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Ripening temperature shapes the flavor metabolite composition in blueberry fruit

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ABSTRACT

High ripening temperatures alter blueberry fruit chemistry, yet the underlying metabolite shifts remain poorly defined. In this study, two blueberry cultivars, southern highbush (*Vaccinium corymbosum* cv. Biloxi) and rabbiteye (*Vaccinium virgatum* cv. Titan™), were grown under two controlled day/night temperature regimes, 20.0/16.0 °C and 30.0/24.0 °C, for two consecutive seasons. The negative impact of higher temperatures on fruit weight, with reductions of up to 34 %, and the sugar-to-acid ratio was more significant in Biloxi than in Titan, highlighting Titan's relative advantage in maintaining fruit quality and taste under heat stress. Higher temperatures also impacted total volatile organic compounds in Biloxi, but not in Titan. Still, changes in key volatile organic compounds, including geraniol, linalool, and methyl 2-methylbutanoate, were detected in both cultivars that could impact the berry flavor. Our results demonstrate how temperature affects blueberry physiology and flavor traits and the adaptability of specific cultivars, i.e., Titan, to higher temperatures. The finding stresses the importance of adapting and optimizing genetic traits to environmental factors under climate change.

1. Introduction

Global climate change poses significant challenges to both natural ecosystems and commercial agriculture. Projections indicate that the global average surface temperature may rise by 1.7 to 4.8 °C by the end of the 21st century. Such warming is expected to profoundly affect crop growth and productivity, with yield losses becoming more pronounced after 2050 compared to the late 20th century (IPCC, 2021).

Blueberry, genus *Vaccinium,* is one of the world's most economically valuable fruit crops (Food and Agriculture Organization, 2020). From 2010 to 2021, global blueberry production more than doubled, increasing from 0.44 million to nearly 1.0 million metric tons (Supplementary Figure 1, Food and Agriculture Organization, 2021). Blueberry cultivars can be divided into three types: highbush (*V. corymbosum*), rabbiteye (*V. virgatum*), and lowbush (*V. angustifolium*, Hancock et al., 2008a). While blueberries are traditionally confined to temperate climatic regions, low and medium-chill cultivars from highbush and rabbiteye have been successfully grown in warmer regions such as Morocco, Spain, and Israel. The effect of growing temperatures on blueberries has been a research topic for many years. In addition, berry quality parameters, including soluble solid content and titratable acidity, are affected by environmental changes, with delayed harvest further affecting these traits (Lobos & Hancock, 2015). More recently, Yang et al. (2019b) presented an adverse effect of high temperature (> 30 °C) on biomass production, fruit set, and fruit ripening in highbush and rabbiteye cultivars, while others showed genotype-dependent effects (Hao et al., 2019). Disruptions in plant development under elevated temperatures can lead to phenology and physiology shifts, adversely affecting crop yield and quality (Zhu et al., 2021). However, no work focused on the effects of ripening temperature on fruit quality and flavor.

Fruit development under higher temperature conditions can impact many aspects of fruit quality, including weight, taste, and flavor (Mattheis & Fellman, 1999). In addition, the increased temperatures expedite fruit ripening in blueberries, which reduces fruit set with smaller fruit size and total yield (Lobos & Hancock, 2015; Yang et al., 2019a, 2019b). Aroma and taste are among the most important sensory attributes that strongly influence consumer acceptance and purchasing

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decisions. Fruit sweetness and tartness are primarily influenced by sugars and organic acids, whereas aroma arises from complex mixtures of volatile organic compounds (VOCs) that impart the characteristic fruit flavors of fresh produce (Forney et al., 2000). The relative proportions of VOCs, sugars, and acids collectively shape the sensory profile of the fruit. Beyond genetic differences among cultivars, environmental factors, including maturity stage, temperature, and light exposure, play significant roles in regulating the production and quality of aromatic compounds in fruits (Forney et al., 2000). Higher temperatures have been shown to increase sugar content while decreasing organic acids and anthocyanins in 'Himekami' apples, whereas in the 'Fuji' cultivar, only anthocyanin levels are reduced under similar conditions (Yamada et al., 1994). In strawberries, sugars decreased, but the effect on organic acids was compound-dependent (increased malic acid, decreased citric acid) (Wang & Camp, 2000). In cucumbers, environmental factors, including temperature, affected C_6 and C_9 volatiles (Li et al., 2019). In grape berries, the effects on VOCs were significant when vines were grown under controlled conditions in the greenhouse (Campos-Arguedas et al., 2022). However, in many cases, temperature was not excluded from other environmental factors, and the responses were strongly genotype-dependent (Kyriacou & Rouphael, 2018).

According to Du et al. (2011), both harvest date and cultivation location significantly affected the accumulation of sugars, acids, and VOCs in several southern highbush blueberry cultivars. In blueberries, glucose, fructose, and sucrose contribute to sweetness, while citric and malic acids are primarily responsible for tartness and acidity (Gilbert et al., 2014; Shi et al., 2023). The sugar-to-acid ratio is a critical determinant of blueberry flavor perception, with an optimal balance leading to a desirable taste profile (Gilbert et al., 2014; Shi et al., 2023). In blueberries, VOCs such as linalool, geranyl acetone, limonene, hexanal, 1-hexanol, decanal, 2-heptanone, β-ionone, 2-nonanone, methyl 2/3-methylbutanoate, and 2-undecanone were shown to play an important role in the flavor acceptability (Du et al., 2011; Ferrão et al., 2020, 2022), demonstrating the interplay between sugars, organic acids, and VOCs in shaping the flavor characteristics of blueberry fruit (Howard et al., 2012). Several studies have assessed changes in the flavor and aroma quality of blueberries under various postharvest storage conditions (Hancock et al., 2008b; Chiabrando et al., 2009; Forney et al., 2022), while only a few studies have examined taste and aroma changes during the pre-harvest stage in blueberries (Du et al., 2011; Lobos et al., 2014, 2018).

While field studies provide valuable insights into the influence of environmental factors on fruit quality, the complex interactions between biotic and abiotic variables make it difficult to isolate the impact of individual factors. Despite extensive field-based research, no previous study has specifically examined how temperature during fruit development and ripening across the season affects taste- and aroma-related compounds in blueberries. Hence, this study aimed to provide new insights into how temperature during fruit ripening influences the flavor and aroma of blueberry fruit. To achieve this, we investigated the effects of ripening temperature and harvest timing on the flavor compounds of blueberries grown under controlled conditions. Both southern highbush and rabbiteye blueberry cultivars were ripened at 20 °C and 30 °C in a controlled environment chamber to assess the response of VOCs, sugars, and organic acids to temperature. To our knowledge, this is the first comprehensive study evaluating the flavor chemical quality of blueberries in relation to ripening temperature and harvest date.

2. Materials and methods

2.1. Plant material and experimental design

The plants used in this study were 4 years old, southern highbush (*V. corymbosum*, cv. Biloxi) and rabbiteye (*V. virgatum*, cv. Titan™) produced from green cuttings. They were grown in a mixture of volcanic tuff and peat (60/40 % v/v, respectively; Tuff Merom-Golan Ltd., Afula, Israel) in 50-L pots. After the 80 % fruit set, plants from both cultivars were transferred to day/night control temperature rooms located on the campus of the Agricultural Research Organization, The Volcani Center, Rishon LeZion, Israel (40 m above sea level). The mean temperatures treatments during the first and second harvest seasons were maintained at 20.0 ± 2.0/16.0 ± 2.0 °C (T20) or 30.0 ± 2.0/24.0 ± 2.0 °C (T30) day/night temperatures. In each temperature treatment, six plants of each cultivar were maintained. The plants were fertilized by commercial fertilizer 5–2–5 + 6 (North Fertilizer Ltd., company, Bet-Shea'an, Israel) using fertigation solutions (fertilization via irrigation system) by drip-irrigation system (discharge of 8.0 L h^{-1} per container; Netafim Ltd., Tel Aviv, Israel). The N, P, and K concentrations in the fertigation solution were 60, 10, and 50 mg L^{-1}, respectively. The plants were watered twice each day. The plants were grown under natural light with no artificial illumination. Throughout the ripening period (10 weeks), the ripe fruits (full purple color) were picked manually, weighed, and counted to determine fruit size. Within 2 h after harvest, 30–60 fruits were sampled from each biological replication, frozen in liquid nitrogen, and kept frozen (−80 °C) until analysis. Before performing the analyses, the fruit from each replicate from each harvest time was grouped into three groups of harvest times (weeks 1–3 for early harvest, weeks 3–6 for mid-harvest, and weeks 7–10 for late-harvest) to examine the effect of harvesting time alongside the temperature effect (T20/T30).

2.2. Chemicals and standards

The chemicals and solvents used in this study were of analytical-grade quality. The water was purified using a Milli-Q® purification system (Millipore Sigma, MA, USA). All the chemicals and standards used in this study were presented in Supplementary Table 1. Chromatography-grade solvents were used in HPLC analysis. Standards of glucose, fructose, sucrose, citric acid, malic acid, quinic acid, succinic acid, octanal, 1-nonanol, 3-hexanol, 2-octanol, hexanal, heptanal, (*E,E*)-2,4-heptadienal, undecanal, 1-phenylethanol, benzaldehyde, 4-hydroxybenzoic acid, methyl hexanoate, ethyl acetate, 6-methyl-5-hepten-2-one, α-pinene, geranyl acetate, linalool, limonene, α-phellandrene, geraniol, α-ionone, citral, damascenone, caryophyllene, and 1,3,5-triisopropylbenzene (internal standard) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Shikimic acid was obtained from Combi-Blocks Inc. (San Diego, CA, USA), and α-terpineol was purchased from Alfa Aesar (Haverhill, MA, USA). 1-Heptanol and benzyl alcohol were acquired from Thermo Fisher Scientific (Waltham, NH, USA).

