Postharvest Evaluation of Blueberry Cultivars for Fruit Quality and Sensory Characteristics

by

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Abstract

Blueberry (*Vaccinium* spp.) production and consumption are a continuously growing market with United States (U.S.) fresh market exceeding $1.02 billion. Rabbiteye (*V. virgatum*; RE) and southern highbush (*V. corymbosum*; SHB) blueberries are commonly cultivated in the southeastern U.S. Research attention has been disproportionately focused on SHB, with extensive characterization of physiochemical, sensory, and volatile traits during ripening and postharvest storage, whereas comparable data for RE, particularly in postharvest contexts, remain limited. RE fruit generally contain fewer volatile compounds than SHB but tend to have higher soluble solids content (SSC), total anthocyanin concentration, and total phenolic content. In contrast, SHB fruit typically maintain firmness more effectively during storage. These differences highlight the need for a broader evaluation of SHB and RE fruit quality traits. Therefore, this study first evaluated 17 genotypes in 2024 over a 28-day storage period for preliminary screening. Based on these results, six genotypes were selected for the 2025 season for sensory evaluation during 14 days of cold storage (4 °C, ~80% RH).

In the first postharvest experiment, seventeen blueberry genotypes (15 RE, 2 SHB) were evaluated in 2024, and five were subsequently selected ('Brightwell', 'Vernon', MS1110R, UGA.RE.9 and 'Legacy' for further study in 2025 across 0, 14, and 28 days (D) of cold storage (4 °C, ~80% RH). Fruit were harvested May–June in both production years from the E.V. Smith Research Center. Across both years, genotype significantly influenced all traits. SSC ranged from 10.4–15.9 °Brix and 0.34-0.61% titratable acidity (Tacid). In 2024, 'Brightwell' and 'Ochlocknee' had the highest SSC, while UGA.RE.9 and 'New Hanover' were lowest. RE genotypes exhibited

greater anthocyanins, phenolics, and antioxidant capacity than SHB. Cluster analysis grouped genotypes by color, phytonutrient content, and reduced composition. In 2025, significant genotype × storage effects were observed for firmness, Tacid, and total anthocyanins, total phenolics and total antioxidant activity. UGA.RE.9 had the highest firmness, while 'MS1110R' and 'Brightwell' exhibited elevated phytonutrient levels. Multivariate analyses separated 'Legacy' from RE genotypes. Overall, RE genotypes exhibited higher SSC and phytonutrient levels, suggesting potential advantages for postharvest quality and sensory related traits

In the second postharvest experiment, six genotypes ('Brightwell', 'Vernon', MS1110, UGA.RE.9, 'Legacy' and 'Optimus') were assessed instrumentally for firmness and general composition (SSC, Tacid and SSC:Tacid) and by a trained panel at 0D and 14D of cold storage. Across all genotypes instrumental firmness ranged from 155.39-251.82 $g \cdot mm^{-1}$ at harvest. SSC declined throughout storage from 12.20 °Brix (0D) to 10.91 °Brix (14D). Tacid ranged from 0.23-0.56% throughout storage with genotype × storage duration interaction. Trained panel evaluation (n = 9) showed significant genotype effect for fruity aroma (3.3-6.1), sweet taste (2.5-4.6) and sour taste (3.1-5.3) on a 15 cm line scale. Panel texture ratings ranged firmness at 2.4-3.3, fibrousness at 4.4-5.0 and seediness at 3.6-7.0. Electronic-nose principal component analysis explained 88.87% of volatile profile variability and separated genotype and storage duration into distinct clusters. Results showed strong genotype dependency over compositional, sensory and volatile profiles and observed shifts throughout storage relevant to breeding and fresh market selection. Together, these results highlight the importance of genotype in shaping fruit quality attributes and demonstrate that integrating instrumental, sensory, and volatile analyses provides a robust framework for postharvest selection and breeding.

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In the preparation of this thesis, the following Artificial Intelligence (AI) tools were used: Microsoft Copilot. This tool was used primarily for grammar checking. The author acknowledges full responsibility for the intellectual content of this work and has ensured that all AI-assisted sections have been reviewed and revised for accuracy and appropriate academic style. All AI-generated content was reviewed and validated for relevance, appropriateness and accuracy before incorporation into the final document to maintain scholarly integrity of this research.

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List of Abbreviations

°Brix	Degree brix (soluble solids content)
%	Percent
ANOVA	Analysis of Variance
AU	Auburn University
°C	Celsius
Cm	Centimeter
D	Day / Days
FID	Flame Ionization Detector
g	Grams
GC	Gas Chromatography
GC-MS	Gas Chromatography Mass Spectrometry
GC-O	Gas Chromatography Olfactometry
$g \cdot mm^{-1}$	Grams per millimeter (firmness unit)
GAE	Gallic Acid Equivalents
HB	Highbush Blueberry
HCA	Hierarchical Cluster Analysis
IRB	Internal Review Board
L*	Lightness (color parameter)
a*	Red Green Color Axis
b*	Yellow Blue Color Axis
µmol	Micromole
mg	Milligram

mL	Milliliter
mm	Millimeter
PCA	Principal Component Analysis
ppm	Parts per million
ppb	Parts per billion
RE	Rabbiteye Blueberry
RE ADV	Rabbiteye Advanced Selection
RH	Relative Humidity
SDA	Spectrum Descriptive Analysis
SE	Standard Error
SHB	Southern Highbush Blueberry
SSC	Soluble Solids Content
SSC:Tacid	Soluble Solids Content to Titratable Acidity Ratio
Tacid	Titratable Acidity
TE	Trolox Equivalent
U.S.	United States
USDA	United States Department of Agriculture
VOC	Volatile Organic Compound
w/v	Weight by Volume

Chapter I Literature Review

1.1 Introduction

Marketing and production of blueberries is expanding not only in the United States (U.S.) but globally. The market for fresh blueberries is on the rise and global production has more than doubled between 2010 and 2019. This resulted in an increase from 439,000 metric tons to almost 1.0 million with the U.S. being the second largest leader in blueberry production following China (Protzman 2021; USDA FAS 2023). In 2023 USDA non-citrus fruits and nuts summary reported that there were 103,000 acres of cultivated blueberries. Overall cultivated blueberry fruit yielded 324,000 tons of fresh fruit and utilized 321,105 tons for wholesale market adding up to $1,028,992,000 worth of blueberries sold (USDA 2024).

Blueberries accounted for 5 percent of the total fruit production value in the U.S. Increased consumer awareness of the health benefits of berries and year-round availability increased domestic demand. Consumption soared from 2002 to 2020 by 574 percent. Imports accounted for over half of U.S. consumption in 2018-2020 (Yeh et al. 2023). Given expected increases in consumer demand for blueberry products in conjunction with Florida and Georgia's early harvest window, there was excellent potential for sustained production expansion (Scherm & Krewer 2003).

Much of the world faces nutritional crises and blueberries are valued for their nutritional content and distinct flavors compared to other fruits (Cho & Moazzem, 2022). Growing scientific evidence has been gathered from clinical research alongside mechanistic research showing the health benefits of blueberries. They contain many phytochemicals, including anthocyanin pigments which make the largest impact on their health functionality (Saini et al. 2024). The fruit contains a great source of flavonoids and flavanols along with folate (vitamin B), potassium,

vitamin C and A (Song et al. 2010; Retamales and Hancock 2018). Blueberries are also rich in chlorogenic acid, ellagic acid (Song et al. 2010) and volatile organic compounds (VOC) (Goff and Klee 2006) which are further known for health promoting properties. Regular intake of blueberries is associated with reduced risk of cardiovascular disease, anti-microbial properties, cancer and type 2 diabetes alongside improved weight maintenance and neuroprotection (Kalt et al. 2020; Lee-Kwan et al. 2017).

While blueberries are produced in mass amounts people still do not get enough of their daily fruit intake as needed. Reports indicated 13.1% of consumers in the U.S. met the daily fruit intake recommendation while only 8.9% met vegetable intake recommendations (Lee-Kwan et al. 2017). Flavor can also have profound health benefits since the volatile organic compounds (VOCs) they contain are dominantly known for antihypertensive, anti-inflammatory, antibacterial, and anticancer properties (Aguiar et al. 2021).

When comparing different ecotypes, RE blueberries are known for their excellent sources of polyphenols and antioxidants. RE cultivars such as 'Climax', 'Powderblue', and 'Brightwell' have significantly higher total anthocyanin content compared to highbush cultivars like 'Crunchie', 'Star', and 'Sharpe' (Lohachoompol et al. 2008). Although when looking at individual VOC content SHB has around 120 different compounds while RE cultivars displayed around 85 (Sater et al. 2020). A study done by Pertuzatti et al. (2021) found that chlorogenic acid contents were higher in SHB cultivars such as ‘Bluecrop’ and ‘Elliot’ and one RE cultivar ‘Florida’ (3.4 – 4.0 g/kg dry weight) (Pertuzatti et al. 2021).

Several studies conducted research on aroma compounds, astringency, and flavor of RE blueberry during ripening and maturity but not throughout postharvest storage timepoints

(Beaulieu et al. 2014; Farneti et al. 2017). This gap in knowledge suggests further research on RE blueberries addressing volatile composition and sensory analysis during postharvest storage is needed. This can be useful towards molecular breeding for improved aroma and developing more in-depth methods for postharvest storage (Sater et al. 2020). As the demand for healthful fruits like blueberry increases, it is necessary to have fruit that can be stored longer without significant losses of flavor and aroma (Takeda et al. 2008).

1.2 Rabbiteye Quality Issues

Blueberry breeders have typically concentrated on traits like larger fruit, higher yields, uniformity, increased resistance to disease and better shipping quality. While flavor and VOCs have not been extensively focused on until more recently (Wang et al. 2017; Klee & Tieman 2018; Lobos et al. 2015). Sensory profiling can help with selecting optimal flavors and volatiles in new blueberry germplasm involving consumers in the process (Gilbert et al. 2015). This sort of selection is critically important as consumers have made it clear they prefer fruits with both high-quality aroma and superior flavor (Sater et al. 2020). Thus, breeding programs will continue to include previously mentioned aspects but also consider flavor and sensory perception as a marketable and important factor for fresh market blueberry (Folta & Klee 2016).

The RE species is largely grown due to its high plant vigor and adaptability to hot and humid climates (Lobos & Hancock 2015). Some RE cultivars such as ‘Alapaha’ and ‘Powderblue’ yield smaller fruit, diminishing their potential to compete with larger fruited highbush (HB) varieties (Johnson et al. 2011). Hand harvesting is a known difficulty with smaller berry size, which often confines it to being machine harvested with fruit then sold as processing instead of to

the fresh market sector. Commercial growers would have an easier time marketing the berry if it was improved for hand harvest efficiency. Pick-your-own operations would greatly benefit from larger fruited cultivars to enhance this experience (NeSmith 2018). The importance of consumers being able to hand harvest their own blueberries, especially in states like Alabama that have a direct u-pick market impact plays a major role in fruit acceptability (DeVetter et al. 2022; NeSmith 2018).

Fruit firmness also plays a significant role in overall fruit quality (Trandel-Hayse et al. 2023). In a study done by Itle et al. (2024) research has observed SHB cultivars have the best maintenance of fruit firmness, and skin strength in postharvest storage. Both SHB and northern HB indicated higher visual ratings and less shrivel/decay incidence and subsequently have higher tissue firmness compared to RE cultivars. Although RE cultivars maintained berry weight the best throughout postharvest storage (Itle et al. 2024).

Other factors that play a role in fruit quality include harvest timing, handling, cell wall structures and other environmental factors such as rainfall (Itle 2021; Marshall et al. 2006). RE cultivars tend to exhibit increased rain-related splitting compared to SHB. Specifically, ‘Climax’, and ‘Tifblue’ varieties had the worst incidence of splitting while ‘Premier’ had the least splitting. Fruit splitting reduces marketable fruit and decreases profit by 3-20% depending on cultivar and environmental factors, with an overall average loss of 14.7%. Splitting also causes significant issues in postharvest storage due to leakage of osmolytes leading to increased rates of decay and spoilage pathogens (Marshall et al. 2006; Marshall et al. 2007).

There are contradictory results with respect to fruit firmness in RE compared to HB cultivars. Silva et al. (2005) found SHB blueberries of ‘Bluecrop’ averaged 254 N where RE cultivar

‘Climax’ averaged at 565 N for shear firmness. When looking at puncture force for SHB ‘Bluecrop’ (0.78 N) and ‘Jersey’ (0.83 N) were lower than the RE ‘Climax’ (1.71 N). This suggests, RE blueberries can withstand storage, harvesting, shipping and handling better than HB blueberries. Moreover, the data from Silva et al. (2005) indicated RE fruit has higher quality regarding firmness and skin strength over HB cultivars. While another preliminary study conducted by Itle (2021), reported that SHB was superior in quality. SHB proved to maintain firmness and better skin strength throughout storage. It was also found consumers preferred SHB over NHB and RE varieties at harvest (Itle 2021). Yet limited research has been done to address the overall liking, texture and flavor of RE compared to SHB during postharvest storge.

1.3 Blueberry Shelf-Life

It is known that the shelf-life of small fruits, such as berries, is short when left in ambient environments. However, if refrigerated properly their shelf-life can be extended significantly (Nunes et al. 2009). SHB can maintain firmness for 4-6 weeks at 4 °C while RE blueberries seem to store better at lower temperatures ranging from 1.5 to 3 °C for 4 weeks (Nunez-Barrios et al. 2005; Schotsmans et al. 2007). A few studies conducted on older RE cultivars indicated shelf-life was as good as or better than HB with fruit maintaining quality up to 6 weeks or longer in storage (Schotsmans et al. 2007).

A two-year shelf-life study conducted by Itle et al. (2024) observed visual ratings of SHB, NHB and RE blueberries over a 30-day period to assess loss of healthy fruit. The ratings indicated SHB fruit showed 25% loss, NHB at 23% loss and RE blueberries displayed the largest amount of loss in healthy fruit at 45%. During the second year SHB showed 29%, NHB at 34% and RE at

49% healthy fruit loss, respectively. This study also reported that SHB had the highest decrease in berry weight at 12% over 30 days in storage while RE stayed mainly consistent throughout storage. The increase in loss of healthy fruit in RE cultivars was likely due to environmental impacts from 2018 to 2019. (Itle et al. 2024).

Basiouny & Chen (1988) conducted a similar study determining differences in harvest date on shelf-life for RE cultivars. Weight loss and visual ratings for loss of healthy fruit were assessed over a 45-day shelf-life study. RE cultivars of 'Tifblue' and 'Bluegem' were reported as healthy with no fruit loss after 15 days. Similarly, after 30-45 days major weight loss was observed at 1.95 g for 'Tifblue' and 2.25 g for 'Bluegem', respectively, for earlier harvest dates (June 15th). While later harvest dates (July 1st) reported larger weight loss in berries at 6.23 g for 'Tifblue' and 6.60 g for 'Bluegem' (Basiouny & Chen 1988).

1.4 Changes in Blueberry Texture Through Storage

Firmness of blueberries is one of the more critical characteristics because it improves the probability that fresh blueberries will have a higher consumer acceptance rate (Sargent et al. 2013). Blueberries are a soft fruit that diminishes in quality as they lose their textural firmness. It is one of the most dramatic changes blueberries undergo during storage postharvest leading to quality issues (Giongo et al. 2013). Since blueberries lose their firmness throughout postharvest storage, harvesting softer blueberries leads to increased unmarketability (Rivera et al. 2022). Softer fruit typically have a shorter shelf-life which can also affect handling, shipping, and storing (Rivera et al. 2023). RE blueberries have firmer and tougher skin than HB blueberries. These textural differences have been related to differences in cell wall constituents of cellulose,

hemicellulose, pectin, and lignin, as well as overall seed size (Silva et al. 2005; Trandel-Hayse et al. 2023). Firmness can also be used to determine other factors such as color and titratable acidity. A study on 'Duke' SHB cultivar conducted by Varaldo et al. (2022) found a positive correlation (0.8313) between firmness and chroma. As firmness decreased L*, b* and chroma also decreased. While firmness was also significantly positively correlated to titratable acidity (0.8673) indicating as firmness decreases so does Tacid (Varaldo et al. 2022).

Firmness tests done on SHB cultivars of 'Meadowlark', 'Sweetcrisp', and 'Farthing' found these cultivars held consistent firmness during 14 days of storage. Subsequently these cultivars also showed increased resistance to weight loss and with lower shrivel incidence (Sargent et al. 2013). NHB tends to have higher firmness traits over SHB and RE blueberries (Itle et al. 2024). RE blueberries showed to have higher fiber traits over SHB and NHB. This suggests that RE has more insoluble fibers than HB which implies they should withstand longer storage than SHB or NHB (Itle & NeSmith 2016).

Consumer panel sensory tests found SHB had the most significant results for overall liking and texture. This indicates consumers do not like blueberries with high fiber content, instead many consumers prefer lower fiber content as the fruit feels less gritty in the mouth (Itle 2021; Itle & NeSmith 2016; Blaker & Olmstead 2014). RE and NHB had similar results in the consumer acceptability panel testing regarding skin toughness, fruit firmness, and seediness (Saftner et al. 2008). Results from consumer panels did not support RE having tougher skin or firmer fruit than HB cultivars. RE cultivars also did not have more seeds than NHB, instead RE have larger/plump, negatively affecting the overall liking and texture (Itle 2021). The conclusions from the Itle (2021) study found SHB fruit was liked by majority of consumers for all traits, while RE was liked just as

much as NHB for their fruit quality, although NHB had more citrus flavor than other blueberry species involved in the study (Itle 2021).

