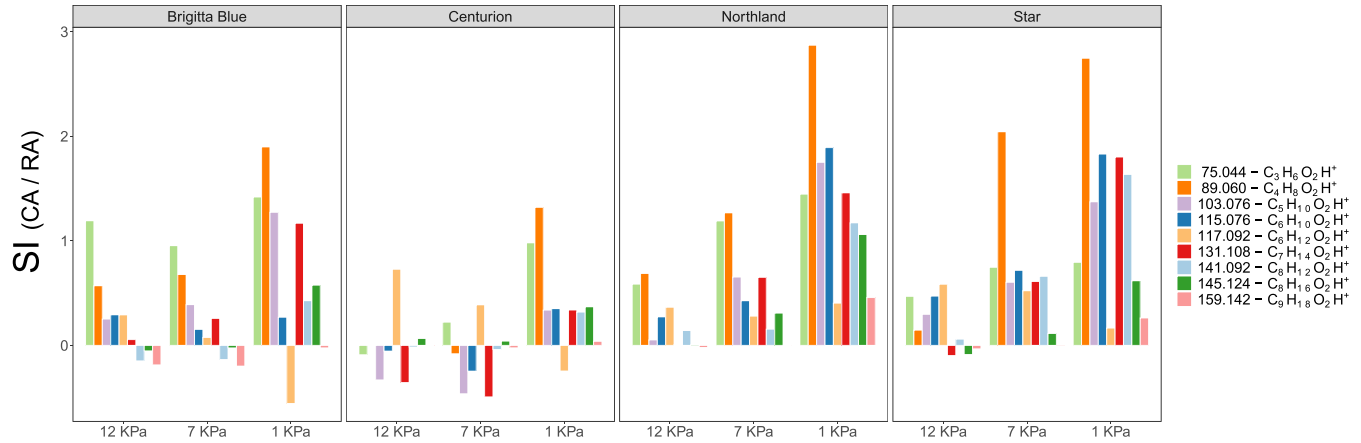

Fig. 8. Fold changes in main ester content during controlled atmosphere (CA) storage under varying oxygen levels (12 kPa, 7 kPa, 1 kPa) in blueberry cultivars "Brigitta", "Centurion", "Northland", and "Star", measured after 42 days of storage using PTR-ToF-MS. Bar plots display Storage Index (SI) values, representing the ratio between measurements taken after CA storage and those after RA storage.

This has been observed in several fruit species, such as apples (Both et al., 2017; Thewes et al. 2020), peach (Ortiz et al., 2010; Maoz et al., 2023), or grape (Maoz et al. 2019) where certain atmospheric hypoxic conditions could promote the synthesis of flavor-enhancing volatile esters.

The varying levels of hypoxicstress induced by different oxygen concentrations in CA storage (1, 7, and 12 KPa) led to different patterns of volatile synthesis in blueberry fruit, particularly for esters (Fig. 8). This observation aligns with findings in other fruit species, such as pears (Lara et al., 2003), strawberries (Yang et al. 2019), apples (Both et al., 2017; Thewes et al. 2020), peach (Ortiz et al., 2010; Maoz et al., 2023), or grape (Maoz et al. 2019), where low oxygen conditions have been shown to modulate ester production. The positive correlation observed between ethanol and acetaldehyde concentrations and certain ester compounds (e.g., ethyl acetate and ethyl isovalerate) suggests a physiological connection between fermentative metabolism and ester synthesis under low oxygen conditions.

However, the differential responses of various ester compounds to hypoxic stress indicate that the relationship between oxygen levels and ester synthesis is not straightforward. Many esters (such as methyl acetate, ethyl acetate, ethyl propanoate, ethyl (2E)-2-butenoate, ethyl 2-methylbutanoate) increased with decreasing oxygen levels, while others (such as methyl isovalerate and 2-methoxyethyl acetate) showed different trends. This variability may be attributed to the complex interplay of different biosynthetic pathways. It may also be due to the varying sensitivity of key enzymes to oxygen levels. For instance, alcohol acyltransferases, which are crucial for ester formation, have shown different oxygen sensitivity levels in various fruit species (Defilippi et al., 2005).