2.3. Analysis of sugars and organic acids by HPLC-DAD-RI

Fruit powder (2 g) was dissolved in 2 mL of HPLC grade water, then vortexed for 15 min, and centrifuged for 10 min at 4000 rpm. Then, supernatants were filtered through a 0.25 μm PVDF disk filter before HPLC analysis. An HPLC system coupled to RI and PDA detectors (LC-2050C, Shimadzu, Kyoto, Japan) and SH-1011 column (8 × 300 mm, 6 μm, Shodex, New York, USA) with sugar SH-G guard column (6 × 50 mm, 10 μm, Shodex, New York, USA), was used. Chromatographic conditions: mobile phase H_2SO_4 5 mM; flow rate 0.7 mL min^{-1} isocratic elution; column temperature 45 °C. PDA detector operated at fixed wavelength 210 nm; analytes were identified based on the retention times of standard compounds. The calibration curves used to quantify sugars and organic acids were constructed by plotting the peak area vs concentration and expressed as mg g^{-1} of standard compound (Supplementary Table 3).

The HPLC method was validated by calculating the limit of detection (LOD), the limit of quantification (LOQ), linearity, accuracy, recovery, and precision. LOD and LOQ were calculated as (3.3×SD)/S and (10×SD)/S, respectively (S, calibration curve slope; SD, standard deviation). The recovery percentage of each standard was calculated as (concentration in spiked sample–concentration in sample)/concentration added to the sample]×100. The intra-day precision was evaluated by triplicate analysis of a single sample; the inter-day precision was calculated by triplicate analysis of the same sample over 3 days. The precision of the methods was expressed as a percentage of relative standard deviation (RSD %).

2.4. Analysis of volatile organic compounds by GC/MS

Frozen tissues of fruits were homogenized with an IKA A11 grinder under liquid nitrogen (IKA, Staufen, Germany). Two grams of sample were immediately transferred to 20 mL amber vials (LaPhaPack, Langerwehe, Germany) that contained 1 g NaCl (Merck, Darmstadt, Germany) and 2 mL of 20 % (w/v) NaCl solution. Then, a 50 µL aliquot of internal standard 1,3,5-triisopropylbenzene 50 mg L^{-1} (Sigma-Aldrich, St Louis, MO, USA) was added. Then the vials were sealed with magnetic screw caps with pre-cut silicone white/polytetrafluoroethylene red LaPhaPack septa (Thermo Fisher Scientific, Waltham, MA, USA) and stored at −35 °C until analysis. The volatile profiles of the samples were examined by headspace solid-phase micro extraction (HS-SPME) and GC/MS (Model 5977B, Agilent, California, USA). Before analysis, samples were placed in an orbital shaker (MRC, Holon, Israel) at 250 rpm for 30 min, followed by static incubation for 30 min at 40 °C to release free volatiles into the headspace. An SPME fiber, assembly 50/30 µm, divinylbenzene/carboxen/polydimethylsiloxane (Supelco, Bellefonte, PA, USA), was introduced into the headspace for 30 min. The fiber was then desorbed for 5 min at 250 °C in splitless mode to the inlet of a 7890A GC (Agilent, California, USA) equipped with an HP-5MS capillary column (30 m × 0.25 mm inner diameter, 0.25 µm film thickness; Agilent), coupled to a 5975C MS detector (Agilent, California, USA). Helium was the carrier gas in constant pressure mode, and the GC temperature was programmed for 1 min at 40 °C, increasing to 160 °C at 5 °C min^{-1} and 2 min at 160 °C, followed by an increase to 250 °C at 10 °C min^{-1} and 1 min at 250 °C. Ionization energy was 70 eV with a mass acquisition range of 40−206 m z^{-1} and a scanning rate of 7.72 spectra s^{-1}. The retention index (RI) was calculated by running C_5–C_{20} n-alkanes. Compounds were analyzed by MSD Chemstation E.02.01.1177, and their MS description was based on the NIST library (Version 2.0). Volatile identification was based on the RI and mass spectra. The identified volatile compounds were quantified using standard calibration curves (Supplementary Table 5). The contribution of each volatile to the blueberry aroma was estimated by calculating the odor activity value (OAV) as the ratio of compound concentration to its water-based odor threshold in the literature (Czerny et al., 2008; Gemert, 2011; Siebert et al., 2018; Niu et al., 2019; Qian et al., 2021, Qian et al., 2022; Xiao et al., 2024). To estimate the potential contribution of each volatile compound to the overall blueberry aroma, compounds with OAVs greater than one were considered to influence sensory perception. The assignment of VOCs to each of the six main flavor types, i.e., green, minty, citrus, fruity, and fatty, was performed based on the odor descriptors from The Good Scents Company (https://www.thegoodscentscompany.com; Supplementary Table 10).

2.5. Statistical analyses

The individual effects of harvest season, ripening temperature, and harvest date, as well as the combined effects of temperature and harvest date on both individual and total contents, were analyzed using two-way analysis of variance (ANOVA). Each data point of sugars, organic acids, and VOCs represents the mean ± standard error (n = 3 plants). The mean values of VOCs results were rounded up if the digit in the first decimal place was more significant than 5 and rounded down if the digit in the first decimal place was <5. Significant differences ($p < 0.05$) among harvest dates in each temperature regime were determined by Tukey's honestly significant difference (HSD) test. The t-test was used to identify the significant difference between the ripening control temperature regimes at each harvest date. All statistical analyses were performed with JMP Pro version 17 (JMP Statistical Discovery LLC, NC, USA). Principal component analysis (PCA) was performed with Metaboanalyst version 6.0 (https://www.metaboanalyst.ca/).

3. Results

3.1. Effect of temperature on berry weight and yield

To evaluate the impact of temperature on average berry weight and yield, only fully matured berries were harvested over two consecutive seasons. Compared to Titan, the ripening temperature significantly affected berry weight in Biloxi. In Biloxi, average berry weight was significantly higher under T20 than T30, declining from 1.36 to 0.89 g (34 %) in year 1 and from 1.30 to 1.00 g (23 %) in year 2 (Fig. 1A). In Titan, a similar pattern was observed in both years between the T20 to T30, but with a smaller magnitude of effect, 2.12 g vs 2.03 g (4 % decrease) and 2.96 g vs. 2.73 g (7 % decrease), respectively (Fig. 1A). We did not observe a clear effect of the temperature on the total yield in both cultivars. In Biloxi, the average yield was slightly higher, but it was not significant. In Titan, a significant effect was recorded in the first year but not in the second, with a lower yield at T30 than T20 (Fig. 1B).

3.2. Differences in sugar profiles and temperature-dependent accumulation

We characterized the three main sugars: fructose, glucose, and sucrose from T20 and T30, across three harvest dates (early, mid, and late; Fig. 2). Principal component analysis (PCA) of temperature and harvest date effects showed that temperature (PC1, X-axis) had a greater influence than harvest date (PC2, Y-axis) in both cultivars (Biloxi: PC1 = 59.30 %, PC2 = 13.50 %; Titan: PC1 = 64.10 %, PC2 = 20.70 %; Fig. 2A).

Glucose and fructose levels accounted for >95 % compared to sucrose in both cultivars (Fig. 2B). In Biloxi, glucose and fructose levels were significantly higher at T20 than T30, particularly during later stages of the season (Fig. 2B). For example, in Biloxi, glucose showed a consistent increasing trend from early to late harvests, rising from 49 to 65 mg g^{-1} at T20 across both years. By contrast, Titan displayed a more stable glucose profile at the initial harvests, with no significant differences between temperatures at late harvest in either year (Fig. 2B). In contrast, sucrose showed inconsistent seasonal accumulation with higher levels under T30 than T20 in both cultivars. Moreover, sucrose levels in Titan were significantly lower than in Biloxi (Fig. 2B).

Analysis of total sugar levels summarizes the effects of the cultivars, temperature, and harvest date. In Biloxi, sugar levels increased throughout the season in both temperatures, with lower levels in T30 than in T20. In Titan, total sugars increased with harvest under T30, while inconsistent trend in T20. However, temperature effects on total sugar levels were inconsistent: early-harvest berries had lower sugars under T30 than T20, whereas mid- and late-harvest berries showed the opposite pattern (Fig. 2B).

We also observed a difference in the ratio of glucose to fructose between the two cultivars. In Biloxi, the average ratio was ~1.0, and generally, the ratio increased along the season, but the effect of temperature was observed in only two time points of the first season (Fig. 2B). In Titan, the average ratio was ~0.8. While the changes along the season were not consistent, the effect of temperature was. The ratio was higher in T30 (0.90–1.00) than in T20 (0.70–0.80). Overall, the ratio was higher in Biloxi than in Titan (Fig. 2B), indicating a higher accumulation of fructose compared to glucose in Titan than in Biloxi.