1.4 Blueberry Volatiles that Impact Aroma

The human olfactory system is one of the largest parts of processing taste that enhances the tongue's ability to perceive flavor (Cho & Moazzem 2022; Chen et al. 2023). Odor and aroma are both responsible for taste and flavor sensations. Odor is the perception of volatile compounds by the olfactory system when they go through nasal membranes outside the mouth (Stone et al. 2020). While aroma is the perception of volatile compounds within the mouth. Flavor is the sum of perceptions resulting from stimulation of multiple senses including both the nose and tongue. Aromatics, tastes, and chemical feelings involve both the tongue and nose for full perception of volatiles (Sánchez-Rodríguez et al. 2018; Stone et al. 2020).

Blueberries from different cultivars can contain different contents of (VOCs) that result in noticeable differences in fruit aroma (Sater et al. 2020). Studies done to identify aromatic volatiles in lowbush (*Vaccinium angustifolium*), HB and RE blueberries have been accomplished. Eighteen terpenes were present of which ten were identified-five being previously identified in highbush blueberries. Newly identified terpenes and terpene esters are y-terpinene, p-cymene, carveol, sabinol, thymol, cineralone, fl-ionone, terpinene4-01, cis-caran3-ol, o-cedrene, geranyl formate and linalool acetate. This class of compounds appears to constitute the preponderance of the aroma that is characteristic of blueberries (Ferrão et al. 2022; Horvat et al. 1983).

Volatiles most associated with aroma are hexanal, limonene, nerol, 1,8-cineole, 1-penten-1-ol, and terpineol. RE and NHB cultivars were more similar for volatiles of limonene (orange

fragrance) and terpineol (lime fragrance). Specifically, 'Austin', 'Brightwell', 'Powderblue' (RE cultivars), 'Bluecrop', 'Elliot', and 'Nelson' (NHB cultivars) had more orange and lime fragrance compared to SHB(Itle 2021). RE blueberries have esters, specifically ethyl acetate, that are dominant within the volatile profiling relating to fruity flavors and aromas (Sater et al. 2020). While the grassy aromas of SHB were associated with linalool and hexanal and minty aromas associated with eucalyptol (Cheng et al. 2020; Sater et al. 2020).

A study done by Du et al. (2014) observed SHB VOCs in four cultivars. 'Jewel', 'Prima Dona', 'Snow Chaser' and 'Kestrel' shared a fresh green, blueberry like, fruity aroma. When employing gas-chromatography olfactometry (GC-O), 43 aroma active volatiles were observed. Five aroma active volatiles remained unidentified/unknown (Du et al. 2014). Within the aroma active volatiles of SHB, nine aldehydes, eight esters, seven terpenes, five ketones, two alcohols, two acids, two sulfurs, and three miscellaneous compounds were identified. While ester VOC composition of the SHB shared similar composition to lowbush cultivars compared to their NHB counterparts (Beaulieu et al. 2014; Du et al. 2014; Horvat et al. 1983). Additionally, the four SHB cultivars shared many common aroma volatiles. Thirty-three volatiles were shared among three out of the four SHB cultivars. Aldehydes were the most abundant volatile group, followed by esters and terpenes. More variation was apparent in the terpene group than any other chemical grouping. Only three aroma active terpenes were observed in 'Prima Dona', but seven terpenes were observed in 'Kestrel' (Du et al. 2014).

1.5 Blueberry Compounds that Impact Taste

The human olfactory system is one of the largest parts of processing taste regardless of the tongue's ability on its own (Chen et al. 2023). Volatiles that stimulate taste in the mouth are defined by three different types of cells in the mouth. These send stimuli to the brain to help process flavors that are either sweet, sour or bitter. Sweet flavors originate from the tongue detecting glucose, fructose, sucrose, sugar alcohols, and starches. Sour flavors are associated with organic acids such as malic, citric, and tartaric acids. Bitter detections are produced by polyphenols such as phenolic acids (hydroxybenzoates and hydroxycinnamates) and flavonoids (Sánchez-Rodríguez et al. 2018).

Breeders have previously observed blueberry genotypes contain berries with unique flavors and aromas that are 'floral' and 'sweet' which will appeal to consumers (Ferrão et al. 2022). Aromas associated with blueberry flavor are linalool, trans-2-hexanal, geraniol, cis-3-hexen-ol, trans-2-hexanol. The three cultivar types similar for blueberry related aroma compounds most associated with taste (e.g., linalool, trans-2-hexenal and geraniol-floral, fruit, citrus, sweet, pungent) were 'Farthing' (SHB), 'Powderblue' (RE), 'Aurora' (NHB). NHB were significantly higher than both SHB and RE for linalool which has a distinct floral, fruity, and citrus flavor and geraniol known for its floral rose smell (Itle 2021).

Sweet and intense blueberry flavors yielded the most positive purchase interest, conversely bad texture associated with seediness were most harmful to interest (Gilber et al. 2015; Itle et al. 2024). Over half the population of consumers are typically most interested in the blueberry flavor itself followed by the health influenced benefits with blueberry consumption (Gilbert et al. 2014). Volatiles in blueberries can vary significantly based on harvest dates,

genotype and environmental impacts. Specifically, flavor volatiles change based on these factors, although varieties like 'Star' experienced the least variation throughout the harvest periods (Gilbert et al. 2013). During 3 seasons, 19 blueberry genotypes were assayed by 217 panelists in 30 sensory panels for overall liking, texture, and perceived sweetness, sourness, and flavor intensities. The study found that certain biochemical compounds were specifically associated with increased or decreased consumer liking and had lower degrees of environmental variation (Gilbert et al. 2015).

1.6 Sensory Analysis using Electronic-Nose and Electronic-Tongue

The term "electric nose" (e-nose) is associated with the detection of odors or the ability to smell with an instrument (Cho & Moazzem 2022; Tibaduiza et al. 2024). It offers a valuable insight that mammalian senses cannot accomplish. While the "electronic tongue" (e-tongue) senses bitter, sour, salt, umami, and astringency the e-nose detects volatiles using flash gas chromatography (GC). Human senses such as the nose or tongue can judge or rate these chemicals, but even consumer or trained panels contain bias depending on the level of sensitivity within the mouth or nose of an individual. The use of e-sensing instruments bypasses risks like toxicity associated with certain VOCs a human nose may not detect upon contact (Cho & Moazzem 2022; Stone et al. 2020; Tan & Xu 2020). For example, some VOCs such as water vapor and carbon dioxide gas do not contain enough aroma or any aroma at all to be detectable by the human nose. While the e-nose can accomplish some tasks humans cannot, it also has its drawbacks such as inability to detect other general items such as or spoilage (Tan & Xu 2020, Röck et al. 2008).

E-senses such as the e-nose and e-tongue can be considered fast and valid alternative instruments compared to other analytical techniques (Cho & Moazzem 2022; Tan & Xu 2020). This includes spectroscopy such as Gas Chromatography- Mass Spectrometry (GC-MS), which is sensitive, selective, and expensive to operate and requires extensive set-up compared to e-sense instruments. Electronic senses were developed to evaluate food and beverage quality alongside assessing human sensory perception more objectively relationships between e-senses and human panel data are still being evaluated (Ares & Varela 2017; Cho & Moazzem 2022). E-tongue and e-nose instruments have tremendous potential to be implemented during harvesting of fruits and vegetables alongside postharvest storage studies. Predicting when aroma and flavor diminish during storage is a potential outcome from these instruments (Ali et al. 2023; Torri et al. 2010; Xu et al. 2018). These predictions could be extremely valuable to postharvest research by allowing more determinations of crop shelf-life during storage studies. They may also provide an impressive prediction regarding human sensory scores of food products by analyzing a product's odor and taste profile (Bleibaum et al. 2002). This would enhance marketability for consumers proving a foods sensory score during harvest to advertise or for postharvest evaluation (Cho & Moazzem 2022).

Over time GC-MS has gotten more sensitive to VOCs. GC-MS has a very low detection limit (>5,000 peak area or >0.01 ppm) which can prove to be useful as there are increasing legal regulations accompanied with food products (Epping & Koch 2023). Although, it does have a weakness in not being able to detect involatile chemicals. GC-MS is massively important in foodstuffs production as it provides the availability to determine food safety with the sensitivity of background contamination. GC-MS data allows the observation of individual analytes selectively

shown as an intensity/time trace (Hübschmann 2015). In the case of e-nose and e-tongue the instruments themselves do not quantify the data for us, instead they use the volatile profiles to separate or categorize samples into clusters (Cho & Moazzem 2022; Tibaduiza et al. 2024). Thus, it is more powerful to use multivariable techniques and sensor data fusion strategies (Tibaduiza et al. 2024).

While e-tongue and e-nose have proven to be impactful in detecting chemicals in liquid substances and gases, they are best paired with other methods of chemical analysis as well. Sensor data fusion strategies can allow for a more in-depth analysis of volatile constituents in a desired sample. The goal is to closely replicate human senses with the additional help of other methods of chemical analysis such as GC-MS and solid phase microextraction (SPME) to get a quantitative profile of analyte composition (Tibaduiza et al. 2024). In a study done by Dong et al. (2019) they summarized both e-nose and e-tongue combined with head space solid phase microextraction gas chromatography mass spectrometry (HS-SPME-GC-MS) could effectively discriminate roasted coffee samples derived from different drying processes. While dried samples of jujube presented similar aroma profiles in GC-MS and flash GC e-nose analysis, they did not show similar results for metal-oxide-semiconductor (MOS) e-nose. Additionally combining all three methods can provide a comprehensive aroma profile, as each instrument has its own valuable information, but MOS e-nose was lacking in analysis where GC-MS and GC e-nose were not (Song et al. 2020).

1.7 Consumer panels vs Trained Panels

Sensory evaluation begins with physiology of the senses and the bias of psychology. Information derived from experiments with the senses has a major influence on test procedures and on measurement of human responses to stimuli. It can largely influence marketing decisions for a product's consumer acceptance and is now a core part of sensory programs (Stone & Sidel 2004). Consumers evaluating sensory characteristics of products is valid within sensory science. Whether to use consumer or trained panels is specific to the study and should be carefully considered regarding the experiment's product. Consumer panels can be used to replace trained panels, but it should be noted that trained panels are necessary in place of consumer panels (Ares & Varela 2017). Consumer and trained panels differ in their level of expertise. Consumer panels are used to determine acceptability on hedonic scales while trained panels are used to gather analytical data on a product for refinement (Stone et al. 2020).

Overall sensory panels consist of three types of data including 1) demographic characteristics of the panel, 2) recruitment and training characteristics, and 3) performance analysis results. The optimal number to have for trained consumer studies is between 8 and 12 panelists. Gender and age are preferable to include alongside health status and nationality (Djekic et al. 2021). In short, trained panels are typically more effective than an untrained panel (Stone et al. 2020; Stone & Sidel 2004). Training a panel is important for sensory evaluation as odor intensity was one of the most difficult for non-trained panelists to distinguish. Training with reference standards can also help a trained panel better determine odor intensity (Losó et al. 2012).

Consumer-assisted selection can help breeders identify the most desirable traits in fruit (Folta & Klee 2016). They are a type of panel designed to be used throughout a process to help study preferences among consumers to determine acceptability (Stone et al. 2020). In the study conducted by Gilbert et al. (2014), several blueberry cultivars were assessed to discover the ideal blueberry VOC profile. Flavor components of intense blueberry flavor and sweetness elicited the highest favor for blueberry sensory components. It was found to be seedy, tough, and dry berry characteristics had an overall negative impact on sensory perceptions and likelihood for purchase. This indicated berry flavor in breeding programs based on consumer panels should be a larger focus because marketing cultivars for flavor traits is highly desirable based on the results from the study (Gilbert et al. 2014).

1.8 Gaps in Flavor and Volatile Information on Rabbiteye Blueberry

Volatile research specifically on RE blueberries is numerous when it comes to the ripening stages and maturity stages. Unfortunately, postharvest research has been neglected when it comes to their volatile content (Beaulieu et al. 2014). When looking at RE compared to HB varieties their quality does differ in volatile content and texture analysis (Itle 2021; Silva et al. 2005). There are studies done on peaches (*Prunus persica*) with the impact of UV-C irradiation on fruit quality during postharvest storage with focus on aroma changes (Zhou et al. 2024). Additionally, studies done on citrus (*Citrus spp.*) exposure to ethylene during postharvest storage were found to impact volatile profiles (Sdiri et al. 2017). Most cultivated fruits have studies done that analyze volatile, flavor, and sensory analysis postharvest. However, native North American fruits including blueberry have limited research done regarding postharvest research.

Postharvest research is significantly lacking in multiple studies including Itle (2021) as fruit quality and volatile analysis comparing NHB, SHB and RE, southern was done only at harvest. Moreover, Beaulieu et al. (2014) only addressed RE volatile analysis throughout different stages of maturity but did not address the postharvest implications of flavor and volatiles.

Postharvest research is needed to address the comprehensive analysis of flavor, volatile, and sensory analysis on RE compared to HB cultivars through postharvest storage. Looking at the postharvest impact of volatiles and flavors along with consumer acceptance is important for marketability, consumer acceptance, and grower considerations (Beaulieu et al. 2014; Itle 2021). Marketers might be able to advertise specific traits for RE blueberries other species within the genus do not have. Higher consumer acceptance means cultivars traditionally excluded from wholesale markets may become more acceptable for their quality and flavor (Gilbert et al. 2014). Knowing how the flavors and volatiles are impacted postharvest will aid breeding programs to select for high quality traits in advanced selections and increase consumer acceptance of the crop. This is also beneficial as growers can add RE genotypes to their selection aiding disease thresholds, drought, and heat tolerance in the Southeastern United States (Folta & Klee 2016; Klee & Tieman 2018).

1.9 Conclusion

Currently there are no detailed papers on RE blueberry flavor or aromatic volatile compounds during shelf-life over a 6-week period (Beaulieu et al. 2014). Since there are papers detailing their volatile compounds during maturity or ripening, postharvest research is needed (Farneti et al. 2017). New guidelines can be made for this specific species based upon research

on their volatile content. Other guidelines for instruments used to analyze blueberries can be updated as well (Folta & Klee 2016).

Studying postharvest storage and its impact on flavor and volatiles can open new doors for more research on different treatments for the RE cultivars. More in-depth analysis can also be done outside of e-nose and e-tongue sensory analysis using GC-O, SPME, and GC-MS methods to extract aromatic volatile compounds to further analyze RE cultivars in postharvest storage (Cho & Moazzem 2022 Tan & Xu 2020, Röck et al. 2008).

Outcomes gained can be seen through firmness, color indexing, soluble solids content (SSC), titratable acidity (Tacid), and pH measurements adding to the already vast literature detailing it in other species of blueberry. Identifying e-senses alongside trained sensory panels in RE compared to SHB cultivars can prove valuable to understand composition, health benefits and quality of RE cultivars. Finding new correlations between firmness, color data, and Tacid can also be explored through statistical analysis. Volatile composition most associated with genetic factors can also be explored by blueberry breeding programs on a global scale. Blueberry breeders can use this data to analyze which factors most strongly impact flavor, and quality in RE cultivars. Consumers will benefit from this study as well having analysis of quality in flavor, firmness, and general composition to guide postharvest, growers, marketability and breeding efforts. It will also allow for a more competitive market value of RE cultivars throughout postharvest storage (Folta & Klee 2016). Recommendations for industry standards based on RE cultivars can be made after analysis of fruit quality, firmness, volatiles, shelf-life, and general composition. Informed education can also be made through dissemination of extension services to improve industry-wide practices (NeSmith 2018).

1.10 Research Hypothesis and Objectives

The main hypothesis for this project is established SHB cultivars will have better overall liking and texture compared to established RE cultivars since SHB has smaller seeds and preferred less gritty composition compared to RE blueberries. Furthermore, this study will take into consideration field location and harvest date. Since RE cultivars have fruit set and maturity dates later into the season over SHB. Thus, we further hypothesize RE fruit quality will deteriorate during postharvest storage due to increased heat exposure during production.

The first objective for this project will be assessing general composition (pH, soluble solids, titratable acidity and firmness) of both RE and SHB blueberry cultivars throughout 6 weeks of postharvest storage at 4 C. This will include both established and advanced cultivars for RE and SHB. Fruit will be harvested from two locations EV Smith and Chilton Research and Extension Center (CREC). A randomized complete block design was used for both research stations blueberry fields. Blueberries will be assessed for postharvest quality, visual indices, colorimeter measurements and texture analysis every 14 days of storage including Day 0, Day 14, Day 28 and Day 42 in a randomized complete block design using 3 or 4 replications.

The second objective is to address the difference in volatile profiling using an e-tongue (E-tongue, Alpha MOS, Toulouse, France) and e-nose instrument of fresh blueberries at harvest (Day 0) and after 2 weeks of cold storage (Day 14). RE and SHB cultivars will be harvested from one field location (EV Smith) and up to three separate harvest dates will be assessed. The e-sense instruments will simulate the human tongue by detecting aromatic volatiles of pureed blueberries after grinding with a 2010 Geno/Grinder to homogenize samples.

The last objective of this experiment will use a consumer panel to assess 6 blueberry cultivars (4 RE and 2 SHB cultivars) after 14 days of cold storage. Both Day 0 (T0) and Day 14 (T2) samples will be used for the consumer panel with two replications. Consumer panels will be used to assess taste and olfactory sensation of RE compared to SHB blueberries to gain a better understanding of what consumers want.