The increase in ester concentrations, particularly under low oxygen storage condition, has been attributed for many fruit species to the activation of enzymatic pathways responsible for ester synthesis, as the reduced oxygen levels can shift the metabolism towards fermentative pathways (Ortiz et al. 2009; Maoz et al. 2019; Maoz et al. 2023; Xu et al. 2020). Prolonged or suboptimal applications of controlled atmosphere (CA) may cause the increased expression of genes involved in fermentative respiration under anaerobic conditions. These genes include pyruvate decarboxylase, aldehyde dehydrogenase, and alcohol dehydrogenase. This gene expression shift can lead to alcoholic fermentation and the development of pyruvate-degradation VOCs, such as ethanol, acetaldehyde, ethyl acetate, and acetone (Maoz et al. 2019). Specifically, the high level of ethyl acetate could be produced due to esterification of ethanol. However, the mechanisms underlying this ester synthesis in blueberries under CA conditions require further investigation.

Our findings expand on the work of Forney et al. (2022) regarding CO2 effects. We demonstrate that when CO2 is maintained at high concentrations, oxygen levels play a crucial role in modifying the aromatic profile of blueberries, particularly affecting esters and alcohols. This finding highlights the complex interplay between oxygen and CO_2 levels in CA storage and their impact on blueberry aroma development.

4.3. *Optimizing genotype-specific storage to enhance blueberry quality*

The varied responses observed among different blueberry cultivars highlight the importance of genotype-specific considerations in optimizing storage conditions. Similar genotype-dependent responses to CA storage have been reported in other fruit species, such as apples (Mditshwa et al. 2018) and peaches (Ai et al. 2024; Obenland et al. 2005). These studies emphasize the importance of considering cultivar-specific responses when developing CA storage protocols. The genetic basis for these differences may lie in variations in the expression or activity of key enzymes involved in aroma compound biosynthesis, such as alcohol dehydrogenases, lipoxygenases, and alcohol acyltransferases (Schaffer et al., 2007).

Our observations provide valuable insights into the potential for fine-tuning storage atmospheres to enhance or maintain desirable aroma profiles in blueberries. However, further research is needed to elucidate the underlying metabolic mechanisms responsible for these cultivar-specific responses to low oxygen environments and to determine the optimal balance between oxygen and CO_2 levels for each cultivar.

The observed variability also suggests the possibility of breeding blueberry cultivars that enhance their aroma profile when stored under CA conditions. By identifying cultivars that exhibit desirable VOC changes during CA storage, breeders could potentially develop new varieties that not only have extended shelf life but also improved organoleptic properties postharvest. To achieve this, it is crucial to deepen our understanding of the genetic regulation of the metabolic pathways involved in the synthesis of both volatile compounds and the key metabolic substrates required for their production (such as sugars, lipids, and amino acids). Investigating these pathways will provide valuable insights into the complex interactions that control the biosynthesis of volatiles, as well as the availability of precursors, which are essential for flavor and aroma development. More information on consumer preferences need to be unveiled and correlated with chemical fruit components.

The enhanced ester production can contribute to the development of different aromatic notes, which may be perceived as desirable or undesirable depending on the cultivar and consumer preferences (Maoz et al. 2019). While the effect of esters on aroma is widely known, the

enhancement of sweetness by several of these compounds is also a known phenomenon for several fruit species (Aprea et al. 2017; Fan et al. 2021a; Fan et al. 2021b; Xiao et al. 2023). Thus, non-sugar sources of sweetness would be highly advantageous for improving the overall liking of blueberry fruit. However, it is crucial to strike a balance between enhancing desirable volatiles and avoiding the accumulation of off-flavors associated with fermentative metabolism and fruit spoilage. The positive correlations observed between ethanol, acetaldehyde, and certain esters highlight the fine line between beneficial and negative anaerobic processes. Careful optimization of CA conditions for each cultivar is necessary to maximize the production of favorable aroma compounds while minimizing the risk of off-flavor development.

Improving fruit quality involves more than just optimizing the concentration of primary and secondary metabolites related to consumer sensory perception. Numerous studies have demonstrated that texture, particularly in blueberries (Giongo et al. 2022, Gilbert et al. 2015), plays a crucial role in shaping the overall quality perception by consumers. Texture attributes, such as firmness and juiciness, have been shown to significantly influence consumer preference, often surpassing the impact of flavour and aroma alone (Gilbert et al. 2015). Thus, enhancing both chemical and physical properties is essential for delivering a superior sensory experience. One of the key findings of this study is the observation that changes in VOC profiles do not necessarily correspond to variations in texture. This highlights the importance of considering both physical and chemical attributes when evaluating fruit quality and developing storage strategies.