3.3. Effect of temperature and different harvests on acids and sugars/acids ratio

We characterized five acids, including citric, succinic, malic, shikimic, and quinic, in the blueberries from two different ripening conditions (T20, T30), and on harvest date (early, mid, and late; Fig. 2C,

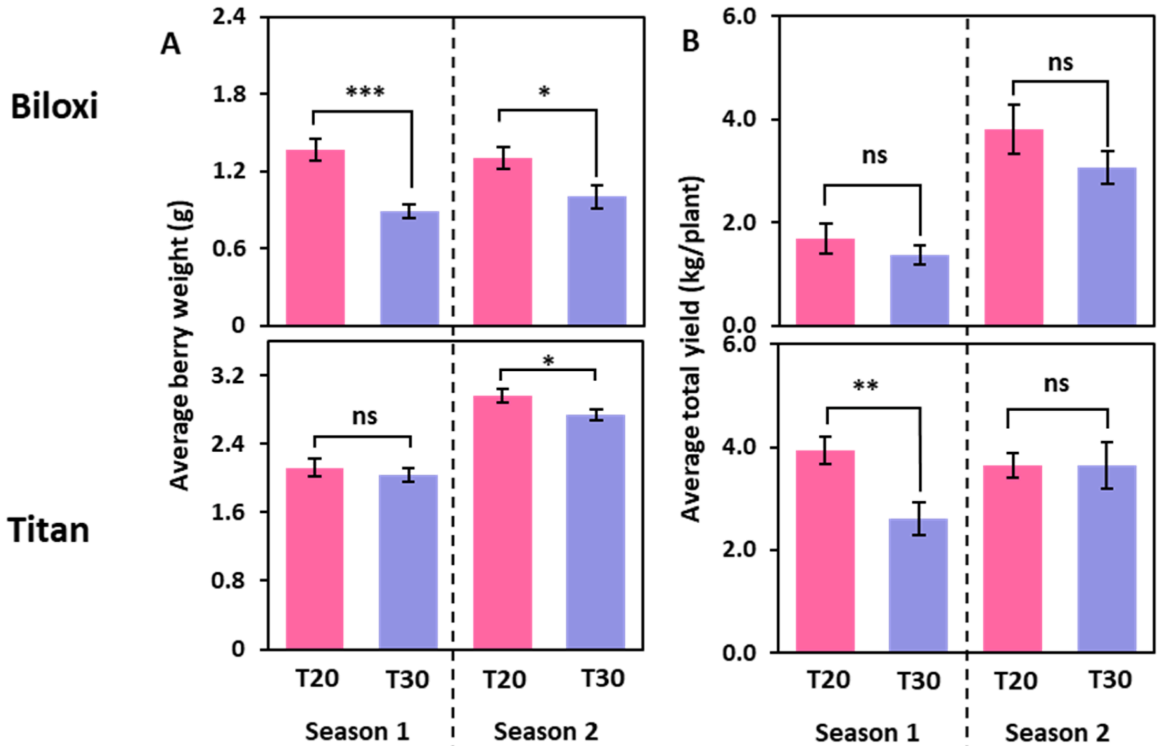

Fig. 1. The Effect of Growing Temperatures on Average Berry Weight (A) and Average Yield per Plant (B) in Biloxi and Titan Cultivars. The bar graphs represent the mean ± standard error for each treatment ($n = 6$ plants). Significant differences between growing temperatures within each season are denoted by *($p < 0.05$), ** ($p < 0.01$), and *** ($p < 0.001$) as determined by a *t*-test, while 'ns' indicates non-significant differences.

Supplementary Table 2). Citric, succinic, and malic acids were the key organic acids in both cultivars. Citric acid accumulation was higher up to 4-fold in Biloxi added higher total acids than in Titan (Supplementary Figure 2). Similarly, succinic and shikimic acids were also higher in Biloxi compared to Titan. Conversely, quinic acid accumulated to higher levels in Titan. We also observe a significant effect of the temperature and harvest date along the season on the accumulation of acids (Fig. 2C). Still, the effects were inconsistent between the cultivars and/or the acids. In Biloxi, the levels of acids were higher in T30 than in T20, while in Titan, an opposite trend was observed. In Biloxi, malic acid increased throughout the season, whereas succinic, shikimic, quinic, and total acid levels declined; citric acid showed no consistent seasonal pattern. In Titan, similar to Biloxi, succinic, shikimic, quinic, and total levels decreased over the season and were higher under T20 than T30.

In both cultivars, the total levels of sugars were lower in T30 than in T20, but different effects were observed on the total acid levels. In Biloxi, the total levels of acids were higher in T30 than in T20, while in Titan, the total levels of acids were higher in T20 than in T30. Therefore, the ratio of sugars/acids in Biloxi was lower in T30 compared to T20, while an opposite trend was observed in Titan. In both cultivars, the ratio increased throughout the season. The ratio in Biloxi was under 4, while in Titan, the ratio exceeded levels of 6, pointing to a 50 % higher level of sugars/acids.

3.4. *Effect of temperature and harvest dates on the accumulation of VOCs*

We identified and quantified 83 VOCs, 68 in Biloxi and 74 in Titan (Fig. 3; Supplementary Tables 4 – 8). The effects of ripening temperature and harvest date on VOCs lead to distinct PCA clusters in both cultivars (Fig. 3B). In both cultivars, PC1 (Biloxi - 41.80 %; Titan - 35.10 %) primarily separated the samples according to temperature, whereas PC2 (Biloxi - 16.30 %; Titan – 16.70 %) reflected the variation associated with harvest date. Overall, a considerable number of VOCs in both cultivars were significantly influenced across seasons by temperature (Biloxi: 44; Titan: 21) and harvest date (Biloxi: 33; Titan: 33; Fig. 3C; Supplementary Table 7). The total VOCs in Biloxi were significantly influenced by temperature ($p < 0.05$), showing consistently higher levels at mid-harvest across both seasons, while Titan did not exhibit significant effects of either temperature or harvest date (Fig. 3D; Supplementary Table 4). Although a consistent trend was observed with higher levels at T30 compared to T20 during mid-season, and lower levels at late-season.

Aldehydes contributed >30 % to the total VOCs in both cultivars (Supplementary Table 8). (*E*)-2-hexenal was the major compound in both cultivars (Biloxi – 194 to 627; Titan - 190 to 756 μg kg^{-1}; Supplementary Table 8). In Biloxi, (*E*)-2-hexenal significantly varied with season, showing similar levels across temperatures during early and mid-harvest (Fig. 4; Supplementary Table 4). In Titan, levels were consistently higher under T30, indicating stronger temperature dependence (Fig. 4; Supplementary Table 4). Hexanal levels were lower than those of (*E*)-2-hexenal and showed a more complex response. In both cultivars, hexanal concentrations were consistently higher under T30 at mid-harvest and lowest at late harvest across both seasons.

Esters were influenced by both factors ($p < 0.01$), with 13 members in Titan and 9 in Biloxi (Fig. 3A; Supplementary Table 6). Methyl 3-methylbutanoate in Biloxi consistently accumulated higher levels at T20 mid (Season I: 6; Season II: 12 μg kg^{-1}) and T30 late-harvests (Season I: 17; Season II: 8 μg kg^{-1}). Despite the higher levels of methyl 3-methylbutanoate (T20: 3 to 17; T30: 4 to 21 μg kg^{-1}) in Titan, the response to temperature was inconsistent between harvest seasons (Fig. 4; Supplementary Table 4). In Biloxi, methyl 2-methylbutanoate consistently accumulated the highest levels during the T20 late harvest (Season I: 5; Season II: 8 μg kg^{-1}) compared to T30 (Season I: 5; Season II: 4 μg kg^{-1}, Fig. 4; Supplementary Table 4). While in Titan, the concentrations decreased with an insignificant difference between temperatures during the harvest season.

In Titan, total ketones contributed 11–30 % to total VOCs and were significantly influenced by temperature, whereas in Biloxi, neither