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Chapter II Postharvest General Fruit Quality Evaluation of Blueberries Grown in Alabama

Abstract

Blueberries (*Vaccinium* spp.) are valued for flavor and phytonutrient content, however, postharvest performance of rabbiteye (RE; V. *virgatum*) cultivars under Alabama conditions remains limited. Objectives were to evaluate physicochemical and phytonutrient attributes of 16 RE and 3 southern highbush (SHB; V. *corymbosum*) genotypes during cold storage. A sub-selection of five common genotypes to both production seasons were selected to assess year-to-year stability. Fruit were harvested in May-June 2024 and 2025 from the E.V. Smith Research Center (Tallassee, AL) and stored at 4 °C and 85% relative humidity for 28 days. Evaluations included color (L*, a*, b*), soluble solids content (SSC), titratable acidity (Tacid) and total anthocyanins (mg/100g), phenolics (mg GAE/100g), and antioxidant capacity (µmol/100g). Genotype significantly influenced all measured traits, while storage effects were evident but genotype-dependent. SSC generally declined by 12.3-16.8 °Brix during storage, whereas Tacid remained stable (0.33-0.77%). Color attributes (L*, a* and b*) varied among genotypes, with limited changes during storage. Phytonutrient content differed significantly among genotypes with MS1110 and UGA.RE.9 exhibiting the highest anthocyanins (32 mg/100g), phenolics (318 mg GAE/100g) and antioxidant (29 µmol/100g) values. All phytonutrients tended to increase in the second harvest by 1-16 mg/100g total anthocyanin, 25-106 mg GAE/100g total phenolics and 0.5-8 µmol/100g total antioxidant capacity. Multivariate analyses (Principal component analysis and hierarchical clustering) identified distinct genotype groupings based on compositional and phytonutrient traits, with several genotypes maintaining consistent trait associations throughout storage. Across years, genotype exerted a stronger influence on fruit quality than environmental or

storage effects. These results highlight promising RE genotypes with stable postharvest quality and support targeted selection for improved fruit quality and nutritional value in southeastern U.S. production systems.

2.1 Introduction

Blueberries (*Vaccinium* spp.) are a major U.S. fruit crop, contributing approximately 5% of national fruit production and generating an estimated $9.1 billion annually (USDA NASS 2024). The southeastern U.S. accounts for ~36% of U.S. blueberry production. Southern highbush (SHB; *V. corymbosum*) cultivars dominate commercial production in the region because of their early flowering, early ripening, high fruit quality, and ability to extend market windows (Phillips et al. 2024; USHBC 2020; USDA NASS 2024b). In contrast, rabbiteye (RE; *V. virgatum*) cultivars are typically produced on a smaller scale for local, organic, and direct-to-consumer markets (Coneva and Singh 2025; Tertuliano et al. 2012). In Alabama, blueberry acreage increased from 788 acres in 2012 to 1,005 acres in 2022, reflecting growing producer interest (USDA 2021; 2022). Unlike larger production regions, Alabama growers primarily favor RE cultivars because of their vigor, disease resistance, delayed flowering, and tolerance to late-spring frosts. Despite these production advantages, RE blueberries are often characterized as smaller and more acidic than SHB cultivars, which may limit their acceptance in wholesale markets (Itle 2021; Itle 2025).

Consumer acceptance of blueberries is largely driven by appearance and flavor, which are strongly influenced by color (L*, a*, b*, chroma and hue angle CIELAB Units), soluble solids content (SSC), and titratable acidity (Tacid) (Brazelton et al. 2013; Canales et al. 2024). Visual attributes such as surface bloom, color uniformity, berry size, and freedom from defects strongly influence purchase decisions, with consumers preferring berries exhibiting greater bloom and higher L* values (Asensio et al. 2025; Wang et al. 2025). Although postharvest changes in color, SSC, Tacid, and phytonutrient content are well characterized in SHB blueberries, similar information for RE cultivars remains limited, particularly across newly released genotypes and

extended storage periods (Carsorzo et al. 2024; Yan et al. 2023; Blaker et al. 2014; Cappai et al. 2018; Giongo et al. 2022; Oh et al. 2024; Sargent et al. 2013; Cheng et al. 2020; Godara et al. 2025; Potter et al. 2011; Trandel-Hayse et al. 2025; Itle et al. 2024; Itle et al. 2025). RE cultivars generally exhibit lower L* and chroma values than SHB cultivars (Sousa et al. 2004), whereas SSC is often 1 to 1.5 °Brix higher with higher Tacid equivalents between 0.5% to 1.1% (Itle and NeSmith 2016; Cheng et al. 2020).

Beyond appearance and flavor attributes, phytonutrient composition represents an additional component of blueberry fruit quality. Comparisons between RE and SHB blueberries remain inconsistent across production regions (Connor et al. 2002). While much research has focused on northern highbush and SHB (Rossi et al. 2022; Wang et al. 2017), studies of RE genotypes have reported contrasting results. Gündüz et al. (2015) found RE cultivars in Northern U.S. exhibited higher anthocyanin content (22 μg Cy-3-glu g^{-1} FW) and higher total phenolics (792 μg GAE g^{-1} FW) than SHB in Georgia, U.S., with slightly lower antioxidant capacity (2.9 μmol TE g^{-1} FW). Similarly, Cheng et al. (2020) reported higher total phenolics in RE cultivars compared with SHB grown in China. However, postharvest quality data for advanced RE selections remain sparse (Carsorzo et al. 2024; Itle et al. 2025; Itle & NeSmith 2016; Potter et al. 2011). Moreover, these contrasting findings likely reflect environmental and genetic influences on phytonutrient accumulation and highlight the need for expanded evaluation of advanced RE germplasm across production regions and storage conditions (Loohachoompol et al. 2008; Ma et al. 2022).

Breeding programs in the southeastern U.S. are dedicated to developing new RE selections with improved fruit quality (Coneva 2021; Stringer et al. 2023). At Auburn University, several RE selections are under evaluation for enhanced nutritional and sensorial traits. Thus, the

objective(s) of this study were to: 1) evaluate physicochemical (color, SSC, Tacid) and phytonutrient attributes (total anthocyanins, phenolics, and antioxidant activity) of a large population of RE genotypes during 28 days of storage and 2) to assess compositional and nutritional traits across a second production season of five sub-selected RE and SHB genotypes to determine seasonal variability in blueberry quality. We hypothesized genotype would be the largest influence for variation in fruit quality traits while advanced selections would exhibit greater stability in SSC, Tacid, color attributes and phytonutrient concentrations from harvest throughout storage conditions.

2.2 Materials and Methods

2.2.1 Experimental Site

Established plants of RE and SHB were harvested from the E.V. Smith Research Station located in Tallassee, AL (32°29'48.7"N, 85°53'33.8"W). The production experiment was set up as a randomized complete block design (RCBD) with 3 blocks (replications) and 3 bushes planted within each block. The bushes were planted between March 2022 and April 2023, therefore age of the plants may differ across cultivars/selections (**Table 2.1**).

The soil type was a loamy sand mixture fitting for blueberry cultivation (USDA Soil Web Survey 2025). Standard fertilization rates and timings followed the guidelines of Smith and Jacobs from University of Georgia's blueberry production guidelines (2019). Specifically, fertigation was applied weekly for 27 weeks starting March through August at a rate of 1.68 N kg/ha during the first year of planting and was increased to 3.69 N kg/ha for following years.

Environmental data including daily temperature (°C), relative humidity (%) and rainfall (millimeters (mm)) were recorded year-round and reported from March 21st until July 1st. The average daily production temperature in 2024 was 21.74°C, while in 2025 daily production temperature was 21.58°C. The minimum and maximum air temperature ranged from -1.1 °C to 37.9 °C in 2024 and from -1.3 °C to 36.3 °C in 2025. Relative humidity in 2024 was 79% and 81% in 2025. Rainfall events were significantly different between the two production years with 2025 receiving 133.4 mm more precipitation than 2024 (**Fig. 2.1)**. The average maximum and minimum temperatures, relative humidity and precipitation from March 21st June 30th, 2024 and 2025 are reported in **Fig. 2.1** and **Table 2.2**.

2.2.2 Experimental Material

A total of seventeen cultivars and advanced selections were studied in 2024, while five cultivars and advanced selections common between both years were tested in 2025. The complete list of utilized genotypes is provided in **Table 2.1** and are listed as follows; SHB cultivars: 'New Hanover' and 'Legacy'. RE cultivars: 'Alapaha', 'Brightwell', 'Krewer', 'Ochlocknee', 'Overtime', 'Titan' and 'Vernon'. RE selections: MS1110, MS1595, MS1221, MS1228, UGA.RE.6, UGA, RE.7, UGA.RE.8 and UGA.RE.9. Blueberry fruit was hand-harvested at commercial maturity, defined by uniform fruit size, full blue surface coloration, and the absence of white or red rings around the pedicel scar (Giongo et al. 2013). Harvesting occurred weekly from 22 May to 24 June in 2024 and 19 May to 23 June 2025. Each cultivar was harvested on two separate harvest dates (harvest 1 and harvest 2) one week apart.

Roughly 400 g of blueberries from each subplot were harvested into labeled plastic bags then placed on ice packs in coolers. Fruit was transported to the postharvest laboratory located in Funchess Hall at Auburn University (Auburn, AL). Upon arrival at the laboratory, fully ripe blueberries ranging from 1.5 to 4 g were used for storage duration study. Blueberry samples were further sorted to remove blemished, bruised or discolored fruit before placing them into cold storage.

Seventeen genotypes were evaluated in 2024, as the first season served as a broad screening to characterize postharvest fruit quality across a diverse set of RE and advanced RE selections. Only a small number of SHB cultivars were selected as these are commonly grown by commercial producers in the southeastern U.S. and served as industry standards for fruit quality and storability comparisons. In 2025, only five genotypes were selected for further evaluation because they represented contrasting SSC and Tacid profiles, allowing assessment of a broad range of fruit quality characteristics (Personal communication with Dr (s). Marlee Trandel-Hayse, Sushan Ru and Penelope Perkins-Veazie). Thus, the 2025 season served as a smaller more manageable focus group for assessing seasonal variability and genotype performance.

2.2.3 Postharvest Storage and Experimental Design

The storage duration study was set up in a RCBD with three replication blocks and three bushes per plot. For postharvest analysis fruit was harvested twice in one-week intervals exactly 7 days apart. Blueberries were only harvested from two out of the three bushes per plot and were combined into one composite sample to represent ~400 g of fresh fruit. In both years, assessments were conducted at harvest (0 days; 0D) and after 14D and 28D of storage. Fruit

were stored at 4 °C and 85% relative humidity (RH). In 2024, this sampling scheme resulted in 17 genotypes × 3 replications × 2 harvests × 3 storage durations, yielding a total of 306 experimental units (n = 306). In 2025, the same sampling protocol was applied to the five selected genotypes and included 5 genotypes × 3 replications × 2 harvests × 3 storage durations (0D, 14D and 28D) resulting in 90 experimental units (n = 90). Fruit from each replication was packed either into clamshells or solo cups depending on the type of assessment. Roughly 20 blueberries per replication were placed into 156 g solo cups for general composition and phytonutrient assays. Meanwhile, a single pint-sized clamshell containing 150 to 200 blueberries per genotype and replication was used to determine visual quality and colorimeter assessments. For color measurements, 10 berries were randomly selected from each clamshell at each storage duration. Following evaluations at each storage duration, fruit designated for compositional analyses were transferred from the solo cups into 50-mL conical tubes and stored at −20 °C until further processing.

2.2.4 Berry Peel Color Measurements

Blueberry peel color was taken from berries at each storage duration and replication by randomly selecting 10 blueberries from the pint sized clamshells. Then, a colorimeter (CM-600, Konica Minolta, Tokyo, Japan) was used to assess L*(lightness), a* (redness/greenness) and b* (blueness/yellowness) chroma (pureness) and hue angle (intensity). Color data was taken on the stem end and perpendicular side to the stem end of each berry. Color data is reported as an average across each genotype, replication, and storage duration in CIELAB units.

2.2.5 General Composition

Compositional blueberry samples (n = 15 fruit per replication and storage duration) were pulled from the -20 °C freezer and thawed to room temperature (~ 25 °C) for about 1 hr. After thawing, two 9 mm steel beads (Grainger, Lake Forest, IL, USA) were placed into the tubes. Homogenization was conducted using a Geno/Grinder 2010 (HG-600, SPEX Sample Prep, Metuchen, NJ, USA) set at 1,600 rpm for 3 minutes; after homogenization steel beads were removed. To determine Tacid content, 0.50 g (± 0.01 g) of blueberry puree was weighed into a 50 mL conical tube using a 5 mL plastic pipette (S-24318, ULINE, Pleasant Prairie, WI, USA). Samples were diluted with 24.5 mL deionized water (DI H_2O) using a Dispensette S (Dispensette S, BrandTech, Essex CT, USA).

SSC and Tacid were taken on an ATAGO refractometer set for blueberry (PAL-BX|ACID, ATAGO Co., Ltd., Bellevue, WA, USA). First, pureed blueberry samples were dispensed onto cheese cloth (approx. 4 cm^2) and squeezed onto the refractometer lens to measure SSC. To measure the Tacid content, diluted blueberry samples were hand mixed and gently poured on the ATAGO meter. After obtaining general composition data all samples were stored at -20°C until phytonutrient extraction occurred.

2.2.6 Phytonutrient Extraction to Quantify Total Anthocyanins, Phenolics and Antioxidant Activity

For total anthocyanins, total phenolics and antioxidant activity assays, fresh frozen samples were extracted from general composition blueberry puree from each replication, following optimized methods of Lee et al. (2005) and You et al. (2011). In short, ~300 mg (± 10 mg) of fresh frozen blueberry puree from each replication were weighed into black 5 mL tubes for

extraction (HS4323K, Heathrow Scientific LLC., Vernon Hills, IL, USA) from all 302 general composition samples in 2024 and the 90 general composition samples in 2025. Universal extraction solvent (3 mL; 60% methanol, 3% formic acid and 37% water, Sigma-Aldrich, St. Louis, Missouri, USDA) was added to each sample to give a 1:10 (w/v) ratio. All samples were vortexed (Vortex Genie 2, Scientific Industries, Inc., Bohemia , NY, USA) for 6 min, sonicated (Ultrasonic cleaner, Model 8845-30, Cole Palmer, Chicago, IL, USA) for 20 min, and centrifuged in a Symphony 2417E benchtop centrifuge (VWR, Radnor, PA, USA) at 10,000 g for 5 min. Samples were then filtered through 0.45 µm nylon filter (30 mm, nylon filter, polypropylene housing, Cytiva Life Sciences, Marlborough, MA, USA), collected, and stored at -20 °C.

Total anthocyanins were measured following Stanciu et al. (2009) on all extracted samples. In short, the samples were pipetted into black 1.5 mL microcentrifuge tubes. Two microcentrifuge tubes were required for each sample. A cold potassium chloride buffer (0.025 M, pH 1) was added to one of the samples and sodium acetate buffer (0.45 M, pH 4.5) was added to the other. Extracted samples were vortexed then stored in a cooler held at 4°C for 2 hr. Absorbance was measured on each sample at 510 and 700 nm on a Shimadzu UV-VIS 2600i spectrophotometer (UV-VIS 2600i, Shimadzu, Kyoto, Japan). Total anthocyanins were calculated using the equation [(Absorbance × Molecular Weight × Dilution Factor × 1000) / 26,900 × 1] and were reported as $g \cdot 100g^{-1}$ cyanadin-3-glucoside equivalents.

Total phenolics were analyzed following Singleton et al. (1974). Sample or calibration standard was added to black microcentrifuge tubes then diluted with DI H_2O and vortexed for 15 sec. Folin-Ciocalteau (0.25 M) was added, and samples were held at room temperature for 3 min. Finally, 1 M sodium carbonate was pipetted, and all samples were held in the dark for 2 h at room

temperature. Absorbance was read at 726 nm. and phenolic concentration was calculated using a gallic acid standard curve ($y = 0.0011x + 0.0034$, $R^2 = 0.9916$). Total phenolic content was calculated as TPC = (C × V) / M and were expressed as g GAE·kg^{-1}, where C is the gallic acid concentration, V is the extract volume, and M is the sample mass.

Brand-Williams et al. (1995) method was followed to determine antioxidant activity on blueberry samples. All steps were performed in a dimly lit room and Trolox was used to construct the standard curve ranging from 100 to 800 nM. The standard curve was run prior to all samples to check for instrument linearity. Additionally, a DPPH control was run just before each sample batch to control for shifts in absorbance. Samples (33.5 mL) and/or universal solvent (DPPH blank, 33.5 mL) were added to 1.5 mL black test tubes then 1 mL of DPPH solution was added. Samples and blanks were vortexed for 30 seconds and incubated in the dark for 15 min. at room temperature. Absorbance was measured at 515 nm. Sample absorbance values were corrected against the DPPH blank at the beginning of each sample batch to account for variation in reagent stability. The corrected absorbance (blank − sample) was used to calculate antioxidant concentration based on the Trolox standard curve ($y = 0.0736x + 0.721$, $R^2 = 0.971$), incorporating sample mass, extraction volume, and dilution factor. Results were expressed as nM·g^{-1}.

2.2.7 Statistical Analysis

All statistical analyses were conducted in JMP Pro 19 (SAS Institute, Cary, NC, USA). Traits analyzed included SSC, Tacid, color attributes, total anthocyanins, total phenolics and antioxidant capacity as previously described. Prior to statical analysis, all residuals were evaluated for normality and homogeneity of variance, and no data transformations were required.