As previously reported (e.g., Concha-Meyer et al., 2015; Chiabrando et al., 2018), CA storage significantly improves the preservation of blueberries, extending their shelf life through various mechanisms. These include a reduced incidence of fungal diseases, minimized fresh weight loss and subsequent shrivelling, and better maintenance of berry texture (Alsmairat et al., 2011). However, the results of our study indicate that not all blueberry varieties respond equally to CA storage. While generally, the texture profile of fruits stored under CA conditions remains more similar to freshly harvested fruits compared to those stored in RA (Fig. 3), the effect of cultivar is pronounced (Fig. 3).

Interestingly, our data reveal that in fruits after CA storage, none of the texture parameters or fresh weight loss correlate significantly with the production of compounds associated with fermentation and senescence processes, such as methanol, acetaldehyde, and ethanol (Fig. 4). This lack of correlation suggests a complex relationship between these metabolites and fruit quality parameters during storage. Our findings further underscore the strong genetic control over blueberry storability. We observed cultivars like “Toro”, “Mondo”, “Legacy”, and “Misty” that exhibited high fold changes in both ethanol and acetaldehyde levels during CA storage, while simultaneously demonstrating lower fold changes in texture parameters. Conversely, cultivars showing higher fold changes in texture parameters, such as “Star”, “Roxy Blue”, “Centra Blue”, or “Sky Blue”, are characterized by smaller concentration changes of these molecules. This differential response among cultivars to CA storage has been noted in previous studies (e.g., Moggia et al., 2017) and highlights the importance of cultivar-specific storage protocols.

5. Conclusion

The study validates the complementary nature of chromatographic and direct-injection spectrometric techniques for analyzing blueberry VOC composition. PTR-ToF-MS demonstrates exceptional reliability in generating VOC fingerprints, primarily due to minimal compound fragmentation and precise quantification. The high correlation between VOC matrices obtained from PTR-ToF-MS and SPME/GC-MS underscores the potential of direct injection mass spectrometry as a robust tool for comprehensive VOC profiling, especially when examining large sample sets. However, compound identification remains a limitation of direct injection mass spectrometry. While PTR-ToF-MS can differentiate many isobaric compounds, numerous isomers still require chromatographic separation for definitive identification. This constraint highlights the continued importance of complementary analytical techniques in detailed VOC characterization.

This study reveals that VOC profile changes in blueberries during CA storage depends on cultivar variability and oxygen partial pressure. Our findings suggest that CA storage has potential beyond mere shelf-life extension, offering opportunities to enhance postharvest fruit quality. The observed increase in fruity ester compounds, typically limited in ripe fruit at harvest, may present an attracting opportunity for improving blueberries’ organoleptic properties during storage.

The variability in responses among cultivars, coupled with the complex relationship between hypoxicstress and VOC production, underscores the necessity for carefully develop tailored storage protocols. These results could significantly impact the blueberry industry, potentially leading to improved postharvest management practices and the development of new cultivars with enhanced flavour retention or development during storage. Moreover, modulating blueberry aroma profiles through tailored management of storage atmosphere composition may have applications in the food processing industry. Brief periods of severe hypoxicstress using various gas mixtures (e.g., nitrogen, argon, ozone, CO_2) prior to freezing could effectively modify and enhance the quality of processed blueberry products (i.e. jam or juices), potentially yielding novel flavour profiles or improved sensory characteristics.

By bridging the gap between postharvest research and breeding programs, future studies can adopt a more holistic approach to improving blueberry quality throughout the supply chain. To fully realize the potential for postharvest quality enhancement in blueberries through optimized storage management, future research should integrate sensory analysis, metabolomics, and genetic studies. This comprehensive and systematic approach, applied across multiple fruit evaluations during extended storage periods, will be crucial in developing tailored storage protocols and breeding strategies that maximize the flavour and quality potential of blueberries in both fresh and processed markets.

CRediT authorship contribution statement

Khomenko Iuliia: Writing – review & editing, Investigation, Formal analysis, Data curation. **Ajelli Matteo:** Investigation. **Degasperi Marta:** Investigation. **Betta Emanuela:** Investigation. **Brian Farneti:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Biasioli Franco:** Writing – review & editing, Supervision. **Giongo Lara:** Writing – review & editing, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Acknowledgements

This study was carried out within the Agritech National Research Center and received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022) and United States Department of Agriculture National Institute of Food and Agriculture, award number 2019-51181-30015, project “VacciniumCAP: Leveraging genetic and genomic resources to enable development of blueberry and cranberry cultivars with improved fruit quality attributes”. This manuscript reflects only the authors’ views and opinions, neither the European Union nor the European Commission can be considered responsible for them. This work was financially supported by the AdP of the PAT (Provincia Autonoma di Trento).

Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.postharvbio.2024.113355.

Data availability

Data will be made available on request.

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