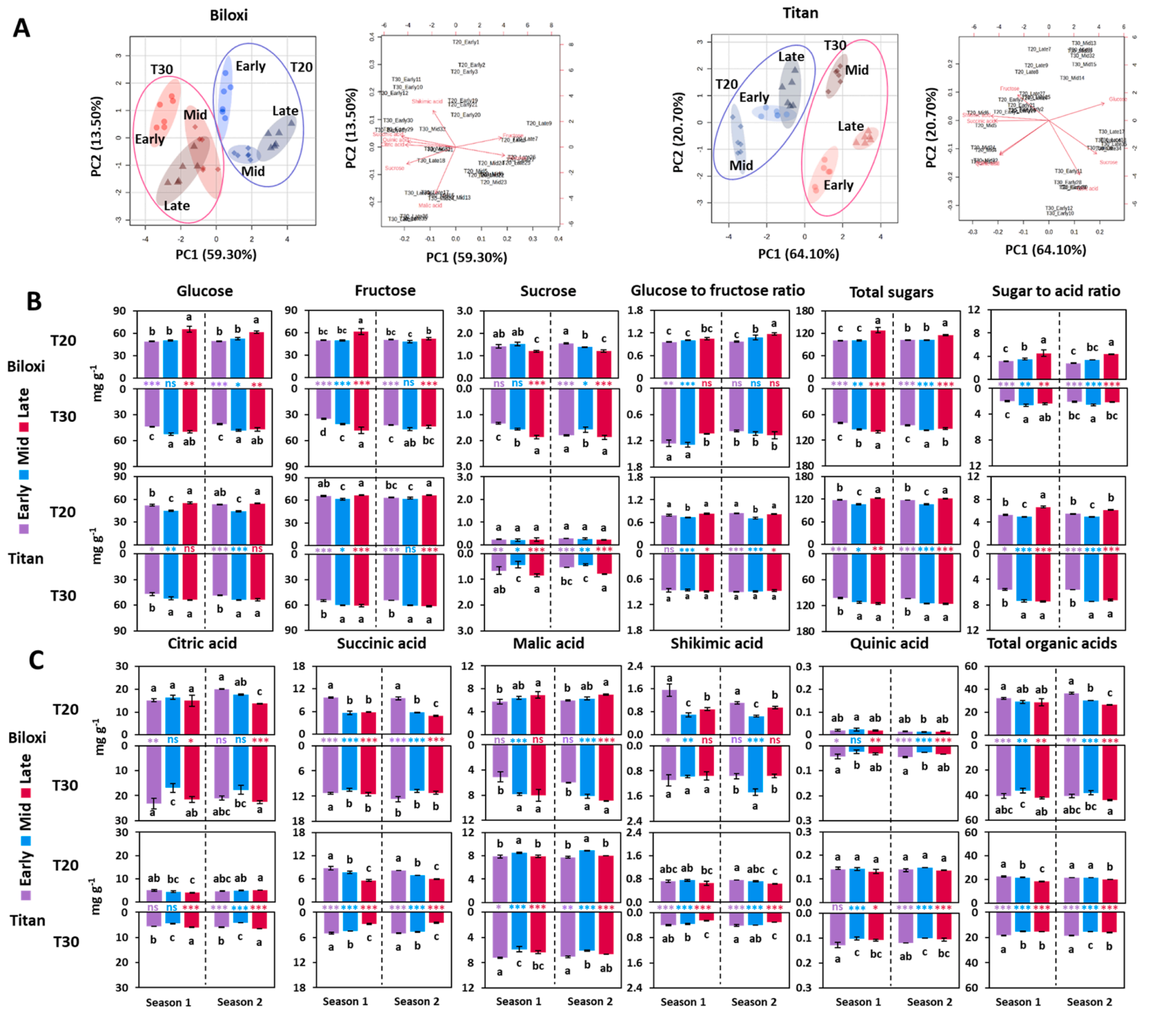

Fig. 2. Effect of Growing Temperature on Sugar and Acids in Biloxi and Titan Cultivars. (A) Principal Component Analysis (PCA) showing variation in sugar and acids profiles of Biloxi and Titan under different growing temperatures (20 °C and 30 °C). Bar graphs showing influence of growing temperature on sugar (B) and acids (C). Each bar graph displays the mean ± standard deviation for each treatment ($n = 3$ plants). Different letters (a-d) denote significant differences ($p < 0.05$) among harvest dates within each growing temperature for individual seasons, as determined by Tukey's HSD test. Asterisks (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$) indicate significant differences between growing temperatures for each harvest date, as determined by a *t*-test, while 'ns' indicates non-significant differences.

temperature nor harvest had a significant effect (Supplementary Table 8). Under T20, Titan consistently accumulated higher total ketone contents across both seasons (Fig. 3D). Among individual ketones, C_4 (2-butanone) and C_5 (2- and 3-pentanone) were significantly affected by temperature, C_9 (2-nonanone) and C_{11} (2-undecanone) were unaffected, while 2-heptanone was significantly affected by both temperature and harvest date (Supplementary Table 4).

Monoterpenes were the largest group (28 compounds) (Fig. 3A; Supplementary Table 6), with 10 showing cultivar-specific accumulation: citral, α-terpinolene, α-phellandrene, camphor, *p*-cymene, and 1,3,8-p-menthatriene in Biloxi, and β-pinene, α-pinene, *m*-cymene, and *trans*-linalool oxide in Titan (Supplementary Table 6). In Biloxi, total monoterpene levels were consistently influenced by temperature ($p < 0.001$) with higher levels at T20 than T30 across both seasons. In Titan, the only consistent effect was observed at late harvest, where T20 also showed higher monoterpene accumulation than T30 (Fig. 3D). In both cultivars, geraniol, linalool, limonene, eucalyptol, and α-terpineol were major contributors to total monoterpene content (Supplementary Table 8). Despite the cultivar dependent significant ($p < 0.001$) effects of harvest date (Titan) and temperature (Biloxi), linalool levels remained consistently higher in Titan (T20: 13–24; T30: 8–22 μg kg^{-1}) compared to Biloxi (T20: 5–14; T30: 3–4 μg kg^{-1}) with similar trends (Fig. 4; Supplementary Table 4). Similarly, eucalyptol levels were higher in Titan, with higher concentrations at T20 during early harvest at T30 (Season I: 6; Season II: 10 μg kg^{-1}, Fig. 4; Supplementary Table 4).

Total norisoprenoid content was not influenced by temperature in either cultivar, while harvest date had a significant effect only in Biloxi (Fig. 3D; Supplementary Table 4). In both cultivars, 6-methyl-5-hepten-2-one (MHO) accounted for 90 % of the total norisoprenoids (Supplementary Table 8) and was not influenced by temperature in either cultivar. In Biloxi, the MHO content was significantly affected by harvest date, whereas in Titan, harvest date had an insignificant effect (Fig. 4; Supplementary Table 4). In season 1, MHO concentrations were highest at early harvest in both temperature regimes, whereas in season 2, they declined as harvest progressed. β-Damascenone (exclusive to Titan) levels (T20: 0 to 1; T30: 0 to 1 μg kg^{-1}) were affected by both

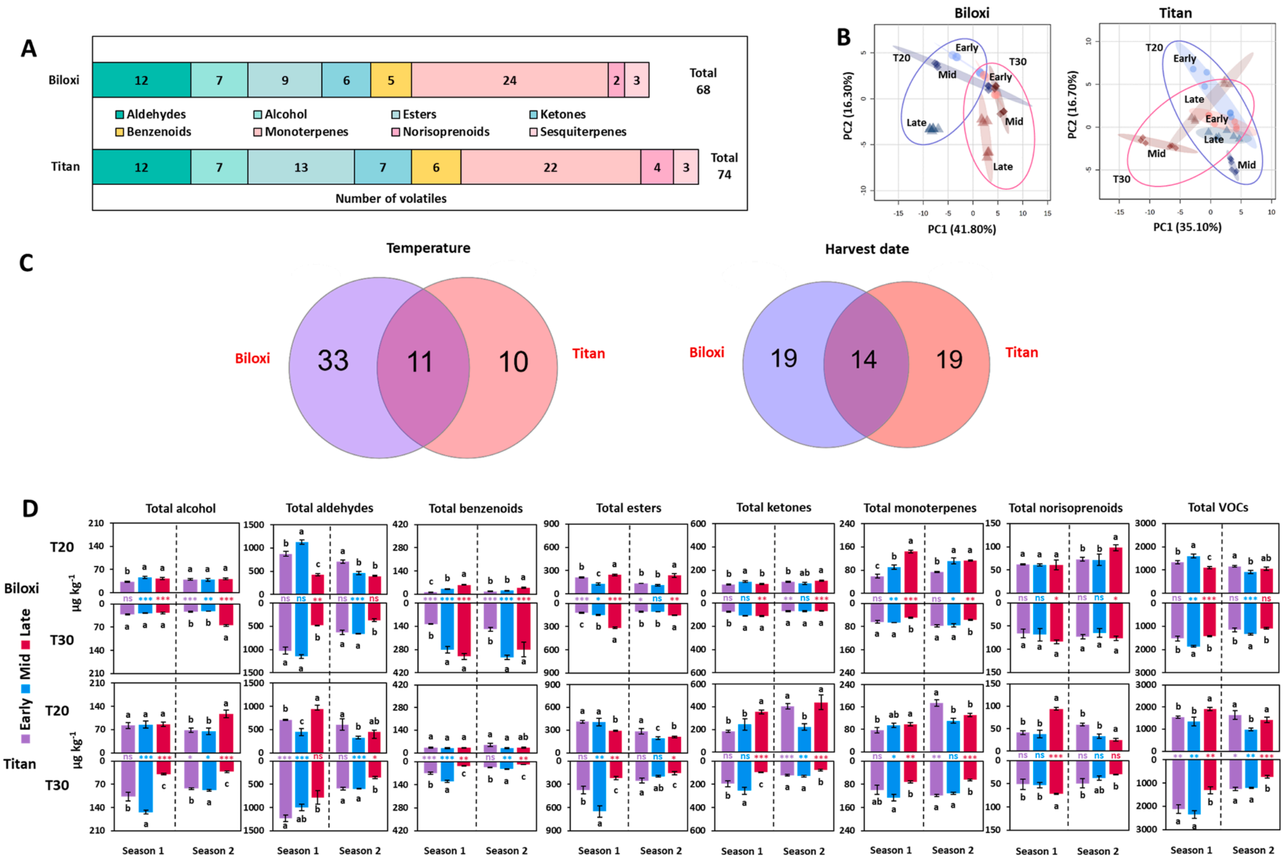

Fig. 3. Overview of Volatile Organic Compounds (VOCs) Identified in Biloxi and Titan Cultivars. (A) Distribution of VOCs across different chemical groups. (B) Principal Component Analysis (PCA) showing variation in VOCs profiles of Biloxi and Titan under different growing temperatures (20 °C and 30 °C). (C) Venn diagram depicting the number of VOCs influenced by different ripening temperatures and harvest dates. (D) Influence of Varying Temperatures on the Total Content of Different Volatile Groups in Biloxi and Titan Cultivars. Each bar graph represents the mean ± standard deviation for each treatment. Different letters (a-c) indicate significant differences ($p < 0.05$) among harvest dates within each growing temperature for individual seasons, as determined by Tukey's HSD test. Asterisks (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$) denote significant differences between growing temperatures for each harvest date, as assessed by a *t*-test, while 'ns' signifies non-significant differences.

temperature and harvest date, though differences were significant only at late harvest (Supplementary Table 4).

Consistently higher benzenoid levels were observed under T30 in Biloxi compared to Titan, and contributed over 20 % of total VOCs content (Supplementary Table 8). This was driven by the consistent effect of temperature on phenylethyl alcohol (T20: 8 to 45; T30: 24 to 243 μg kg^{-1}) and methyl salicylate (T20: 1 to 8; T30: 57 to 194 μg kg^{-1}) across both seasons in Biloxi, whereas Titan did not show a similar pattern (Fig. 4; Supplementary Table 4).