For Model 1, a two-way fixed-effects ANOVA evaluated genotype, storage duration (0D, 14D and 28D), and their interactions using only the 2024 dataset focusing on the 17 genotypes analysis. Mean separation for significant fixed effects utilized Tukey's Honestly Significant Differences (HSD) at $\alpha = 0.05$. To evaluate multivariate patterns for the postharvest traits measured on the 2024 data set, principal component analysis (PCA) was used to assess genotype-level associations among SSC, Tacid, color attributes (L*, a* and b*), and phytonutrient compounds (total anthocyanins, total phenolics and total antioxidant capacity). PCA was performed on the correlation matrix using standardized variables and genotype means. Analyses were conducted at 0D and 28D to capture the beginning and end of storage and to identify genotype-driven shifts in trait expression during cold storage.

For the five common genotypes harvested across the 2024 and 2025 seasons Model 2 analyzed all dependent variables using the four-way ANOVA with fixed effects including year, genotype, harvest number, and storage duration. Year was treated as a fixed effect, and replicates were considered independent observations. Model 2 included the same dependent variables and observations as Model 1. Multivariate analysis was also conducted for the 5 common genotypes using hierarchical cluster analysis (HCA) at 0D and 28D to represent the beginning and end of storage. HCA was performed using standardized variables, and clustering patterns were used to characterize genotypes exhibiting enhanced or suppressed postharvest trait expression across years and storage duration.

2.3 Results and Discussion

2.3.1 General Composition Differed Across the Seventeen Blueberry Genotypes

To support selection of the five common genotype across the 2024 and 2025 production season, a two-way ANOVA (Model 1) was conducted on the general composition across 1 genotypes (**Table 2.3**). The genotype × storage duration interaction was not significant for SSC but was significant for Tacid (**Fig. 2.2a and b).** SSC decreased during storage across most genotypes and was influenced by genotype ($P = 0.0249$) and storage duration ($P < 0.0001$). In contrast, Tacid exhibited significant effects of genotype ($P = 0.0026$), storage duration ($P < 0.0001$), and their interaction (P = 0.0003), demonstrating genotypes differed in their ability to maintain acidity during storage.

To better characterize quality difference, SSC responses were examined across individual genotypes at harvest and after 14D and 28D storage. At harvest (0D), the RE cultivar 'Brightwell' and RE selection MS1228 exhibited the highest SSC values at 16.63 ± 1.34 and 16.65 ± 1.07, respectively (**Fig. 2.2a**). In contrast, the SHB genotypes 'Legacy' and 'New Hanover', had the lowest SSC values, ranging between 12.53 to 12.43 °Brix. By 28D, 'Legacy' and 'New Hanover' maintained the lowest SSC values at 11.33 ± 0.30 and 11.45 ± 0.21 °Brix, respectively. In comparison, RE cultivars such as 'Brightwell' (15.30 ± 1.43°Brix), and RE advanced selections MS1228 (16.83 ± 1.38 °Brix) and MS1234 (14.00 ± 1.02 °Brix) maintained high SSC values through 28D of storage (**Fig. 2.2a)**.

Unlike SSC, Tacid exhibited a significant genotype × storage duration interaction, with several RE genotypes maintaining higher acidity throughout storage (**Fig. 2.2b**). 'Vernon' had consistently high Tacid values ranging from 0.56–0.66%, while the advanced selections MS1228 and MS1234 showed similar Tacid content across the storage duration (0.50–0.69% and 0.53–

0.77%, respectively). In contrast, SHB ‘Legacy’ and ‘New Hanover’ maintained the lowest and most stable Tacid values, ranging from 0.31–0.35% across storage duration.

The 2024 general composition dataset served as an effective first-year screening tool for evaluating blueberry fruit composition and general quality. It distinguished genotype and storage duration related differences in SSC and Tacid while identifying genotypes with contrasting postharvest responses during storage. All evaluated genotypes in this study remained within or above the typical SSC range reported for commercially acceptable blueberries (10–14 °Brix) (Beaudry et al. 1998). These findings further align with reports that SHB genotypes frequently show lower SSC under southeastern U.S. production conditions (Cheng et al. 2020; Xu et al. 2025). Similar trends have been observed in previous studies showing RE genotypes often exhibit higher SSC than SHB due to genetic differences (Itle & NeSmith 2016; Gündüz et al. 2015). While these observations are based on a single season, they are consistent with breeding goals that prioritize stability in SSC and Tacid (Coneva 2021; Itle et al. 2025; Potter et al. 2011) and suggest genotype plays a substantial role in regulating general composition traits.

In addition to SSC, Tacid results were also consistent with previous studies demonstrating that RE genotypes typically exhibit higher organic acid concentrations than SHB genotypes (Gündüz et al. 2015; Loohachoompol et al. 2008). These trends further align with genomic studies identifying major genes associated with organic acid metabolism, including those regulating citric acid synthesis and transport supporting genotype as an important driver of acidity variation (Oh et al. 2025). Tacid also remained relatively stable during cold storage, consistent with findings in SHB and northern highbush blueberries where changes in acidity were minimal throughout storage (Cheng et al. 2020; Xu et al. 2025). These results indicated soluble

sugars and acidity are relatively stable postharvest traits largely governed by genotype, making both useful parameter for identifying cultivars and/or selections with desirable flavor profiles and storage performance.

2.3.2 Principal Component Analysis of Physiochemical Traits at 0 and 28 Days Storage

Principal component analysis (PCA) was used to summarize overall patterns and variation in general composition, color quality and phytonutrient traits across the 17 genotypes at 0D and 28D. This multivariate approach enabled visualization of genotype groupings, identification of trait correlations and evaluation of how storage influenced overall fruit quality (Trandel-Hayse et al. 2023).

At harvest (0D) the PC1 (40.4%) and PC2 (28.1%) explained 68.5% of the total variation among genotypes (**Fig. 2.3a**). Genotypes separated into three distinct groups reflecting genotypic differences between SSC, Tacid, color (L*, a* b*, Chroma and Hue) and phytonutrient concentrations. Group I included the UGA RE selections, ‘Titan’, and ‘Vernon’, and was associated with elevated Tacid, L* and hue angle values. Group II consisted of MS1234, MS1228, and ‘Overtime’ and was highly correlated with chroma (saturation of color). Group III included MS selections,‘Alapaha’, ‘Brightwell’ and ‘Ochlocknee’, and were correlated with high phytonutrient concentrations. While group IV included SHB ‘Legacy’ and ‘New Hanover’ which were correlated with enhanced a* (redness) and b* (blueness) values. These patterns are consistent with previous blueberry PCA studies, which identified genotype as a major driver of variation in postharvest quality, phenolic composition, and anthocyanin content (Xu et al. 2025; Ochmian et al. 2025).

After 28D of storage, the PCA explained 68.9% of the total variability, with PC1 and PC2 accounting for 48.4% and 20.5%, respectively (**Fig. 2.3b**). Although similar genotype groupings were observed after storage duration, the differences among groups became less pronounced over time, suggesting a storage reduced trait discrimination among the genotypes and dependent variables. Group I, consisted of MS1110, 'Brightwell', and 'Ochlocknee' and was most correlated with high SSC and phytonutrient concentrations. Group II included most RE cultivars and selections and was correlated with hue angle, pH and Tacid. Group III consisted of UGA.RE.6 and UGA.RE.7 and remained correlated with higher L* values. Group IV included the SHB cultivars 'Legacy' and 'New Hanover', which were associated with a* and b* values. These genotypes remained separated from all RE genotypes, indicating SHB blueberries maintain a distinct quality profile throughout storage. These findings further support previous reports showing storage can reduce the magnitude of quality differences among genotypes while preserving overall clustering patterns (Xu et al. 2025; Ochmian et al. 2025).

Overall, PCA results demonstrated genotypes exerted a stronger influence on fruit quality than storage duration and informed the selection of five representative genotypes for multi-year evaluation: 'Brightwell', 'Vernon', MS1110, UGA.RE.9, and 'Legacy'. Selection was based on both contrasting quality attributes at harvest and the degree of stability observed during storage. Specifically, 'Brightwell' served as the Alabama industry standard (East 2019; NeSmith 2004), 'Legacy' represented a high-quality SHB genotype with a distinct quality profile characterized with vibrant color, high SSC and low Tacid, 'Vernon' and UGA.RE.9 represented intermediate fruit quality, and MS1110 represented a lower quality genotype due to its comparatively high Tacid and less favorable color attributes.

2.3.3 General Composition Among Five Sub-Selected Genotypes

The five selected genotypes ('Brightwell,' 'Vernon,' MS1110, UGA.RE.9, and 'Legacy') were evaluated across the 2024 and 2025 seasons to determine year-to-year consistency in fruit composition. Results from the mixed model analysis are reported in **Table 2.4**. No differences were found in the year × genotype × storage duration interaction for SSC (P = 0.5069), Tacid (P = 0.5804) or SSC:Tacid ratio (P = 0.5096) However, a significant genotype × year interaction was detected for both SSC (P = 0.0075) and Tacid (P = 0.0008), indicating genotypes did not respond uniformly across years. Genotypic differences were also found for SSC (P = <.0001) and Tacid (P = <.0001; **Table 2.4**). While harvest number did not affect any general composition traits (P > 0.05) except Tacid. Similarly, storage duration affected Tacid (P = 0.0161) but not SSC (P = 0.0797) or the SSC:Tacid ratio (P = 0.8788)

Within the genotype × year interaction clear differences were observed in SSC across both production seasons. 'Brightwell' exhibited the highest SSC in 2024 (15.89 ± 0.56) and 2025 (13.47 ± 0.19), while 'Vernon' had moderate SSC in 2024 (12.39 ± 0.32) but was lowest in 2025 (10.37 ± 0.35) (**Fig. 2.4a**). MS1110, UGA.RE.9 and 'Legacy' had moderate SSC in both years ranging from 11.59 ± 0.35 to 12.87 ± 0.66. These results align with previous reports describing 'Brightwell' as having consistently high SSC relative to other RE genotypes (Gilbert et al. 2013; 2015). While 'Vernon' indicated a decline in SSC in 2025, suggesting greater sensitivity to environmental variation (Itle et al. 2025). When SSC was averaged across storage duration in 2024 and 2025, SSC did not differ among 0D, 14D, and 28D ranging from 12.32 ± 0.32 to 12.98 ± 0.30 to (**Fig. 2.4b**). These results suggest SSC is relatively stable through storage duration while genotype and year exerted a stronger influence on SSC (Cao et al. 2025).

For Tacid, ‘Vernon’ consistently exhibited the highest acidity in both 2024 (0.61 ± 0.02) and 2025 (0.51 ± 0.02), while ‘Legacy’ had the lowest Tacid across both years (0.34 ± 0.02and 0.38 ± 0.04, respectively; **Fig. 2.4c**). ‘Brightwell’, MS1110 and UGA.RE.9 had moderate acidity between both years ranging from 0.43 ± 0.02 to 0.50 ± 0.02, respectively. These differences align with previous reports of ‘Legacy’ as a lower acid SHB genotype (Gilbert et al. 2013; 2015) and ‘Vernon’ as comparatively more acidic RE genotype (Itle et al. 2025). When averaged across years, Tacid was highest at harvest (0D; 0.48 ± 0.01) and lowest after 14D of storage (0.43 ± 0.01), indicating a modest decline in acidity during storage. This decline is consistent with previous reports of organic acid depletion during blueberry storage as organic acids are consumed during respiration (Guo et al. 2023)

Across production seasons, genotype was the primary determinant of fruit composition. Importantly, SSC and Tacid values for all genotypes remained within established quality standards. Blueberries with SSC values near 10 °Brix are considered acceptable and anything higher than 14 °Brix are exceptional, while commercial Tacid should range between 0.3% -1.0% (Beaudry et al. 1992; Saftner et al. 2008). Seasonal environmental variation likely contributed to the significant genotype × year interactions observed for SSC and Tacid. Water availability is known to influence blueberry fruit composition, with reduced precipitation generally increasing SSC through concentration effects and greater rainfall diluting sugars during berry development (Ortega-Farias et al. 2021). Consistent with this relationship, rainfall was 121 mm lower in 2024 compared to the 2025 season (**Fig. 2.1**). Ultimately, the decreased precipitation likely contributed to the generally higher SSC observed relative to 2025. Previous studies have also shown that reduced water availability can alter fruit acidity through concentration effects

associated with smaller berry volume (Shireman et al. 2009). Unlike SSC, Tacid was not higher in 2024 and other factors beyond precipitation, including temperature, solar radiation, and organic acid metabolism, also contributed to year-to-year variation in fruit acidity (Lopes et al. 2026).

2.3.4 Colorimeter Characteristics Among the Five Sub-Selected Genotypes

Mixed-model results for blueberry peel color are presented in **Table 2.4**. A significant year × genotype × storage duration interaction was detected for b* ($P = 0.0089$), indicating that storage-related changes in blue-yellow coloration varied among genotypes between production years. Among two-way interactions, genotype × year effects were significant for L*, a*, and b* ($P < 0.001$), while the genotype × storage duration interaction was significant only for a* ($P < 0.001$). No genotype × harvest number interactions were detected ($P > 0.05$). For main effects, genotype significantly influenced L* ($P = 0.0060$), a* ($P < 0.0001$), and b* ($P < 0.0001$). Year affected L* ($P < 0.0001$) and a* ($P < 0.0001$), but not b* ($P = 0.5519$). Storage duration influenced only a* ($P = 0.0003$), whereas harvest number affected only b* ($P = 0.0331$).

To facilitate interpretation of peel color responses in this study, values of L*, a* and b* are presented as the year × genotype × storage duration interaction are presented in **Table 2.5**, although the three-way interaction was significant only for b*. In 2024, b* values at harvest (0D) ranged from −4.04 ('Vernon') to −0.61 (MS1110) and shifted negatively during storage. Similar genotype-specific patterns were observed in 2025, where b* values ranged from −3.80 ('Vernon') to −0.90 (MS1110) at harvest and from −4.33 ('Brightwell') to −1.60 (MS1110) after 28D of storage. Although not significant in the three-way interaction, genotype x year differences were evident for L* and a*. Lightness (L*) was highest in ‘Legacy’ during the 2024 season (28.3–35.4),

whereas L* was lower in 2025 at harvest (26.2) before increasing to 33.6 after 28D storage. In contrast, ‘Brightwell’ and ‘Vernon’ exhibited relatively similar L* values between years, while UGA.RE.9 had the highest L* value at harvest in 2025 (29.6). For a*, year-to-year differences were most evident in ‘Legacy’, which had increased a* in 2025 (3.7) compared to 2024 (1.6). Similarly, MS1110 and ‘Vernon’ indicated higher a* in 2025, whereas UGA.RE.9 and ‘Brightwell’ showed smaller differences between years, respectively.

Although chroma and hue were measured, both parameters are calculated directly from a* and b* and therefore did not provide additional biological interpretation beyond the primary CIELAB color coordinates (Cinko and Becerir, 2024). Thus, L*, a*, and b* were used as the primary metrics for evaluating blueberry peel color and quality in this study. Across the three-way interaction, the lowest b* across both years were seen in RE genotypes ‘Brightwell’, UGA.RE.9, and ‘Vernon’, indicating a greater shift toward the blue during storage (Yan et al. 2023). Conversely, MS1110 consistently exhibited the highest b* values, suggesting a lighter fruit surface appearance (Yan et al. 2023; Yan et al. 2024). Similar storage-associated changes in blueberry surface color have been reported previously and are attributed to changes in fruit surface characteristics and pigment expression (Chu et al. 2018; Grohar et al. 2025). Genotypic differences in b* likely reflect underlying variation in anthocyanin accumulation, wax bloom intensity and epicuticular wax deposition, all of which are under strong genetic control and contribute substantially to blueberry appearance (Qi et al. 2019; Yan et al. 2023; Yan et al. 2024; Lopes et al. 2026).

Similar to b*, lightness (L*) is commonly associated with fruit surface brightness and epicuticular wax bloom. In blueberry, greater L* values generally reflect a lighter fruit appearance

and increased wax expression (Yan et al. 2023; Yan et al. 2024), characteristics that consumers often associate with freshness and overall market quality (Aensio et al. 2025; Yan et al. 2023). Beyond visual appeal, epicuticular waxes also help reduce water loss and protect fruit during postharvest storage. However, all cultivars evaluated in this study maintained acceptable shelf-life through 28 d of storage. The significant genotype × year interaction further suggests seasonal growing conditions influenced wax expression. ‘Legacy’ had greatly L* in 2024 compared to 2025, whereas ‘Brightwell’ and ‘Vernon’ maintained relatively consistent values. This implies wax expression in ‘Legacy’ was more responsive to seasonal growing conditions. In contrast, RE cultivars appeared to maintain a more stable fruit surface appearance despite environmental variation between years (Barney et al. 2024; Cao et al. 2025).

The largest year-to-year differences were observed in ‘Legacy’ followed by MS1110 and ‘Vernon’, with higher a* recorded in 2025 than 2024. In contrast, ‘Brightwell’ and UGA.RE.9 maintained consistent a* between years. Positive a* values indicate increased red coloration and are strongly related to anthocyanin accumulation and pigment development. Anthocyanin biosynthesis in blueberry is under both strong genetic control as well as environmental factors including temperature and light exposure during fruit maturation, resulting in cultivar-specific responses across growing seasons (Lopes et al. 2026; Yan et al. 2024). Changes in a* during storage were relatively small, indicating that peel pigmentation remained generally stable across all genotypes following harvest and that color development was primarily established during fruit growth rather than during postharvest storage (Grohar et al. 2025).