3.5. Examine the effect of temperature on VOCs by OAV and odor descriptors

To evaluate the potential impact of temperature and harvest date on the changes in berry flavor, we collected the aroma descriptors and their odor threshold (μg kg^{-1}) of identified VOCs in both cultivars (Supplementary Table 9). We calculated odor active value (OAV) and identified 28 compounds (OAV>1), including two or four, particularly in Biloxi or Titan, as possible contributions of VOCs to the blueberry aroma (Table 1). Among them in both cultivars, hexanal, ethyl acetate, geraniol, octanal, and methyl 2-methylbutanoate were notable due to their high OAVs and distinctive accumulation patterns (Table 1).

Ten aldehydes exhibited OAVs>1, including heptanal, 2,4-hexadienal, octanal, decanal, (*E*)-2-hexenal, nonanal, (*E*)-2-pentenal, 2-octenal, undecanal, and hexanal. Among these, six showed OAVs between 2 and 9 in both cultivars. Notably, heptanal exceeded its threshold to a greater extent in Titan than in Biloxi, while hexanal, octanal, and undecanal exhibited OAVs above 10. Hexanal, known for its fresh, green, and fruity notes, showed similar ranges in both Biloxi (T20: 22.0 to 92.2; T30: 19.2 to 106.1 OAV units) and Titan (T20: 24.5 to 72.4; T30: 26.6 to 101.2 OAV units), with higher values in T30 than T20 across seasons. Interestingly, octanal (citrus, fatty notes) was higher in T20 compared to T30, with higher OAVs in Titan (T20: 12.1 to 57.9; T30: 7.2 to 16.8 OAV units) than in Biloxi (T20: 7.7 to 29.3; T30: 7.6 to 17.1 OAV units, Table 1).

Seven monoterpenes had OAV>1, including α-terpineol, limonene, eucalyptol, linalool, geraniol, citral (exclusive to Biloxi), and α-pinene (exclusive to Titan). Geraniol, with sweet, fruity, and berry notes, had higher OAVs in T20 (Biloxi: 18.9 to 71.9; Titan: 16.3 to 63.1 OAV units) than in T30 (Biloxi: 19.6 to 43.7; Titan: 15.3 to 37.3 OAV units) in both cultivars. Among monoterpenes, geraniol contributed 80 % to 90 % of the cumulative OAV units in Biloxi, whereas it contributed 45 % to 80 % in Titan (Data not shown – Supplementary Table 9). Additionally, three terpenes — α-terpineol, limonene, and linalool — contributed 5–10 % cumulative OAV units despite their relatively low absolute concentrations due to their low thresholds. Although eucalyptol (minty, woody, herbal notes) was a common monoterpene to both cultivars, its OAV unit's contribution was lower in Biloxi (<5 %) than in Titan (<29 %, Data not shown – Supplementary Table 9). Eucalyptol levels remained stable across harvest dates in Biloxi, with lower OAVs at T30 (0.7 to 1.8 OAV units) than at T20 (1.0 to 2.2 OAV units). In Titan, eucalyptol levels were higher than in Biloxi, showing a different trend at T30 (3.0 to 11.5 OAV units), with an increase during the early and mid-season.

Of the six esters identified, methyl 2-methylbutanoate and ethyl

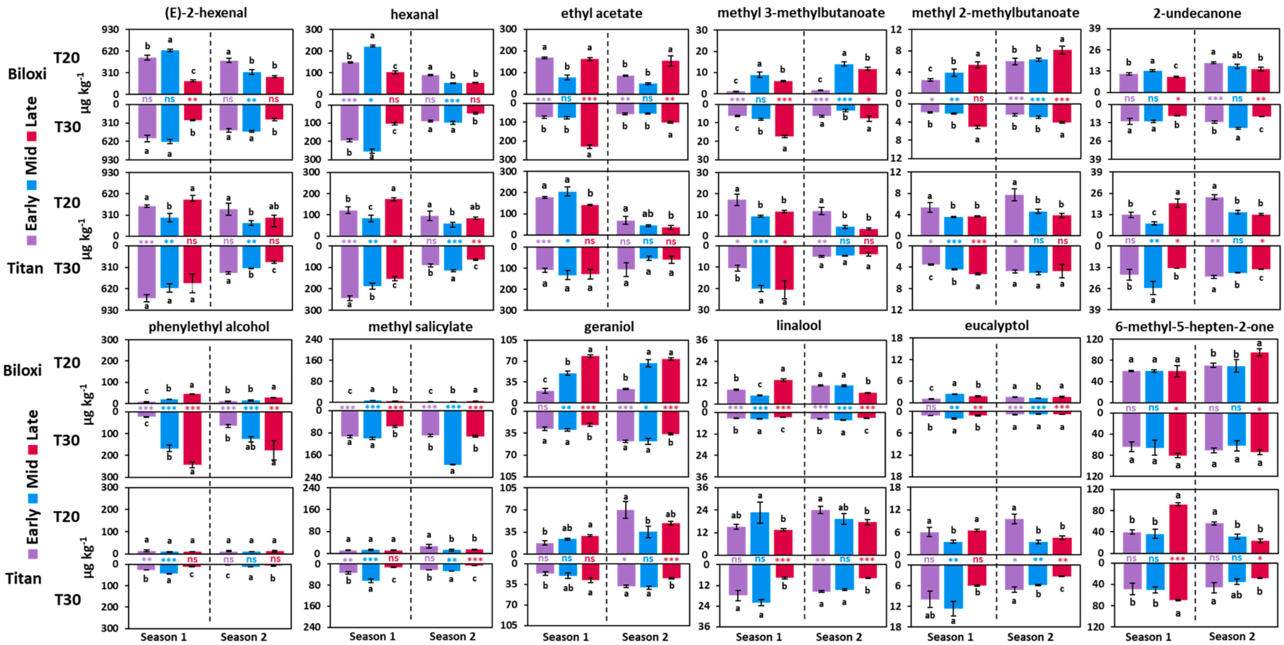

Fig. 4. Influence of Varying Temperatures on Volatile Organic Compounds (VOCs) in Biloxi and Titan Cultivars. Each bar graph presents the mean ± standard deviation for each treatment (n = 3 plants). Different letters (a-c) indicate significant differences ($p < 0.05$) among harvest dates within each growing temperature for individual seasons, as determined by Tukey's HSD test. Asterisks (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$) denote significant differences between growing temperatures for each harvest date, as assessed by a t-test, while 'ns' signifies non-significant differences.

acetate had OAVs greater than 10 in both cultivars. Methyl 2-methylbutanoate with fruity notes OAV increased with harvest progression (early < mid < late). However, lower ripening temperatures (Biloxi: 10.1 to 32.7; Titan: 14.2 to 31.0 OAV units) led to higher OAVs than T30 (Biloxi: 7.6 to 20.4; Titan: 14.1 to 21.1 OAV units) in both cultivars. Apart from these, ketones (5), benzenoids (3), and norisoprenoids (2) also contributed to the flavor profile, with OAVs >1. Notably, β-damascenone, specific to Titan and associated with fruity, citrus, and bittersweet notes, exhibited a higher range of OAVs under T20 (30.7–79.9) than T30 (15.6–66.9) (Table 1).

Compounds contributing to blueberry aroma (OAV >1) were assigned a single odor descriptor, and their OAVs were summed into six typical aroma categories: fruity, green, minty, citrus, fatty, and floral (Supplementary Tables 10 and 11). The radar map of summarized aroma categories (Fig. 5) revealed a 2.5-fold increase in cumulative floral OAV at the T20 late harvest in Biloxi (73.4 vs. 30.4 OAV units at T30). In Biloxi, the minty aroma showed the opposite trend to floral, with a 2.5-fold higher accumulation under T30 than T20, while citrus, green, fruity, and fatty aromas did not exhibit significant temperature effects (Fig. 5; Supplementary Table 11). In Titan, citrus aroma was 2.1-fold higher at T20 late harvest (66.9 OAV units) than at T30 (31.2 OAV units). Fruity aroma remained stable across harvest dates (40–50 OAV units) in both temperature regimes. Minty aroma, though low in intensity, increased nearly 2.7-fold at the T30 mid-harvest (10.8 vs. 3.9 OAV units in T20). Similarly, fatty aroma was 2.1-fold higher in T30 mid-harvest berries compared to T20 (Fig. 5; Supplementary Table 11).

4. Discussion

4.1. Increased temperature resulted in smaller berries

Berry weight and yield are essential quality traits for growers and consumers (Alan Erb, 1993). They are considered critical parameters for analyzing stress impacts on blueberry fruit quality (Mingeau et al., 2001). While the tendency is to increase fruit berry size by breeding or applications (Maoz et al., 2019), a berry weight of around 1.3 g is not exceptional in blueberries. In Israel, Titan berry weight is consistently larger than Biloxi, with a difference of about 0.7 g represents local conditions. This decrease was significant in Biloxi, while it was insignificant in Titan over the two years. Although southern highbush and rabbiteye cultivars are generally better adapted to warmer climates (Yang et al., 2019a), Biloxi berries under high temperatures exhibited reduced weight, likely reflecting decreased photosynthesis and a source–sink imbalance, where water and carbohydrates are diverted from the fruit to vegetative tissues, as previously reported in blueberries (Lobos & Hancock, 2015) and other fruit crops (Kumudini, 2004; Sun et al., 2012). The positive correlation between larger fruit size and lower temperatures is likely due to an extended ripening period with prolonged carbohydrate accumulation and cell expansion, as shown in strawberries (Miura et al., 1994; Ledesma et al., 2008). In contrast, Titan maintained relatively stable fruit size and cultivar-specific resilience to heat stress. To minimize the temperature effect on fruit load/yield potential, pollination was performed before moving the plants to the greenhouses in both years. However, significant variation in yield was observed only in Titan during the first year, likely due to the plants being moved too early, before pollination was fully completed. Alternatively, it can result from a lower fruit set percentage at higher temperatures, as previously demonstrated in rabbiteye (Yang et al., 2019b). Titan exhibited more consistent berry weight and yield, maintaining larger berries under elevated temperatures, suggesting that its greater tolerance to high-temperature conditions may be a heritable trait and a promising target for selective breeding.

4.2. Sugar accumulation was negatively affected by increased temperature

Sugars and organic acids contribute to the basic taste of fruits, providing sweetness and tartness/sourness sensations (Viljakainen et al., 2002). This study revealed sugar and acid profiles in blueberries consistent with previous findings (Ehlenfeldt et al., 1994; Mikulic-Petkovsek et al., 2012; Zhang et al., 2020). In fully matured blueberry fruit, glucose and fructose are the main sugars with fold-changes of lower sucrose levels, corresponding to the reduction in invertase activity (Kader et al., 1993). The lower levels of sugar at T30 compared to T20

Table 1

A Comprehensive List of Volatile Organic Compounds (VOCs) with estimated Odor Activity Values (OAVs) calculated as compound concentration/odor threshold (OT) in the literature ([$]Czerny et al. 2008; [§]Gemert, 2011; [#]Siebert et al., 2018; [¥]Niu et al., 2019; [€]Qian et al., 2021; *Xiao et al., 2024) greater than 1: Assessing the Impact of Varying Growing Temperatures on Biloxi and Titan Cultivars.

Compounds	Odor descriptor	Odor threshold (μg kg^{-1})	OAV																							
			Biloxi												Titan											
			Season 1						Season 2						Season 1						Season 2					
			T20			T30			T20			T30			T20			T30			T20			T30		
			Early	Mid	Late	Early	Mid	Late	Early	Mid	Late	Early	Mid	Late	Early	Mid	Late	Early	Mid	Late	Early	Mid	Late	Early	Mid	Late
In both Biloxi and Titan																										
methyl salicylate[§]	minty	40	0.0	0.2	0.2	2.4	2.5	1.5	0.1	0.1	0.2	2.3	4.9	2.4	0.3	0.4	0.4	0.9	1.6	0.4	0.7	0.4	0.4	0.6	0.7	0.2
phenylethyl alcohol[$]	roses, floral	140	0.1	0.2	0.4	0.2	1.2	1.8	0.1	0.2	0.2	0.5	0.9	1.3	0.2	0.1	0.1	0.2	0.4	0.1	0.1	0.1	0.1	0.0	0.1	0.1
methyl hexanoate[¥]	ether-like, fruity	10	0.2	0.4	0.6	0.4	0.7	0.3	0.2	0.4	0.7	0.2	0.4	0.3	0.7	1.1	1.0	2.1	4.2	0.3	2.0	1.0	0.7	1.4	1.1	0.3
methyl butanoate[#]	pungent, ethereal, fruity, fumy	10	0.3	0.5	0.8	0.3	0.3	0.5	0.6	0.3	1.0	0.4	0.6	0.4	0.6	0.6	1.0	1.1	0.7	0.2	1.0	0.5	0.7	0.8	0.4	0.2
α-terpineol[§]	woody, piney, citrus	4.6	0.3	0.2	0.8	0.1	0.2	0.1	0.4	0.7	0.4	0.2	0.2	0.2	1.3	1.5	2.6	1.7	1.9	0.9	2.5	1.5	1.7	1.7	1.2	0.5
methyl 3-methylbutanoate[€]	fruity, apple, pineapple	4.4	0.3	2.0	1.4	1.5	1.9	4.0	0.4	3.2	2.7	1.5	0.8	1.8	3.9	2.2	2.7	2.4	4.6	4.7	2.7	1.0	0.8	1.2	1.1	1.0
phenylacetaldehyde[$]	floral, honeysuckle, rosy	1	0.4	0.8	2.0	1.7	1.9	2.5	0.5	1.1	2.2	0.7	2.0	1.4	2.0	2.6	3.0	4.3	3.8	1.1	3.8	2.3	1.4	3.9	1.6	1.1
heptanal[§]	green, herbal	2.8	0.5	0.9	1.0	1.3	1.9	1.7	0.6	0.5	0.7	0.3	0.6	0.6	1.6	2.0	4.2	3.9	4.2	1.3	2.2	1.4	1.3	1.5	1.2	0.5
limonene[§]	citrus, fresh, sweet	4	0.9	0.9	1.7	0.7	2.0	0.6	1.3	1.2	1.0	0.6	0.9	0.5	1.2	1.9	1.0	1.9	2.4	1.2	2.8	1.9	1.8	2.0	1.6	0.8
eucalyptol[$]	minty, woody, herbal	1.1	1.0	2.2	1.7	1.0	1.8	1.0	1.5	1.2	1.5	0.8	0.7	0.8	5.5	3.2	6.0	9.2	11.5	5.5	8.7	3.2	4.2	6.5	5.4	3.0
1-hexanol[§]	fruity, sweet green	5.6	1.2	1.9	1.3	1.7	1.0	0.5	0.6	0.7	1.1	0.5	0.3	0.4	4.5	3.4	1.1	1.5	2.5	0.0	2.4	1.3	1.0	1.3	1.0	0.2
6-methyl-5-hepten-2-one[§]	fatty, green, citrus, bittersweet	50	1.3	1.2	1.2	1.3	1.4	1.7	1.5	1.4	2.0	1.5	1.3	1.5	0.8	0.8	1.9	1.0	1.0	1.4	1.2	0.7	0.5	1.0	0.8	0.6
linalool[§]	green, rosy, floral	6	1.4	0.9	2.3	0.6	0.7	0.5	1.8	1.8	1.1	0.7	0.8	0.6	2.6	3.8	2.3	3.0	3.7	1.3	4.0	3.3	3.0	2.6	2.5	1.4
methyl decanoate[§]	oily wine fruity floral	4.3	1.9	0.9	1.4	2.3	1.8	1.6	1.1	0.9	1.3	0.9	1.3	1.2	1.9	0.8	1.3	1.5	4.4	1.0	2.0	1.2	1.0	1.2	1.0	0.6
2-undecanone[§]	fruity, floral	5.5	2.1	2.5	1.8	2.2	2.3	1.5	3.3	3.0	2.6	2.3	3.1	1.6	2.4	1.4	3.7	3.2	4.7	2.5	4.4	2.7	2.4	3.4	3.0	2.6
2,4-hexadienal[§]	leaf, fatty, green	60	2.4	3.6	1.0	3.3	3.5	1.1	1.2	0.8	0.6	1.2	1.3	0.6	1.7	0.9	1.8	2.9	2.4	0.4	0.8	0.7	0.8	1.2	1.9	0.5
2-nonanone[§]	fruity, earthy	5	2.4	3.0	2.3	3.0	4.3	3.1	3.8	4.4	1.8	3.0	3.0	2.3	4.3	4.8	9.1	6.7	8.2	3.7	10.1	4.2	7.0	7.4	5.0	3.0
(*E*)-2-pentenal[§]	pungent, green, citrus	1.5	3.0	2.6	2.5	2.5	2.5	2.4	2.6	2.5	2.4	2.4	2.4	2.4	2.6	2.7	3.2	3.2	3.0	2.5	2.9	2.6	3.6	2.9	2.4	2.4
decanal[§]	fruity, citrus, orange	0.4	3.1	4.4	3.7	4.0	2.6	3.0	3.4	2.8	3.2	2.4	2.3	2.3	2.5	3.5	7.8	4.1	5.5	5.6	7.8	5.5	5.5	5.5	5.4	3.4
2-octenal[§]	green-leafy, less fatty	4	4.6	5.7	5.6	7.3	6.7	4.4	5.5	4.5	4.8	3.3	3.1	2.8	1.0	1.2	9.2	2.8	2.5	7.2	2.5	1.9	2.2	2.2	2.6	1.4
(*E*)-2-hexenal[$]	grassy, pungent	110	4.8	5.7	1.8	5.2	5.7	2.5	4.5	3.0	2.4	4.0	4.2	2.4	4.0	2.5	4.9	6.9	5.6	5.0	3.7	1.8	2.5	3.6	3.0	2.2
undecanal[§]	sweet, fatty odor, rose	0.09	5.3	11.4	12.6	11.8	16.5	11.9	6.2	4.6	7.6	5.0	4.7	6.9	5.7	3.6	10.4	7.2	8.1	10.6	13.5	9.2	10.4	11.0	10.4	8.5
nonanal[$]	roses, fresh	2.8	7.0	7.1	8.2	7.7	5.7	7.2	8.9	6.0	8.9	4.3	4.7	4.7	4.8	4.0	7.0	2.0	3.7	2.8	5.5	3.6	4.4	3.5	4.5	2.7
methyl 2-methylbutanoate[€]	fruity	0.25	10.2	15.6	21.5	7.6	8.4	20.5	24.1	25.4	32.7	9.7	11.6	16.3	21.6	14.2	14.6	14.2	17.8	21.1	31.0	18.5	15.4	19.3	20.5	19.1
octanal[€]	citrus, fatty	0.7	17.4	29.3	28.0	14.5	15.8	17.1	17.4	8.4	7.7	7.6	8.2	10.3	23.8	12.2	57.9	15.5	13.5	16.9	15.8	13.6	21.6	9.9	11.1	7.3
geraniol[$]	sweet, fruity, berry	1.1	18.9	45.5	71.9	25.3	26.9	19.6	21.6	60.6	67.1	43.7	43.7	32.9	16.3	21.6	26.7	15.3	19.0	25.6	63.1	32.0	44.6	35.1	37.3	23.0
ethyl acetate[§]	fruity, tart	8.5	19.8	9.0	19.2	8.6	9.0	27.1	10.0	5.7	18.2	6.7	6.5	11.9	20.9	24.2	16.6	12.9	15.7	15.2	8.1	5.2	4.2	12.6	6.4	7.1
hexanal[$]	fresh, green, fruity	2.4	61.8	92.2	42.7	81.1	106.2	43.0	37.3	22.0	22.9	37.0	40.9	19.3	51.0	35.0	72.4	101.2	78.0	63.7	40.0	24.5	35.2	37.7	48.0	26.6
Only in Biloxi																										
citral*	sweet, fruity	12	0.9	1.4	1.1	0.9	0.8	0.8	0.9	0.7	0.9	0.8	0.6	0.5	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
1-penten-3-one[§]	pungent, mustard odor	13	2.1	2.5	1.7	1.0	1.0	2.3	1.2	0.8	1.0	0.6	0.4	0.9	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Only in Titan																										
3-pentanone[§]	ethereal, pungent, sweet	82	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.3	0.8	0.9	0.2	0.2	0.2	1.0	0.7	1.3	0.2	0.3	0.1
α-pinene[$]	herbaceous, spearmint	2.2	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.4	0.4	1.2	1.6	1.6	0.9	1.0	0.6	0.7	1.0	1.0	0.4
2-pentanone[§]	ethereal, fruity odor	70	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.5	0.7	0.8	0.4	0.9	0.2	0.7	0.6	1.2	0.2	0.4	0.2
β-damascenone[$]	fruity, citrus, bittersweet	0.013	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	38.8	46.7	30.7	34.0	43.1	15.7	79.9	69.5	56.2	66.9	32.6	36.6