2.3.5 Sub-Selected Genotypic Differences in Total Anthocyanins, Phenolics and Antioxidant Capacity

Significant year × genotype × storage duration interactions were detected for total anthocyanins ($P = 0.0060$), total phenolics and total antioxidant activity ($P = 0.0030$). Among the two-way interactions, total anthocyanins were only influenced by the genotype × harvest number ($P = 0.0025$), while total antioxidant activity was affected by both genotype × storage duration ($P = 0.0033$) and genotype × year ($P < 0.0001$). Total anthocyanins ($P = 0.0001$), total phenolics ($P < 0.0001$) and total antioxidant activity ($P < 0.0001$) differed among genotypes. While only total antioxidant activity ($P < 0.0001$) and total phenolics ($P = 0.0486$) with minimal storage duration effects (**Table 2.4)**

In the year × genotype × storage duration interaction, total anthocyanins were highest in 2024 in 'Brightwell' (0D 13.96 ± 2.15, 14D 22.14 ± 4.46 and 28D 17.32 ± 2.18 mg·100 g^{-1} FW) and MS1110 (0D 29.68 ± 5.21, 14D 20.95 ± 1.44 and 28D 25.45 ± 4.39 mg·100 g^{-1} FW) across all storage durations. Conversely, 'Vernon' (0D 6.48 ± 0.49, 14D 18.23 ± 3.41 and 28D 11.03 ± 0.85 mg·100 g^{-1} FW) and UGA-RE.9 (0D 6.77 ± 1.22, 14D 6.72 ± 1.19 and 28D 23.24 ± 1.54 mg·100 g^{-1} FW) had the lowest total anthocyanins, with 'Legacy' (0D 24.40 ± 5.27, 14D 4.63 ± 0.63 and 28D 7.12 ± 0.80 mg·100 g^{-1} FW) showing intermediate concentrations. In 2025, 'Brightwell' (0D 11.77 ± 2.96, 14D 17.51 ± 2.21, and 28D 9.51 ± 2.72 mg·100 g^{-1} FW) and 'Vernon' (0D 22.46 ± 7.58, 14D 11.94 ± 3.62, and 28D 13.51 ± 7.52 mg·100 g^{-1} FW) exhibited trends opposite to 2024 concentrations. In contrast, MS1110 (0D 19.55 ± 10.92, 14D 28.09 ± 7.43, and 28D 30.27 ± 10.17 mg·100 g^{-1} FW) maintained high total anthocyanins. Similarly, 'Legacy' (0D 5.86 ± 1.38, 14D 15.60 ± 1.48, and 28D 17.96 ± 4.87 mg·100 g^{-1} FW) displayed a trend opposite to that observed in 2024 (**Fig. 2.5a**).

Total phenolic content was also significantly influenced by the genotype × year × storage duration interaction (**Fig. 2.5b**). In 2024, MS1110 consistently had highest phenolic content across all storage durations (0D 344.24 ± 24.50, 14D 318.89 ± 24.50, and 28D 337.57 ± 24.52 mg GAE·100 g^{-1} FW), while UGA.RE.9 (0D 169.53 ± 21.84, 14D 167.08 ± 19.89, and 28D 296.55 ± 24.85 mg GAE·100 g^{-1} FW) and 'Vernon' (0D 186.90 ± 19.89, 14D 299.68 ± 19.89, and 28D 226.68 ± 19.90 mg GAE·100 g^{-1} FW) showed lower phenolic concentrations. In 2025, total phenolics were generally lower at harvest but increased during storage for several genotypes. MS1110 again had the highest phenolic content (0D 189.32 ± 19.89, 14D 224.81 ± 19.89, and 28D 256.22 ± 19.90 mg GAE·100 g^{-1} FW), whereas UGA.RE.9 (0D 143.98 ± 30.49, 14D 161.95 ± 30.68, and 28D 177.79 ± 30.25 mg GAE·100 g^{-1} FW) had the lowest concentrations. While 'Brightwell' (0D 223.46 ± 19.89, 14D 233.53 ± 19.89, and 28D 217.51 ± 19.90 mg GAE·100 g^{-1} FW) and 'Legacy' (0D 177.19 ± 29.78, 14D 232.16 ± 29.78, and 28D 232.36 ± 29.81 mg GAE·100 g^{-1} FW) exhibited intermediate values, respectively.

Antioxidant capacity was also significantly affected by the genotype × year × storage duration interaction (**Fig. 2.5c**). In 2024, antioxidant capacity followed similar genotype-dependent trends. MS1110 had the highest content (0D 29.71 ± 2.10, 14D 27.56 ± 2.10, and 28D 24.42 ± 2.11 μmol TE·g^{-1} FW), while UGA.RE.9 (0D 10.03 ± 1.87, 14D 12.35 ± 1.70, and 28D 16.57 ± 2.13 μmol TE·g^{-1} FW) and 'Vernon' (0D 13.68 ± 1.70, 14D 19.34 ± 1.70, and 28D 20.51 ± 1.71 μmol TE·g^{-1} FW) were lowest. In 2025, antioxidant capacity was lower overall and exhibited minimal changes throughout storage. Across all genotypes, antioxidant capacity ranged from 13.93 to 16.44 μmol TE·g^{-1} FW with limited genotype-specific variation. 'Legacy' exhibited the highest values, ranging from 15.35 ± 2.56 to 16.44 ± 2.55 μmol TE·g^{-1} FW, whereas MS1110

exhibited the lowest values, ranging from 13.93 ± 1.87 to 14.62 ± 1.70 μmol TE·g^{-1} FW. Intermediate values were observed for 'Brightwell' (14.55 ± 1.71 to 15.27 ± 1.70 μmol TE·g^{-1} FW), 'Vernon' (14.53 ± 1.86 to 15.08 ± 1.70 μmol TE·g^{-1} FW), and UGA.RE.9 (14.54 ± 2.59 to 15.11 ± 2.60 μmol TE·g^{-1} FW), confirming the minimal changes observed throughout storage.

The strong year × genotype × storage duration interaction for phenolics and antioxidant capacity aligns with recent literature showing phytonutrient composition is highly genotype dependent and impacted by environmental conditions (Ochmian et al. 2025). Although anthocyanins are often considered stable during storage their concentration depends widely on genotype as a baseline (Oh et al. 2025). Across both years, genotypes with higher anthocyanin concentrations generally exhibited higher antioxidant capacity reflecting the well-established relationship between anthocyanin content and antioxidant activity in blueberries. This positive association has been reported by Ochmian et al. (2025) where total anthocyanins strongly correlate with DPPH radical scavenging capacity (r = 0.93).

Significant genotype × harvest number interaction was seen for total anthocyanins and phenolics but not for antioxidants (Anthocyanins: $P = 0.0025$ and Phenolics: $P = 0.0031$; **Table 2.4**). Phytonutrient content was generally higher at later harvests, except for 'Vernon', specifically, in harvest 2 'Brightwell' (15.96 mg·100 g^{-1} FW), and MS1110 (32.19 mg·100 g^{-1} FW) were highest while in harvest 1 'Vernon' 'Vernon' (16.32 mg·100 g^{-1} FW) was highest. Conversely advanced selection UGA.RE.9 exhibited the lowest content between both harvests. A similar pattern was observed for total phenolics, with 'Brightwell' among the highest in harvest 1 and harvest 2 (264.35 and 302.78 mg GAE·100 g^{-1} FW), while UGA.RE.9 was consistently the lowest (174.19–209.39 mg GAE·100 g^{-1} FW) across harvests. Antioxidant capacity generally increased

from harvest 1 to harvest 2, with MS1110 having the greatest increase (17.04 to 21.49 μmol TE·g^{-1} FW). In contrast, UGA.RE.9 was the only genotype to decline, decreasing from 13.93 to 12.68 μmol TE·g^{-1} FW between harvests (**Table 2.6**).

Environmental conditions, particularly temperature and precipitation, are well known to influence sugar accumulation and phytonutrient composition in blueberries, including anthocyanins and phenolics (Correia et al. 2016). In this study, seasonal differences between 2024 and 2025 likely contributed to variation in phytonutrient responses. The 2025 season exposed blueberry plants to greater cumulative precipitation (518 mm) compared to 2024 (397 mm), with more concentrated rainfall events occurring during April and May, alongside slightly cooler late spring maximum temperatures (**Fig. 2.1, Table 2.2**). These environmental differences may have influenced fruit development and compositional dynamics, as increased water availability can affect metabolite concentration through dilution effects while also altering plant physiological responses (Ehret et al. 2012).

Anthocyanin accumulation in blueberry fruit has been shown to vary with cultivar, harvest timing, and storage conditions (Connor et al. 2002; Kalt et al. 2003; Kårlund et al. 2014; Godara et al. 2025; Itle et al. 2025). Previous work has also demonstrated later harvests are often associated with increased anthocyanin, phenolic, and antioxidant concentrations (Cvetković et al. 2022), which is generally consistent with trends observed in this study. Together, these findings suggest both preharvest environmental conditions and postharvest storage contribute to variability in phytonutrient content, with genotype mediating the magnitude and direction of these responses. While increased precipitation in 2025 may have contributed to variability in concentration dynamics, the general trend of enhanced phytonutrient content at later harvests

and during storage supports the role of maturation and storage-related physiological processes in shaping blueberry nutritional quality (Kalt et al. 2003; Mallik & Hamilton 2017).

2.3.6 Hierarchical Cluster Analysis of Five Sub-Selected Genotypes

Hierarchical cluster analysis (HCA) was analyzed at harvest (0D) and after 28D of storage to further characterize multivariate relationships among the five genotypes (**Fig. 2.6a**). At 0D three primary clusters were identified. Cluster I indicated 'Legacy' and MS1110 had enhanced phytonutrient content including anthocyanin, phenolic and antioxidant concentrations. Cluster II contained UGA.RE.9 which exhibited enhanced SSC and Tacid concentrations and Cluster III indicated suppressed a* and b* values in 'Vernon' and MS1110. Consistent with previous multivariate analysis methods these results showed blueberry cultivars naturally separate into quality-based groups strongly associated with genetic influence on general composition and phytonutrient traits (Ochmian et al. 2025; Casorzo et al. 2024). The clustering designated at harvest also aligned closely with the PCA results which similarly separated genotypes based on SSC, Tacid and phytonutrient concentrations. Together this suggests initial fruit quality is strongly associated with genotype dependency (Connor et al. 2002).

After 28D of storage two main clusters were identified showing cluster structure shifted during storage (**Fig. 2.6b**). Cluster I included 'Brightwell' and MS1110 with enhanced phytonutrient concentrations for phenolics and antioxidants. Cluster II exhibited suppressions in SSC, Tacid, all phytonutrient traits alongside lower L* and a* values. These results reinforce the role of genotype in determining postharvest quality and support the identification of cultivars with promising postharvest quality traits. This reduction in cluster strength further suggests dilution of

quality traits throughout storage where SSC, Tacid, color and phytonutrient concentrations become weakened as fruit undergoes metabolic changes. Similar storage related conditions have been observed in SHB and northern highbush blueberries where long term cold storage conditions reduce overall quality traits (Xu et al. 2025; Cheng et al. 2020).

The clustering observed in both storage durations highlighted genotypes with enhanced and suppressed postharvest qualities. Genotypes that clustered high for SSC, elevated phenolics and strong antioxidant capacity show as strong candidates for breeding programs targeting improved flavor and nutritional quality. Genotypes that consistently clustered with lower values may require targeted improvement in breeding programs or deemed as less suitable for long term storage markets. HCA successfully integrated multiple quality traits providing to be a useful tool for identifying multidimensional quality traits important for breeders to target focusing on consumer preference, nutritional value and postharvest performance (Itle et al. 2025; Canales et al. 2024).

2.4 Conclusion

Across a broad 2024 screening (17 genotypes) and the 2024 and 2025 sub-selections (5 common genotypes) postharvest quality of RE and SHB blueberries was primarily genotype dependent while storage time, harvest number and year also impacted fruit quality. In the 2024 study SSC and Tacid differed mainly among genotype and storage duration where SSC generally declined with a slight increase after prolonged storage due to water loss. Tacid held steady or slightly increased across storage duration and was again dependent largely on genotype. These patterns observed were supported by the 2025 sub-selection where genotype remained

dominant in being the source of variation for general composition traits (SSC and Tacid), all color attributes and all phytonutrient traits. Color attributes further emphasized genotype uniqueness which is important for market appearance. Genotypes consistently differed in b*, a* and L*with modest shifts throughout storage. Phytonutrients were consistently higher in fruit harvested later in the season showing the importance of harvest timing for maximizing nutritional quality. For Alabama production systems and southeastern U.S. breeding programs, these findings identified promising RE genotypes based on their ability to maintain fruit appearance, compositional attributes, and phytonutrient content throughout storage. Observing how environmental variability throughout the season and across years impacts specific traits highlights the need for multi-year evaluation prior to new genotype releases.

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2.6 Tables

Table 2.3. Blueberry genotypes and advanced selections used for postharvest quality and shelf-life evaluations. All genotypes were planted and harvested at the E.V. Smith Research Center in central Alabama (Tallassee, AL).

Type	Genotype	Breeding Program	Planting Date	Year of Evaluation
SHB Cultivars	'New Hanover'	NCSU[1]	3/29/2022	2024
	'Legacy'	USDA, New Jersey[2]	4/3/2023	2024, 2025
RE Cultivars	'Alapaha'	UGA[4]	3/29/2022	2024
	'Krewer'	UGA	3/29/2022	2024
	'Vernon'	UGA	3/29/2022	2024, 2025
	'Titan'	UGA	3/29/2022	2024
	'Ochlocknee'	UGA	3/29/2022	2024
	'Brightwell'	UGA	4/14/2023	2024
	'Overtime'	Fall Creek[3]	3/29/2022	2024
RE Selections	MS1110	USDA, Poplarville	4/3/2023	2024, 2025
	MS1595	USDA, Poplarville	4/3/2023	2024
	MS1221	USDA, Poplarville	4/3/2023	2024
	MS1228	USDA, Poplarville	4/3/2023	2024
	UGA.RE.6	UGA	3/29/2022	2024
	UGA.RE.7	UGA	3/29/2022	2024, 2025
	UGA.RE.8	UGA	3/29/2022	2024, 2025
	UGA.RE.9	UGA	3/29/2022	2024, 2025

Cultivars are those that are released to growers, while advanced selections are not yet released to stakeholders. [1]NCSU: North Carolina State University, [2]USDA; New Jersey: United States Department of Agriculture, Agriculture Research Service, New Jersey Agricultural Experimental Station; [3]Fall Creek: Fall Creek Farm and Nursery Inc; [4]USDA, Poplarville: United States Department of Agriculture, Agriculture Research Service, Southern Horticultural Research Unit: Poplarville, MS; UGA: University of Georgia. SHB = Southern highbush; RE = Rabbiteye.

Table 2.2. Environmental data of monthly averages including March, April, May and June with minimum temperature in Celsius (C), maximum temperature in C, relative humidity % and precipitation in centimeters for 2024 and 2025.

Month	Minimum Temperature (°C)	Maximum Temperature (°C)	Relative Humidity %	Precipitation (mm)
		2024 Data		
March	8.33	21.67	59	20.1
April	11.11	25.56	65	75.4
May	17.78	29.44	74	178.8
June	20	33.33	79	122.9
		2025 Data		
March	6.67	22.78	58	29.5
April	12.22	26.67	67	176.8
May	17.22	28.33	72	161.3
June	20.56	31.67	77	150.6

Values represent monthly average minimum and maximum temperature and relative humidity, and total monthly precipitation for each year.

Table 2.3. Significance (*P*-Values) of main effects and interactions based on dependent variables including general composition, color and phytonutrients measured from 17 genotypes representing the 2024 dataset.

	Dependent Variable								
Independent Variable	SSC	Tacid	SSC:Tacid	L*	a*	b*	Total Anthocyanins (mg·100 g^{-1})	Total Phenolics (mg GAE·100g-1)	Total Antioxidants (µmol·100g^{-1})
Genotype	0.0249	0.0026	<.0001	0.8858	0.0074	0.4158	0.4792	0.0006	0.0720
Storage Duration	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Genotype × Storage Duration	0.9988	0.0003	0.0135	0.7306	0.558	0.094	<.0001	<.0001	0.0004

Cultivars are those that are released to growers, while advanced selections are not yet released to stakeholders. [1]NCSU: North Carolina State University, [2]USDA; New Jersey: United States Department of Agriculture, Agriculture Research Service, New Jersey Agricultural Experimental Station; [3]AU: Auburn University, [4]Fall Creek: Fall Creek Farm and Nursery Inc; [5]USDA, Poplarville: United States Department of Agriculture, Agriculture Research Service, Southern Horticultural Research Unit: Poplarville, MS; UGA: University of Georgia. SHB = Southern highbush; RE = Rabbiteye.

Table 2.4. Significance (*P*-Values) of main effects and interactions based on dependent variables including general composition, color and phytonutrients measured from 5 genotypes representing the 2024-2025 sub-selection.