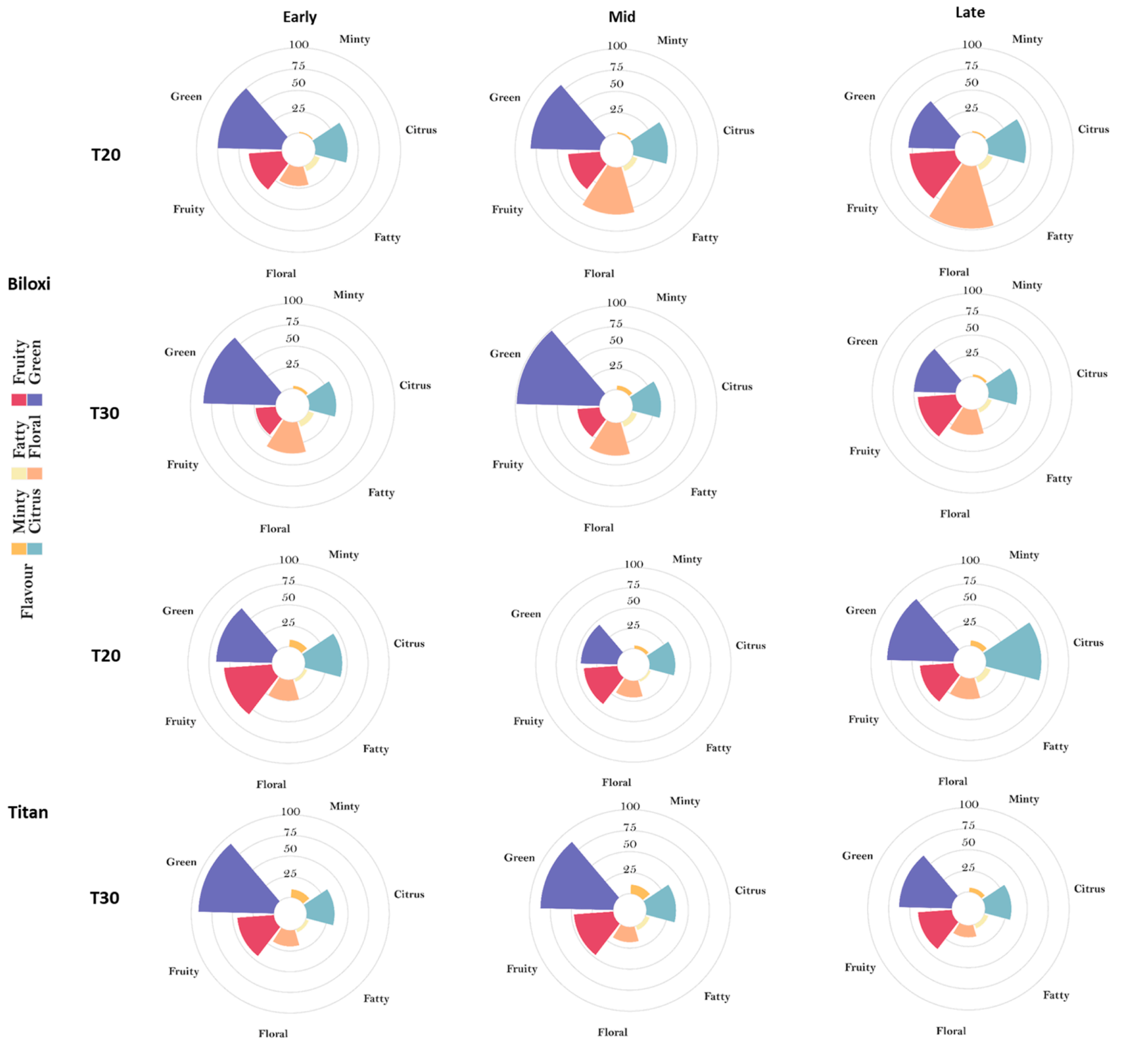

Fig. 5. Radar graph depicting the average flavor accumulation profile of Biloxi and Titan across harvest stages (Early, Mid, Late) under varying growing temperatures (20 °C and 30 °C). A compilation of total Odor Active Values (OAVs) for diverse flavor types from two harvest seasons.

can be explained by the negative effect of increased temperature on photosynthesis and the production of sucrose in the leaves (Sage & Kubien, 2007; Ruelland & Zachowski, 2010). The negative effect of higher temperatures on sugar levels is correlated with a decrease in fruit size, which likely explains the higher sugar levels observed at lower ripening temperatures due to a slower fruit development period. Interestingly, we have characterized a lower glucose-to-fructose ratio in Titan compared to Biloxi, regardless of temperature or harvest date. This reflects a higher fructose content in Titan, which may represent a favorable trait for maintaining the sweetness level under high temperatures, as fructose is more than twice as sweet compared to glucose (Da Conceicao Neta et al., 2007). Previously, it was shown that rabbiteye cultivars have lower glucose-to-fructose ratios, but these results were not significant (Itle & NeSmith, 2016). The decrease in sucrose levels at higher temperature and the distinct glucose to fructose ratio in Titan may reflect altered phloem unloading, invertase activity, or vacuolar sugar partitioning mediated by transporters such as SWEETs, sucrose transporters, and tonoplast sugar transporters (Chen, 2014; Shammai et al., 2018; Salvi et al., 2022). Therefore, Titan and potentially more widely, the rabbiteye cultivars interesting sugar chemotype that can be utilized in future breeding into sweeter cultivars. Still, molecular players leading to higher fructose levels are poorly understood in plants.

4.3. Organic acids demonstrate a species-dependent accumulation, resulting in a significant impact on sugar to acid ratio

We characterized five key acids, citric, malic, and succinic acids, with citric acid playing a more significant role in highbush compared to rabbiteye, whereas malic acid showed the opposite trend (Ehlenfeldt et al., 1994; Mikulic-Petkovsek et al., 2012; Zhang et al., 2020). The effect of temperature on the total acids had a greater impact considering the maturation index (i.e., total sugars/ acids), a key parameter influencing basic taste and sweetness perception (Harker et al., 2002). Moreover, the higher fructose levels relative to glucose are also expected to enhance the sweetness perception in Titan compared to Biloxi. This further supports that Titan is better adapted to higher temperatures than Biloxi.

In grapes, elevated temperature has been shown to increase ABA accumulation in the pericarp, thereby modulating ripening and decreasing overall acidity by reducing malate levels (Carbonell-Bejerano et al., 2013). Similarly, in blueberries, we observed cultivar-dependent acid responses: acids increased in Biloxi but decreased in Titan under high temperature. Together with changes in the glucose-to-fructose ratio, these results suggest that ABA or ABA-related signaling pathways may mediate cultivar-specific metabolic acclimation to heat. Though ABA was not directly measured in our study, future work integrating ABA quantification with expression profiling of key biosynthetic (NCEDs), signaling, and regulatory genes will be valuable to clarify its role in temperature-driven sugar and acid partitioning in blueberry.

Additionally, we characterized shikimic and quinic acids with health benefits rather than for taste, especially when their concentration is 10-fold lower than the other acids. In contrast to quinic acid, shikimic acid was higher in Biloxi than in Titan. Quinic acid concentrations fluctuated between harvests under T30 compared to T20 in both cultivars, whereas shikimic acid decreased in Titan with harvest under T30 conditions. Both acids are biosynthesized from 3-dehydro-quinate as a direct precursor in the case of quinate (by quinate dehydrogenase) or by two enzymatic reactions in the case of shikimate (Alcázar Magaña et al., 2021). These acids play critical intermediates in the biosynthesis of aromatic amino acids (phenylalanine, tyrosine, tryptophan), which are precursors in the biosynthesis of volatile aromatics and phenolic compounds, and their levels may reflect broader shifts in the downstream phenylalanine metabolism to phenylpropanoids under temperature stress (Maeda & Dudareva, 2012). It is a direction we intend to explore in the future.

4.4. Identification of key VOCs affected by increased temperature

Consequently, a comprehensive analysis of VOCs was conducted to assess the effect of temperature on blueberry flavor. Recently, the contribution, importance, and inter-cultivar variation of VOCs within the same species have been extensively discussed. Key volatiles, including terpenes, aldehydes, and esters, have been identified as potential targets for flavor improvement by breeding (Du & Rouseff, 2014; Sater et al., 2020). Analysis of the aromatic profile of the two cultivars under the experimental conditions resulted in identifying 83 compounds. We and others have shown that only a small subset of the VOC profile potentially contributes to the flavor in fruits, such as table grapes (Maoz et al., 2020) and tomatoes (Ruiz et al., 2005; Piombino et al., 2013). Here, of the 83 compounds, only 34 had OAV>1, 30 in Biloxi, 32 in Titan, and 28 compounds with OAV>1 that were common to both cultivars. Among the volatiles detected, limonene, geraniol, linalool, methyl 2-methylbutanoate, methyl 3-methylbutanoate, eucalyptol, and β-damascenone consistently showed OAVs greater than 1, indicating a substantial sensory impact. These compounds are associated with fruity, floral, and sweet notes and are well-documented as key contributors to blueberry aroma in prior studies (Du et al., 2011; Du & Rouseff, 2014; Sater et al., 2020). Only 6 or 9 compounds in Biloxi or Titan had OAV>10, respectively. These allowed us to examine the effect of temperature and spotlight the VOCs that could influence berry flavor.

The C_6 aldehydes hexanal and (*E*)-2-hexenal were the most abundant VOCs by concentration (Cheng et al., 2020; Qian et al., 2021) and remained stable across both cultivars. Hexanal and (*E*)-2-hexenal impart distinct green/grassy/fresh odor notes (Du & Rouseff, 2014), but can also be associated with the basic fruity flavor (Maoz et al., 2020). In Biloxi, temperature had a significant effect on C_5 [1-penten-3-ol, (*E*)-2-pentenal, 1-penten-3-one], C_7 [heptanal, (*E*)-2-heptenal], and C9 (nonanal and nonanol) volatiles, whereas these compounds were unaffected by temperature or harvest timing in Titan. The C_5 ketones (2-pentanone and 3-pentanone), identified only in Titan, were temperature-sensitive, highlighting cultivar-specific differences in volatile responses to the environmental conditions. These findings are consistent with previous studies by Spicher et al. (2016) and Almeida et al. (2021), which reported that higher temperatures affect linolenic acid availability, redirecting volatile production from C_6 compounds toward C_5 volatiles. Additionally, temperature-affected, high-OAV compounds, like phenylacetaldehyde and phenylethyl alcohol, originate from phenylalanine. In petunia (*P. axillaris*) flowers, the phenylacetaldehyde emission increased >2-fold when the temperature increased from 20 to 30 °C (Sagae et al., 2008).

Esters are important for the flavor of many fruits, including strawberries, bananas, apples, and blueberries. Methyl 2-methylbutanoate was the main ester by OAV, affected by temperature, and derived from the amino acid leucine or de novo biosynthesis of its keto acid (Maoz et al., 2023). We identified a large group of methylated esters, including methyl 2/3-methyl butanoate, methyl hexanoate, methyl salicylate, methyl butanoate, and methyl decanoate. Methylation of such compounds has been suggested to be involved in plant stress responses (Chang et al., 2021). Supporting this, methyl salicylate levels positively correlated with temperature. The temperature effect on methyl salicylate was fold change higher in Biloxi than in Titan, providing initial insights into how temperature-induced stress may also result in an effect on the berry flavor.

Monoterpenes and norisoprenoids accounted for nine VOCs with OAV>1, primarily contributing to fruity and floral aromas in blueberries (Sater et al., 2020). Terpenoids exhibit diverse concentration ranges and odor profiles in plants and fruits (Dudareva et al., 2013; Schwab et al., 2008). In blueberries, eucalyptol, linalool, geraniol, and limonene were shown to enhance floral and fruity notes, influencing consumer preference (Du et al., 2011; Du & Rouseff, 2014; Ferrão et al., 2022). In Biloxi, total monoterpenes were influenced by temperature, though Titan showed no significant change. Linalool was consistently higher at T20 than T30 in both cultivars, while eucalyptol accumulated more in T30 compared to T20 in Titan. Limonene and geraniol followed similar trends, with higher accumulation at T20 than T30. The C_{13} norisoprenoid β-damascenone, derived from carotenoid degradation, remained stable across temperatures, peaking at late harvest and contributing to OAV ranges of 15–70 under different temperatures.

Further, we represented the changes in key VOCs by radar plots with a cut-off of OAV > 1, categorizing them into six typical flavor groups previously identified in blueberries (Du & Rouseff, 2014; Cheng et al., 2020). In Biloxi, floral, fruity, and citrus notes were more abundant in T20 late harvests, while green notes dominated in T30 mid-harvest. Conversely, Titan showed a 2.1-fold increase in citrus, green, and minty flavors, reflecting cultivar-specific VOC profiles (Du & Rouseff, 2014; Cheng et al., 2020). These variations underscore the significant quantitative impact of temperature and cultivar on aroma profiles (Farneti et al., 2017; Cheng et al., 2020). Floral aromas were more prominent at lower temperatures in Biloxi, while citrus aromas prevailed in Titan under similar conditions. It is worth mentioning that calculating OAV from the water-based system threshold from the literature may reflect the perception thresholds in the blueberry fruit matrix or potential volatile interactions (Plotto et al., 2004). Moreover, we expect that the taste of the berries, i.e., the sugar-to-acid ratio, which defines the sweetness-to-sourness levels, will play a role in the potential impact of VOCs on the overall flavor of the berries. The sensory studies in tomato puree showed that sugars and acids shape not only sweetness and sourness perception but also aroma expression through their interaction with volatiles (Tandon et al., 2000; Baldwin et al., 2008). Sugar addition to the tomato puree reduced sourness and bitterness while enhancing ripe and sweet notes, whereas acids shifted perception toward sour, citrus, and green flavors. The combined addition of sugars and acids also modified volatile partitioning, amplifying green or fruity or floral notes depending on the volatile context (Baldwin et al., 2008). The observed results under elevated temperature support that blueberry fruit flavor cannot be attributed to sugars, acids, or volatiles separately, but rather from the integrated effects. Therefore, sensory analysis can further help to understand if the changes in the chemical flavor profiles translate into perceived differences in berry flavor.

Regarding the overall response of the Titan berries to the increased temperature compared to the Biloxi, several hypotheses can explain the differential response to the increased temperature. One associated differential response to cuticle thickness in blueberries. Despite similar chemical composition, rabbiteye cultivars exhibit nearly a two-fold higher total wax content compared to highbush cultivars (Chu et al., 2017; Yang et al., 2019a). Future exploration of cultivar-specific wax differences in relation to their biosynthesis during blueberry ripening (Yan et al., 2024) may provide a valuable selection criterion for breeding programs aimed at enhancing blueberry adaptation to warmer climates.

Apart from contributing to aroma, VOCs in fruits may also play functional roles in plant stress responses. Although whole-plant or fruit emission data were not collected in this study, volatile emissions have been shown to protect cells from oxidative damage, stabilize membranes, and modulate antioxidant activity under abiotic stress (Sharkey et al., 2008; Schwab et al., 2008; Dudareva et al., 2013). Interestingly, cuticular waxes and fatty acid-derived volatiles share common biosynthetic origins in long-chain fatty acid metabolism (Dudareva et al., 2013). Fatty acid–derived aldehydes, alcohols, and esters responded differently to elevated temperatures in a cultivar-dependent manner, with Biloxi showing significant changes while Titan remained stable. Future studies combining volatile profiling with transcriptomics could clarify the links between aroma production and heat resilience in blueberries.

Alternatively, a more established hypothesis will be the involvement of heat shock proteins (HSPs) and their transcriptional regulators (HSFs) (Wang et al., 2004). HSPs function as molecular chaperones, stabilizing proteins and modulating downstream signaling pathways that influence carbon allocation and secondary metabolism (Feder & Hofmann, 1999; Schoffl et al., 1998; Wang et al., 2004). The molecular characterization and expression of HSPs have been reported in blueberry (Shi et al., 2017). Investigating cultivar-specific activation of HSPs in future studies could provide mechanistic insights into the observed flavor shifts under heat stress and guide the development of heat-tolerant, high-flavor blueberry cultivars.

5. Conclusion

Blueberry flavor-related metabolites have been systematically characterized during two consecutive years under two ripening temperatures, at three time points along the season, and in two cultivars, Biloxi and Titan. Elevated temperatures (30 °C) reduced fruit weight by greater than 20 % in Biloxi, leading to significant declines in aldehydes, esters, and terpenes. Titan exhibited greater thermal resilience, minimal fruit weight loss, and a more stable sugar-acid profile, and a lower glucose-to-fructose ratio under higher temperatures. These findings highlight the need for genotype-by-temperature interaction and harvest timing studies to inform breeding strategies for climate-resilient, flavor-retentive cultivars.

Ethical statement - studies in humans and animals

The authors declare that in this study we have not used any **humans and animals** based experiments in this paper.

CRediT authorship contribution statement

Kasipandi Muniyandi: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Krishna Kumar:** Investigation. **Guy Tamir:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Daniel Chalupowicz:** Methodology, Investigation. **Mirko De Rosso:** Methodology. **Riccardo Flamini:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization. **Nir Dai:** Writing – review & editing, Methodology, Investigation, Funding acquisition, Conceptualization. **Itay Maoz:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Data curation, 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.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.afres.2025.101371.

Data availability

Data will be made available on request.

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