	Dependent Variable								
Independent Variable	SSC	Tacid	SSC:Tacid	L*	a*	b*	Total Anthocyanins (mg/100g)	Total Phenolics (mg GAE/100g)	Total Antioxidants (µmol/100g)
Year	<.0001	0.8004	0.0084	<.0001	<.0001	0.5519	0.9386	<.0001	<.0001
Genotype	<.0001	<.0001	<.0001	0.0060	<.0001	<.0001	0.0001	<.0001	<.0001
Storage Duration	0.0797	0.0161	0.8788	0.3100	0.0003	0.3999	0.8295	0.0486	0.9206
Harvest Number	0.1028	0.0036	0.0093	0.0331	0.0775	0.9056	0.0666	0.0213	0.1955
Genotype × Year	0.0075	0.0008	0.4802	<.0001	<.0001	0.0086	0.1821	0.0019	<.0001
Genotype × Storage Duration	0.6890	0.0407	0.1627	0.1409	<.0001	0.1483	0.4858	0.0003	0.0033
Genotype × Harvest Number	<.0001	0.0003	<.0001	0.0011	0.1604	0.3698	0.0025	0.0031	0.8064
Year × Genotype × Storage Duration	0.5069	0.5804	0.1834	0.4449	0.2484	0.0089	0.0060	<.0001	0.0030

P-values were obtained from mixed-model ANOVA performed in JMP Pro, Version 19 (SAS Institute Inc.) evaluating the main effects of Year, Genotype, Storage Duration, Harvest Number and their interaction. Significance was defined as $P < 0.05$ ($\alpha = 0.05$).

Table 2.5. Colorimetric attributes (L*, a*, b*) of five sub-selected blueberry cultivars measured at 0, 14, and 28 days (d) of storage from the 2024 and 2025 production seasons.

			Colorimeter	Metric	(CIELAB	Units)			
		L*			a*			b*	
Genotype	0D	14D	28D	0D	14D	28D	0D	14D	28D
					2024				
'Brightwell'	27.9c-e	27.5a-e	33.8a-e	0.9c-g	1.2d-g	0.4d-g	-3.5e-i	-1.2d-h	-3.3d-h
'Vernon'	29.8ab	33.9a-d	32.54a-c	0.9e-g	1.07g	0.56fg	-4.0f-i	-2.1f-i	-3.9h-i
MS1110	29.8a-d	30.79a-e	32.23a-e	0.9e-g	0.4e-g	1.0c-g	-0.6d-i	-3.9g-i	-2.42hi
UGA.RE.9	33.1a-d	28.7a-d	30.2a	0.4bc	0.8c-g	1.0c-g	-3.7b-e	-2.9c-f	-0.9b-d
'Legacy'	33.9c-e	35.39a-e	28.3a-e	1.6c-f	0.4c-g	0.8c-g	-2.1a-c	-3.7a	-3.0ab
					2025				
'Brightwell'	29.4a-e	25.5a-c	27.7a-e	0.6e-g	2.8f-g	0.8e-g	-2.8d-h	-1.2d-h	-4.3d-h
'Vernon'	26.8a-e	30.9a-e	32.7a-d	1.1c-g	2.5c-g	1.3c-e	-3.8hi	-2.4d-i	-3.1d-h
MS1110	27.1a-e	25.4e	28.2de	1.7d-g	1.1c-g	2.6c-g	-0.9i	-3.97h-i	-1.6g-i
UGA.RE.9	29.6a-e	31.7a-e	27.3a-e	1.0a	0.5ab	1.0ab	-3.1a-c	-2.9a-d	-2.0b-g
'Legacy'	26.2a-e	28.7a-e	33.6b-e	3.7a-b	1.2b-d	0.5c-g	-1.0a-c	-3.6a-c	-3.0a-d

Values represent least squares means for the genotype*year*storage timepoint interaction. Different letters within each column and storage timepoint indicate significant differences based on Tukey's HSD (α = 0.05). L*, a*, and b* values are reported in CIELAB color space, where L* indicates lightness, a* red–green, and b* blue–yellow coordinates.

Table 2.6. Total Anthocyanin, phenolics and antioxidants between the genotype × harvest number averaged across the 2024 and 2025 production season.

Genotype	Anthocyanin (mg/100g)	Phenolics (mg GAE/100g)	Antioxidants (µmol/100g TE)
		Harvest 1	
'Brightwell'	14.78b	264.35a-c	18.55a-c
'Vernon'	16.32b	221.84cd	15.98bc
MS1110	16.02b	211.31bd	17.04a-c
UGA.RE.9	5.62b	174.19d	13.93c
'Legacy'	11.83b	214.28cd	15.77bc
		Harvest 2	
'Brightwell'	15.96b	302.78ab	19.44ab
'Vernon'	11.54b	205.28cd	16.7a-c
MS1110	32.19a	318.18a	21.49a
UGA.RE.9	12.88b	209.39cd	12.68bc
'Legacy'	13.59b	239.53bd	20.04a-c

Values represent least squares means for each genotype within harvest (Harvest 1 and Harvest 2), averaged across the 2024 and 2025 production seasons. Different letters within each column and harvest indicate significant differences among genotypes based on Tukey's HSD ($\alpha = 0.05$). Total phenolics are expressed as mg gallic acid equivalents (GAE) per 100 g fresh weight, and total antioxidant activity as µmol Trolox equivalents (TE) per 100 g fresh weight.

2.7 Figures

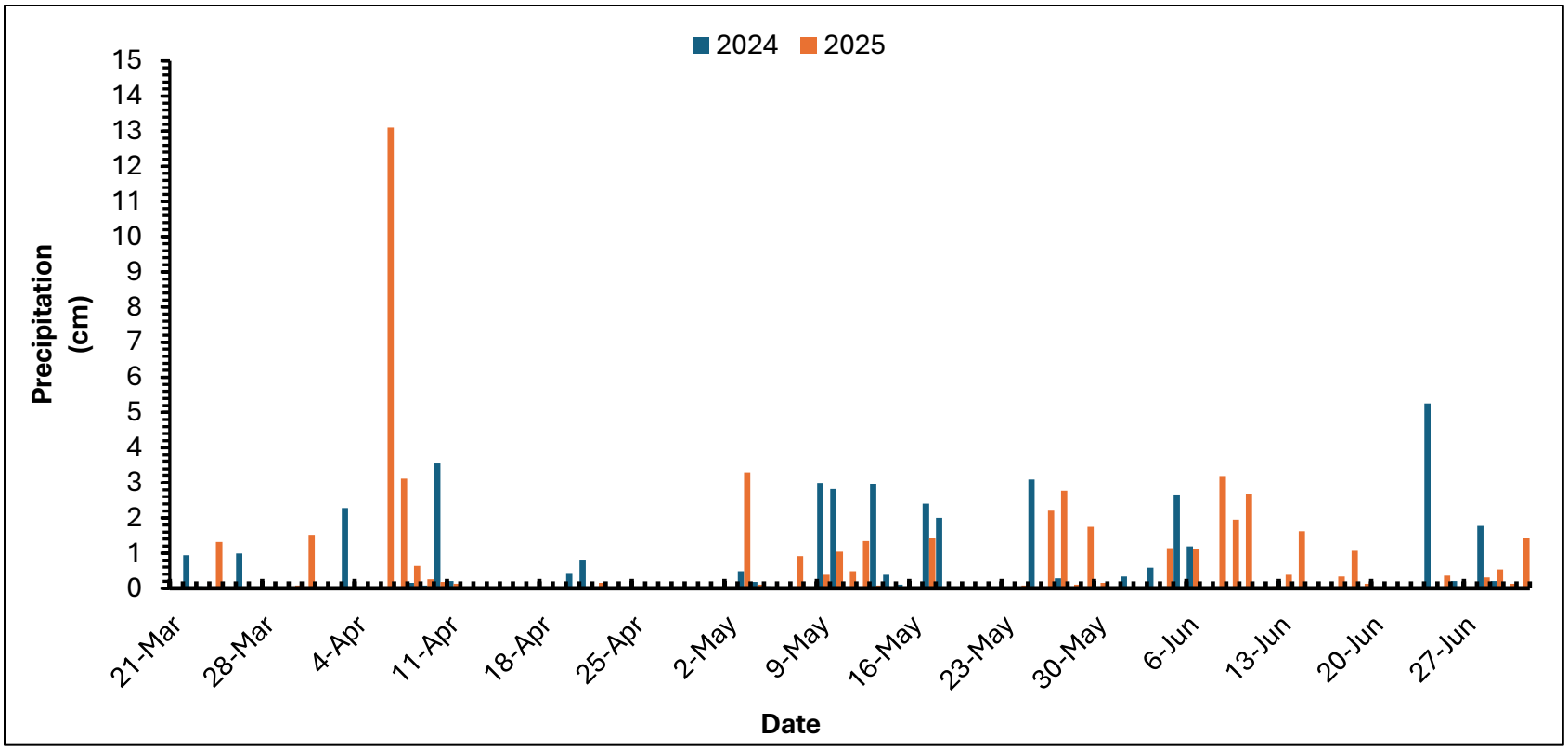

Figure 2.1. Daily recorded precipitation at the E.V. Smith Research Center during the 2024 and 2025 growing seasons (cm). Bars represent total rainfall recorded on each sampling date. Two extreme rainfall events occurred in 2025, one in early April and another in late June, contributing to elevated precipitation variability between years.

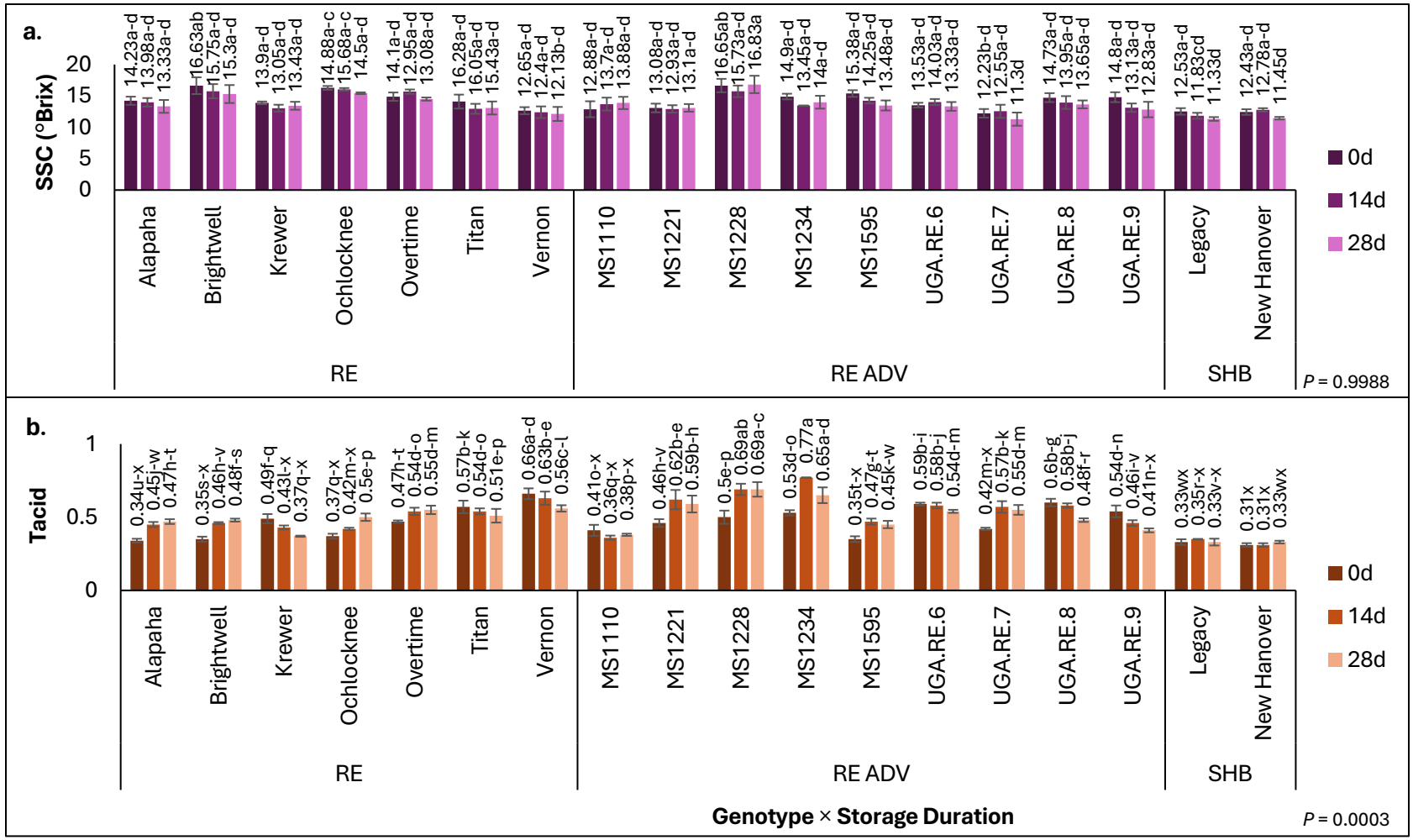

Figure 2.2. Mean soluble solids content (SSC, °Brix) (a) and titratable acidity (Tacid) (b) of rabbiteye (RE), rabbiteye advanced selections (RE ADV), and southern highbush (SHB) genotypes harvested at E.V. Smith in 2024. Error bars represent ± standard error of the mean. Data labels show the mean and Tukey's HSD (α = 0.05). Different letters indicate significant differences among means.

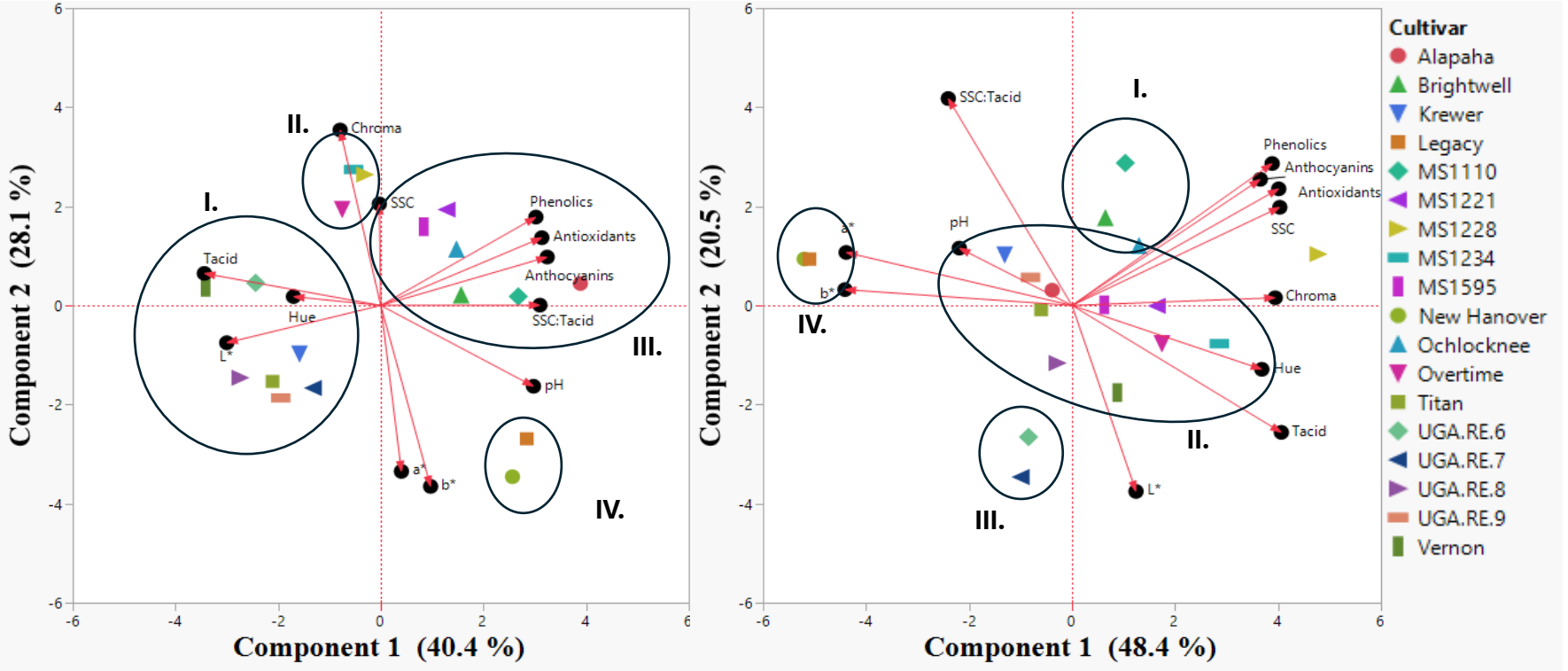

Figure 2.3. Principal component analysis of all physiochemical traits measured in 2024 across 17 genotypes at 0D (a) and 28D (b) including soluble solids content (SSC), titratable acidity (Tacid), color values (L*, a*, b*, chroma and hue), total anthocyanins, total phenolics and total antioxidant capacity. Circled regions indicate clear separations (I, II, and III) highlighting groupings of genotypes associated with similar physicochemical trait profiles at 0D and following 28D of storage. These groupings illustrate shifts in trait associations over storage time rather than discrete statistical groupings.

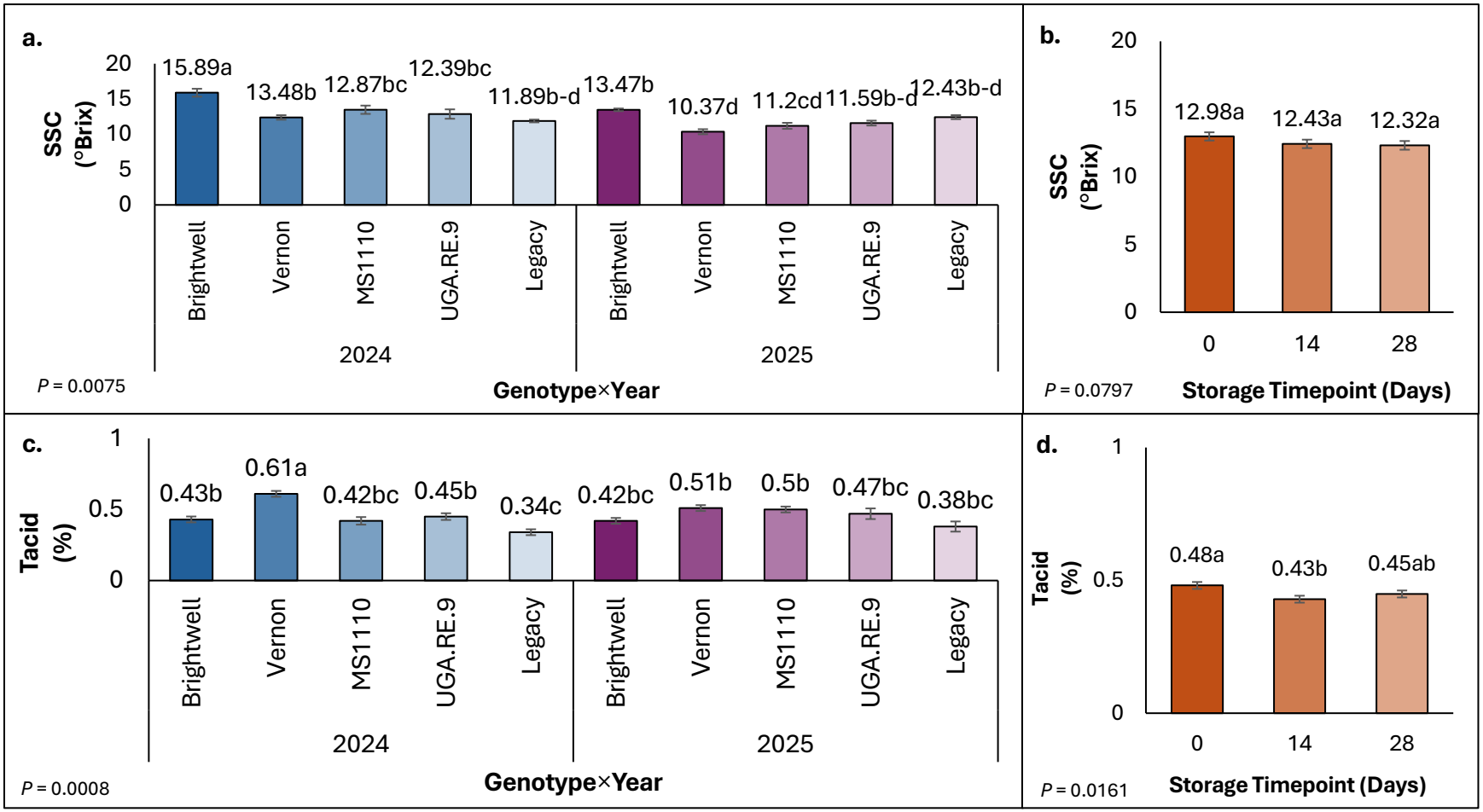

Figure 2.4. Soluble solids content (SSC) in the cultivar×year interaction (a) and across storage timepoint (b), and titratable acidity (Tacid) measured across genotype and year (c) and mean Tacid across storage timepoint (d). Error bars represent ± standard error of the mean. Data labels show the mean values, and different letters indicate significant differences according to Tukey's Honest Significant difference test, α = 0.05.

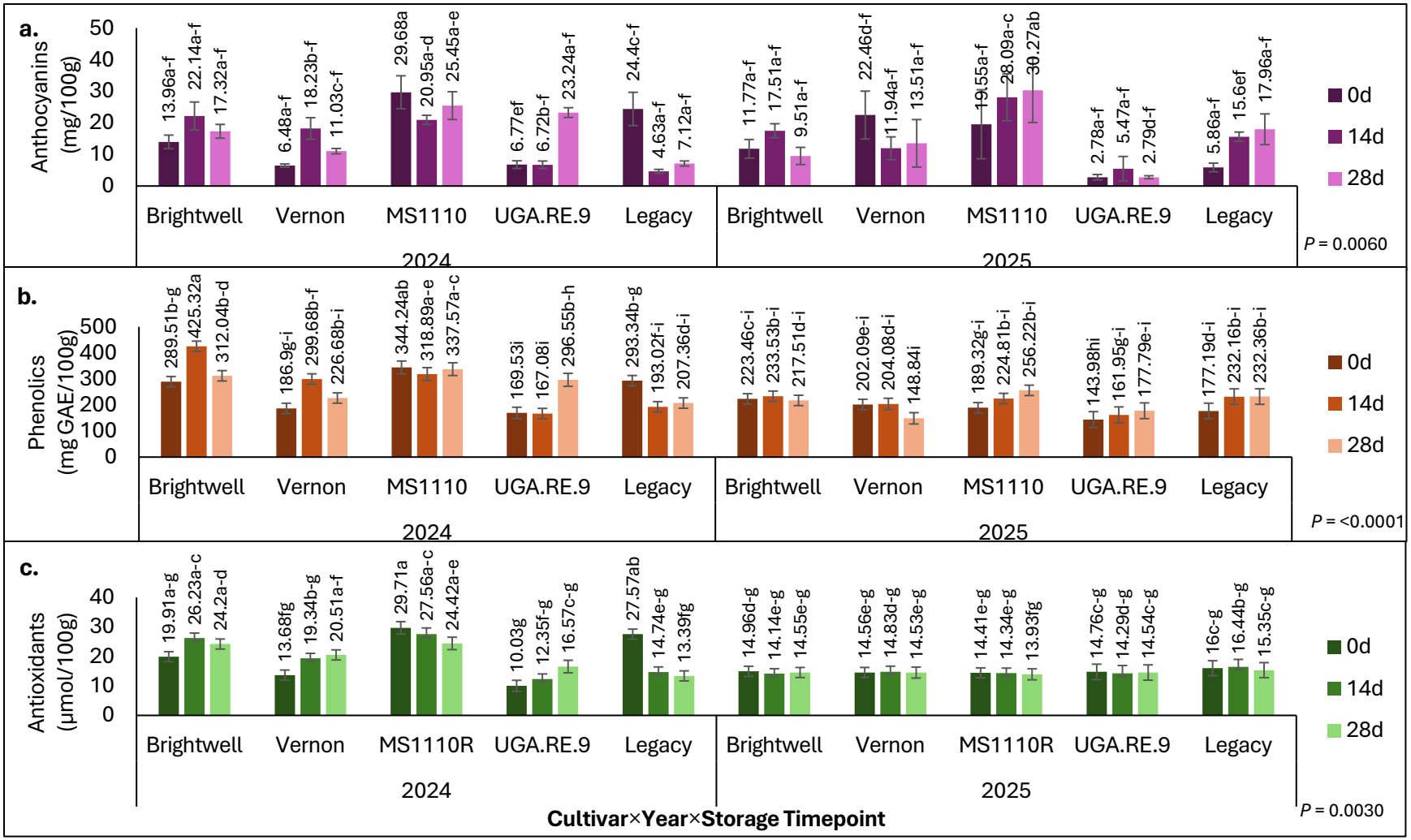

Figure 2.5. Total anthocyanins (a), total phenolics (b), and total antioxidant capacity (c) measured across each genotype as a function of the genotype×year×storage timepoint (0, 14, and 28 days) interaction. All phytonutrient values are reported on a fresh-frozen weight basis. Error bars represent ± standard error of the mean. Data labels show mean values, and different letters indicate significant differences according to Tukey's Honest Significant Difference test, α = 0.05.

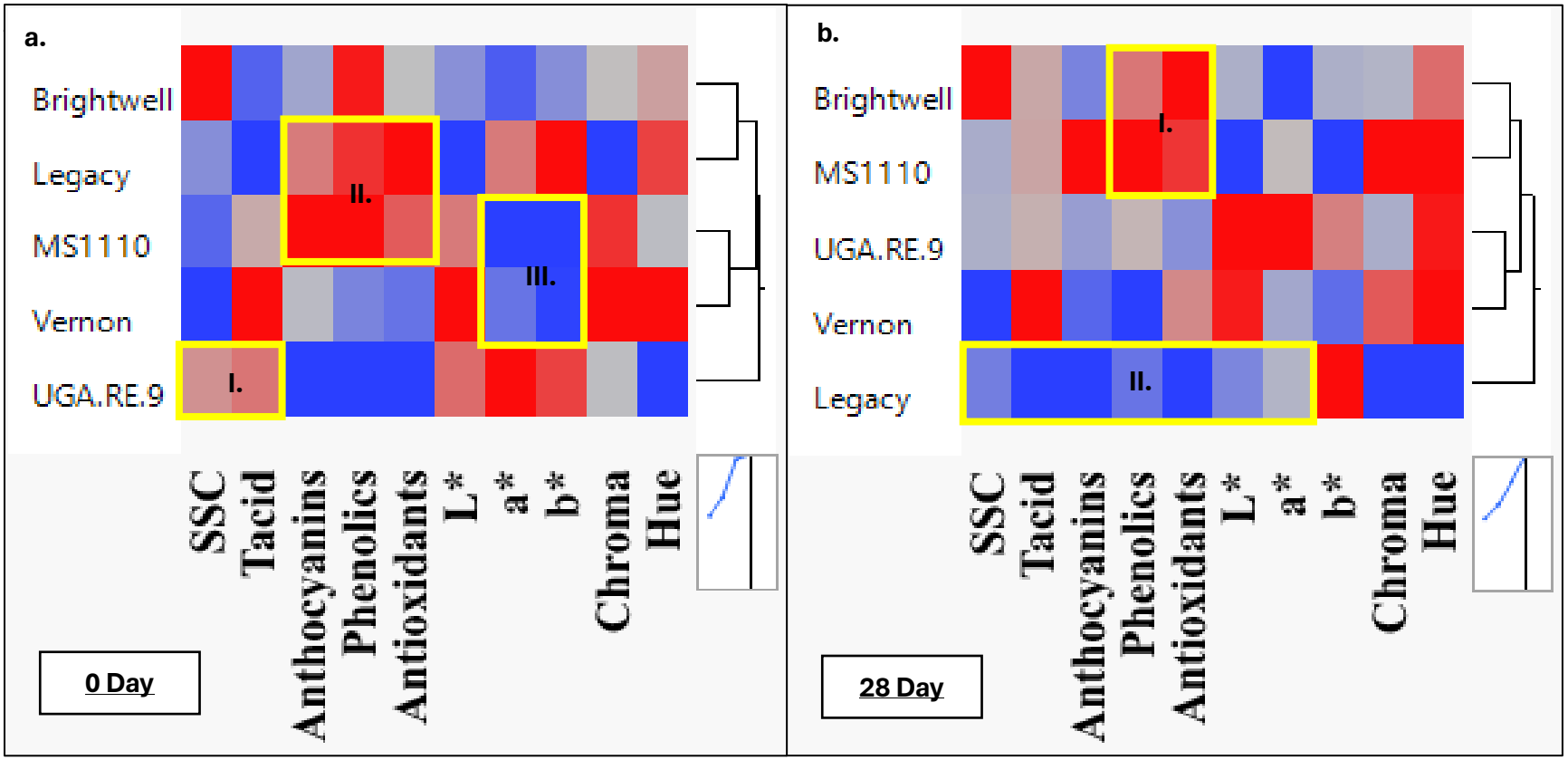

Figure 2.6. Hierarchical cluster analysis (HCA) of all physiochemical traits measured in 2025 across 5 sub-selected genotypes at 0D (a) and 28D (b) including soluble solids content, titratable acidity, L*, a*, b*, chroma, hue, total anthocyanins, total phenolics and total antioxidant capacity. Clusters (I–III) shown in both dendrograms represent groupings of genotypes with similar physicochemical trait profiles at 0D and after 28D of storage. These clusters are descriptive and illustrate shifts in trait associations over storage time rather than statistically discrete groups. Red indicates enhanced traits while blue indicates suppression of traits.

Chapter III Postharvest Sensory Evaluation of Blueberries

Abstract

Blueberry fruit quality is influenced by physicochemical, sensory, and volatile traits, however, limited research has integrated these measurements to evaluate rabbiteye (RE; *V. virgatum*), southern highbush (SHB; *Vaccinium corymbosum*), and RE advanced selections under Alabama conditions. Six blueberry genotypes ('Brightwell', 'Vernon', MS1110, UGA.RE.9, 'Legacy', and 'Optimus') were harvested at two harvest intervals separated by 7 days (D). Following harvest, fruit were evaluated at harvest (0D) or after 14D of cold storage (4 °C, 80% relative humidity). Fruit quality was assessed through soluble solids content (SSC, °Brix), titratable acidity (Tacid, %), SSC:Tacid ratio, and instrumental firmness ($g \cdot mm^{-1}$). Sensory attributes were evaluated using Spectrum Descriptive Analysis by a trained nine-member panel, and volatile profiles were characterized using an electronic e-nose. UGA.RE.9 maintained the highest firmness throughout storage, increasing from 251.82 $g \cdot mm^{-1}$ (0D) to 264.28 $g \cdot mm^{-1}$ (14D), whereas MS1110 was lowest (0D 160.93 and 14D 162.57 $g \cdot mm^{-1}$). SSC differed among genotypes and harvest number, with the lowest (9.67 °Brix, 'Optimus') and highest (13.70 °Brix, 'Brightwell') observed during Harvest 1. Across all genotypes SSC declined by 1.29 °Brix during 14D storage. Tacid was lowest in 'Optimus' (0.22%) and highest in 'Vernon' (0.51%), and declined by 0.10% during storage across all genotypes. Sensory texture, floral, sweet, green, and overripe aromas, and astringent, musty/earthy, malty, honey, green, and overripe flavors were unaffected by genotype, storage duration, or harvest number. Fruity aroma, sweet taste, and sour taste differed among genotypes and storage durations. Fruity aroma ranged from 2.9 in UGA.RE.9 to 6.1 in 'Optimus' at harvest and declined during storage. Sweet taste ranged from 2.9 in 'Vernon' (D0) to 4.6 in 'Brightwell'

(D0), while sour taste ranged from 3.1 in ‘Brightwell’ (D0) to 7.3 in MS1110 (14D). E-nose principal component analysis explained 89.9% (0D) and 95.1% (14D) of the variation and revealed distinct volatile-profile separation among blueberry genotypes, harvest numbers and storage durations. In this study, genotype exerted a stronger influence than storage duration and harvest number on physicochemical, sensory, textural, and volatile traits, highlighting opportunities for breeding improved flavor and storage performance in southeastern U.S. blueberry production.

3.1 Introduction

Blueberries (*Vaccinium* spp.) are a widely cultivated crop, accounting for approximately 5% of the United States (U.S.) fruit market (USDA NASS, 2024). Production and consumption have expanded rapidly over the past two decades due to increasing consumer demand and the adaptability of both southern highbush (SHB) and rabbiteye (RE) cultivars to diverse growing regions, particularly in the southeastern U.S. (Rodriguez-Mateos et al. 2016). As the market becomes increasingly competitive, fruit quality has emerged as a primary factor influencing purchasing decisions (Diehl et al. 2013). Consumers not only expect visually appealing fruit, but also seek products with balanced sweetness and acidity, characteristic aroma profiles, and favorable textural attributes such as firmness and juiciness (Asensio et al. 2025; Gilbert et al. 2015). Consequently, understanding the factors influencing these sensory-driven quality traits across blueberry ecotypes and genotypes has become critical for maintaining market competitiveness and meeting consumer expectations (Gilbert et al. 2015; Mengist et al. 2021).

Blueberry quality assessments have heavily relied on instrumental measurements such as soluble solids content (SSC) to indicate sweetness, titratable acidity (Tacid) to represent sourness and firmness to evaluate textural traits (Kader 1997; Sanchez-Ballesta et al. 2023). These instrumental measurements are often utilized in horticulture as an inexpensive and rapid way to assess standardized ranges (Islam et al. 2024). Standard quality benchmarks reported by Beaudry (1992) suggested SSC should be maintained between 10 and 14 °Brix, with Tacid ranging from 0.3 to 1.3% citric acid equivalents. Since sweetness and acidity are perceived simultaneously, the SSC:Tacid ratio is often used as an indicator of flavor balance. In blueberry research, this ratio has been associated with consumer preference, with Canales et al. (2024)

reporting greater willingness to pay for fruit exhibiting SSC:Tacid ratios between 35 and 45. However, the SSC:Tacid ratio can be misleading, as equivalent values may arise from differing absolute concentrations of sugars and acids (e.g., low SSC with low acidity versus high SSC with high acidity) (Ikegaya 2024; Schwieterman et al. 2014). Moreover, these instrumental metrics do not fully capture the multidimensional nature of blueberry aroma, flavor, and texture (Yan et al. 2020), and therefore fail to reflect key sensory attributes that ultimately influence consumer perception (Farneti et al. 2020; Oh et al. 2025; Saftner et al. 2008).

A comprehensive understanding of blueberry quality integrates aroma, taste, texture, and visual cues that collectively define consumer experience and product acceptability (Gilbert et al. 2015). Aroma is experienced through volatile compounds that stimulate the olfactory system and is perceived through both orthonasal (before consumption) and retronasal (during consumption) (Taylor & Roberts 2004). Retronasal olfaction plays a dominant role in defining flavor profiles and differing intensities. Flavor is a complex sensory trait encompassing both aroma and taste to create a combined experience, with aroma playing a large part in flavor perception over taste (Lawless & Heymann 2010). Texture strongly influences consumer acceptance through firmness, juiciness, seediness and fibrousness (Blaker et al. 2014).

To better capture sensory-related attributes, complementary analytical approaches have been developed to bridge the gap between conventional instrumental measurements and human perception (Pavagadhi & Swarup 2020; Aouadi et al. 2020). Electronic sensing technologies, such as electronic-nose (e-nose) systems, utilize sensor arrays to detect complex mixtures of volatile compounds and interpret them as collective signal patterns rather than discrete chemical identities (Baldwin et al. 2011). These systems provide a rapid and reproducible method for

volatile profiling by generating aroma “fingerprints” that can be associated with sensory perception. Although e-nose instruments cannot identify or quantify individual volatile compounds, they are effective for comparative analyses and pattern recognition across samples (Baietto & Wilson 2015; Moazzem 2023). While e-nose technology has been applied to blueberry (Jin et al. 2015), few studies have integrated volatile fingerprinting with traditional horticultural measurements to evaluate rabbiteye (RE), advanced RE selections, and southern highbush (SHB) blueberries within a unified framework.

In contrast, sensory science provides direct evaluation of human perception and overall eating quality. Consumer panels assess overall liking, whereas trained descriptive panels quantify specific sensory attributes. Spectrum Descriptive Analysis (SDA) is a standardized, quantitative method enabling the development of reproducible sensory profiles across harvest dates, storage timepoints, and genotypes (Civille & Lawless 1989; Lawless & Heymann 2010). Despite its rigor, the application of SDA in blueberry research remains limited, particularly in postharvest studies, resulting in gaps in understanding how flavor and texture attributes vary across harvest conditions, storage durations and genetic backgrounds (Blaker et al. 2014; Lippi et al. 2023; Liu et al. 2023; Moazzem 2023).

To address these gaps, this study integrates instrumental, sensory, and electronic sensing approaches to evaluate six blueberry genotypes, including RE, SHB, and advanced selections. The objectives of the study were to 1) assess instrumental quality through SSC, Tacid, and firmness and 2) evaluate sensory attributes using trained SDA in conjunction with volatile profiling via electronic-nose (e-nose) systems across 6 blueberry genotypes, two storage timepoints (0 and 14 days) and two harvest dates. We hypothesized genotypes would influence sensory

profiles independent of SSC and Tacid due to breeding driven differences in genetic background affecting volatile composition and physicochemical properties of blueberry fruit. We further hypothesized storage duration will alter sensory attributes, resulting in softening and overripe aroma and flavor due to enzymatic degradation and changes in volatile stability across all genotypes.

3.2 Materials and Methods

3.2.1 Ethics Statement

This project for the sensory evaluation of blueberries (e.g., descriptive panel utilizing SDA) throughout postharvest storage was approved by the Institutional Review Board (IRB) of Auburn University with Exempt Protocol #STUDY00000534. Everyone involved as a trained panelist participated voluntarily by signing an informed consent document prior to any training or participation in sensory evaluation.

3.2.2 Experimental Site and Fruit Harvest

Fruit utilized for sensory and electronic-nose analyses were harvested from the same established RE and SHB blueberry plantings described in Chapter 2, located at EV Smith Research Station in Tallassee, AL (32°29'48.7"N, 85°53'33.8"W). In short, the production experiment was planted between March 2022 and April 2023 and was set up as a randomized complete block design (RCBD) with 3 blocks (replications) and 3 bushes planted within each block. Six genotypes as reported in **Table 3.1** (RE Cultivars: ‘Brightwell’, ‘Vernon’; RE advanced

selections: MS1110, UGA.RE.9; SHB cultivars: ‘Legacy’, ‘Optimus’) were harvested between 12 May and 24 June 2025.

Bushes were maintained utilizing standard commercial management practices, following the practices of Smith and Jacobs from University of Georgia’s blueberry production guidelines (2019). Fruit was harvested at commercial maturity defined by uniform blue coloration and absence of red or green coloring (USDA 2024; Giongo et al. 2013; Trandel-Hayse et al. 2023). For each genotype fruit was harvested from the first bush within each replication. Harvest occurred on two separate harvest dates (harvest 1 and harvest 2) spaced one week apart. Fruit was harvested into labeled plastic bags. Directly after harvest, berries were stored on ice in coolers and were immediately transported to the postharvest laboratory at Auburn University. Upon arrival at the laboratory fully ripe blueberries (1.5 to 4 g) were selected and then further sorted to remove defective fruit and placed into cold storage for shelf-life evaluation.

3.2.3 Postharvest Storage and Experimental Design

Postharvest storage conditions and sampling design followed the shelf-life study described in Chapter 2. In short, fruit was stored at 4 °C and ~80% relative humidity. A randomized complete block design (RCBD) with three blocks was used for sorting and storage. Ten berries from each replication/block were placed into 9 oz ventilated Solo cups for each genotype and storage duration for firmness evaluation (n = 30 berries per storage duration period). Following firmness sorting, remaining fruit from all three replications were combined into a single composite sample and re-sorted into two separate clamshells for sensory treatments. Approximately ~120 berries were placed into two labeled 1-pint clamshells for sensory

evaluation at harvest (0D) and after 14 days of cold storage (14D), resulting in one composite sample per genotype and storage duration. E-nose analysis was conducted on subsamples taken from these clamshells concurrent with, or immediately following, sensory panel evaluations. Subsamples (≈2 g) were collected for each genotype, storage duration, and harvest number and analyzed in triplicate for volatile profiling. A total of nine trained panelists (n = 9) participated in each sensory session, with nine sessions conducted over the course of the experiment (May–July 2025). Each genotype and storage duration combination was evaluated across two harvest events (one week apart), resulting in duplicate assessments and a total of n = 18 observations per genotype and storage duration.

3.2.4 Blueberry Firmness Measurements

Firmness was assessed using the FruitFirm1000 texture analyzer (CVM Inc., Pleasanton, CA, USA) following sampling for each genotype across three replications and two storage durations (0D and 14D) (n = 30 berries per storage duration). Ten intact blueberries were brought up to room temperature (~25 °C) approximately 30 minutes prior to measurement. Berries were loaded onto the machine on their perpendicular side to the stem end to allow the machine to take their diameter in mm and distance force firmness in (g mm^{-1}). After running the berries through the machine, they are placed into 50 mL conical tubes (89039-660, VWR, Radnor, PA, USA) to be stored in -20 °C (for up to 6 mo.) or -80 °C for up to 18 months to conduct general composition at a later date.

3.2.5 Compositional Analysis of Soluble Solids Content and Titratable Acidity

SSC and Tacid were measured utilizing blueberries from firmness evaluations. Prior to SSC and Tacid readings, fruit were brought to room temperature and then homogenized. During the thawing stage, two 9mm beads (Grainger, Lake Forest, IL, USA) were placed into 50 mL conical tubes while thawing occurs. Homogenization is done using a Geno/Grinder 2010 (HG-600, SPEX Sample Prep, Metuchen, New Jersey) with settings: Run Time: 1:30 minutes, Rate: 1000, Rest: 0:15 seconds, Cycle: 2 cycles. After homogenization beads were removed and approximately 0.5 g of puree was diluted with 24.5 mL of deionized water and mixed thoroughly to create a solution for Tacid measurements. SSC was measured utilizing an ATAGO PAL-BX|ACID refractometer (Calibrated for blueberries) by filtering a small portion of undiluted puree through cheesecloth and applying the juice to the lens. Tacid was measured by utilizing the same instrument with diluted puree. All measurements were performed in triplicate for each genotype across all storage durations.

3.2.6 Sensory Panel Training for Fresh Blueberry Assessment

A trained panel utilizing nine panelists (n = 9) ages 23 to 63 were trained according to Spectrum Descriptive Analysis method for one hour a day across 3 days for one week. Panel training occurred one to two weeks prior to evaluating harvested blueberries. During the first training session panelists were presented with universal standards for aroma and taste including: vegetable oil (Great Value™ Vegetable Oil, Walmart Inc., Bentonville, AR, USA), apple sauce (Mott's, Williamson, NY, USA), orange juice (Great Value™ Orange Juice (Frozen), Walmart Inc., Bentonville, AR, USA) and grape juice (Welch's, Waltham, MA, USA) for aromatic intensity and dilutions for: sucrose, caffeine, citric acid, and salt for taste attributes. Caffeine (bitter intensity)

and salt (salt intensity) were removed from the study due to lack of presence in blueberry samples. Flavor attributes included musty/earthy (methyl isoborneol; 2-Methylisoborneol ≥98.0%, GC), astringency (Great Value™ Alum, Walmart Inc., Bentonville, AR, USA), honey (Great Value 100% Honey, Walmart Inc., Bentonville, AR, USA), malty (turbinado sugar; Sugar In the Raw® Granulated, Cumberland Packing Corp., Brooklyn, NY, USA), green (Cis-3-Hexanal Solution, 50% in triacetin, Stabilized) and overripe (blueberry puree overripened for 24 hr at room temperature)standards. Texture attributes included firmness (olive; Great Value™ Medium Pitted Black Olives, Walmart Inc., Bentonville, AR, USA), fibrousness (almond; Great Value™ Whole Natural Almonds, Walmart Inc., Bentonville, AR, USA and pineapple; Great Value™ Pineapple Chunks, Walmart Inc., Bentonville, AR, USA) and seediness (yogurt; Great Value™ Original Vanilla Low-fat Yogurt, Walmart Inc., Bentonville, AR, USA and dried fig; Bettergoods, Walmart Inc., Bentonville, AR, USA) (**Table 3.2**). During training panelists discussed all sensory attributes associated with blueberry from a previous study conducted at Auburn University's food science lab under Moazzem et al. (2023). Panels were conducted for 9 weeks in total during the 2025 growing season from May 2025 until July 2025.

3.2.7 Aroma Training

Aroma training focused on calibrating panelists to the aromatic attributes most relevant to blueberry fruit (**Table 3.2**). Universal aroma intensity references were defined using standard products: vegetable oil (intensity 2), applesauce (intensity 5), orange juice (intensity 7), and grape juice (intensity 10), based on perceived aroma intensity. Fruity aroma (intensity 8.5) was trained utilizing Blackberry WONF (LorAnn Oils Blackberry Flavor, LorAnn Oils, Inc., Lansing, MI, USA))

with one drop placed on a cotton ball inside a solo cup with lid. Floral aroma (intensity 9) was represented by geraniol (100% Pure & Natural Geranium Essential Oil, Therapeutic Grade, Majestic Pure Cosmeceuticals, San Diego, CA, USA) diluted in 1 L of distilled water. Sweet aroma (intensity 4) was represented by a vanillin solution prepared by dissolving 0.5 g vanillin (Fisher Scientific, Geel, Belgium) in 500 mL of water. Green aroma (intensity 9) was represented by water infused parsley which was prepared by steeping 25 g of chopped parsley in 300 mL of water for 15 minutes and filtering the liquid. Overripe aroma (intensity 8.5) was represented by blueberries that were pureed the day before and held at room temperature (~ 25°C) overnight. Panelists evaluated each standard utilizing olfactory senses and were advised to smell their own skin between standards to reduce sensory fatigue. Discussion was held for each standard, and intensities were adjusted from a study conducted by Shakir (2023) at Auburn University.

3.2.8 Taste and Flavor Training

Taste and flavor training utilized both basic tastes and retronasal flavor attributes (**Table 3.2**). Sweet taste was trained using sucrose (Great Value™ Granulated Sugar, Walmart Inc., Bentonville, AR, USA) solutions at 2% (intensity 2), 5% (intensity 5), 7% (intensity 7.5) and 10% (intensity 10) to give a range from low to high sweetness. Sour taste was trained using citric acid (Milliard™ Citric Acid, Milliard Brands, Lakewood, NJ, USA) solutions at 0.05% (intensity 2), 0.08% (intensity 5), 0.11% (intensity 7.5) and 0.15% (intensity 10) following pre-established intensities set by Gunness et al. (2009). Astringency (intensity 7) was represented by utilizing a 0.125% alum solution to display the drying, puckering sensation associated with tannins commonly found in fruit commodities. The reference solution was prepared by dissolving 1.25 g alum in 1 L of water.

Additional flavor attributes were trained utilizing reference standards including musty/earthy flavor (intensity 4) represented by methyl isoborneol at 50 parts per billion (ppb). Malty flavor (intensity 8) was represented by 50 g of turbinado sugar in 1 L of water. Honey flavor (intensity 8.5) was represented by 75 g of honey in 1L of water. Green flavor (intensity 9) utilized cis-3-hexenal at 0.3 – 3 parts per million (ppm) in water. Overripe blueberry puree (intensity 10) was created as listed previously. Panelists were instructed not to consume orally the musty/earthy, green flavor and overripe flavor standards. While considered flavors, utilizing olfactory senses for these standards is adequate for recognizing these flavor profiles in real samples. Panelists evaluated each standard by holding samples in mouth and exhaling through the nose to perceive retronasal aroma followed by group discussion to adjust standards as needed based upon Shakir (2023) previously utilized standards.

3.2.9 Texture Training

Training was conducted to calibrate panelists with sensations expected in fresh and stored blueberries using reference foods that represented differing textures (**Table 3.2**). Firmness was demonstrated using soft canned pitted olives (intensity 3). Firmness was defined as the force required to compress a berry between the front teeth. Fibrousness was trained using raw almonds (intensity 1) and canned pineapple chunks (intensity 15) providing the low and high scale for fibrous texture. Seediness was represented by utilizing yogurt (intensity 1) and dried figs (intensity 15) to demonstrate the range of seed density that may be perceived during mastication or chewing. Panelists evaluated each standard through oral consumption, discussed the

intensities and adjusted calibration according to discussion based upon Shakir (2023) previously utilized standards.

3.2.10 Sensory Panel Evaluation on Blueberry Fruit

Panelists (n = 9) evaluated each genotype ('Brightwell', 'Vernon', MS1110, UGA.RE.9, 'Legacy' and 'Optimus') during the first day of storage (0D) and two weeks later (14D). Prior to panel assessment standards, blueberry purees and whole berry samples were prepared fresh and provided at each evaluation. Pureed and whole berry samples utilized a combined clamshell of all three field replications that were harvested. Panelists were provided with a pureed sample to assess aroma, taste and flavor while whole berry samples were provided for the texture and visual assessment portion of the questionnaire. Participants were provided with more material upon request if supply ran low. Pureed and whole samples were served together per genotype and storage duration. Differing genotypes and storage durations were labeled with a 3-digit code and served one by one so bias between samples was minimized. Samples were served in 4 oz solo cups with lids at ambient temperature (~25 °C) with a tray, napkins, unsalted saltines (Great Value, Walmart Inc., Bentonville, AR, USA) and filtered water. Panelists were instructed to cleanse their pallet between samples for taste and flavor with the saltines and water provided. During the aroma portion of evaluation all panelists were advised to sniff standards and their own skin in between switching from reference standards and pureed samples to cleanse the nose. For taste attributes, panelists were advised to pinch their nose and consume the pureed sample to minimize retronasal olfaction interference. For flavor attributes, panelists were advised to consume the puree and experience both taste and retronasal olfaction to evaluate the sample. All

reference standards provided during training were also available for use during each evaluation across 9 weeks of evaluation. All samples were rated on a 15 cm line scale (0 = lowest; 15 = highest) utilizing all reference standards provided. Puree from the blueberries was set aside for e-nose analysis and utilized in triplicate.

3.2.11 Electronic-Nose Analysis

E-nose analysis was conducted utilizing methods from Moazzem et al. (2023). Blueberry volatile profiles were quantified utilizing the Heracles Neo e-nose (Alpha MOS, Toulouse, France). Triplicate e-nose measurements were obtained by dispensing the homogenized puree into three separate vials from the same puree utilized for trained panel evaluation. In short, two grams of homogenized sample was transferred to a 20 mL glass vial (202-0050, Alpha MOS, Toulouse, France) and capped with 20 mm magnetic crimp cap with septa (202-0060, Alpha MOS, Toulouse, France). Vials were then placed into the e-nose instrument and incubated at 50 °C with 500 ppm continuous stirring for 20 minutes inside the e-nose incubation chamber to produce volatiles for the headspace. Headspace (5,000 mL) volatiles are then transferred via robotic arm into the trap at 125 µL/s. Initial trap conditions are set for 40 °C for 50 seconds on 1 mL/min at a constant flow and 10 mL/min split mode until 240 °C trap desorption temperature was reached. Chromatographic analysis consisted of H_2 gas as the carrier at 1 mL/min flow rate for separation of volatiles inside the non-polar MXT column (10 m*180 µm) with FIDs. Compound identification was carried out utilizing the AroChemBase. All samples were run and analyzed in triplicate (n = 3) from pureed blueberry fruit that were provided on the same day of panelist evaluations.

3.2.12 Statistical Analysis

Statistical analysis for general composition, firmness and sensory evaluation data was conducted in RStudio Version 2026.07.0 and R (Posit IDE, Vienna, Australia). Two separate models were utilized to evaluate instrumental and sensory data. Volatile data were analyzed using the AlphaMOS software package and the AroChemBase database, with principal component analysis (PCA) used for multivariate evaluation. Model 1 included general composition and firmness traits analyzed using a three-way fixed-effects ANOVA. Genotype, harvest number, and storage duration were treated as independent variables. Two-way interactions (genotype × storage duration and genotype × harvest number) were included, whereas the three-way interaction was run as genotype × storage duration × harvest number. When fixed effects were significant, mean separation was performed using Tukey's HSD at $\alpha = 0.05$.

Model 2 included sensory attribute analysis (aroma, taste, flavor, and texture intensities) obtained from the trained panel and analyzed using a three-way fixed-effects ANOVA with genotype, harvest number, and storage duration as independent variables. As in Model 1, two-way interactions (genotype × storage duration and genotype × harvest number) were included to assess whether storage or harvest-related changes differed among genotypes, while the three-way interaction was excluded to maintain model parsimony and avoid difficult-to-interpret higher-order effects. Tukey's HSD was used for mean comparisons when significant effects were observed at $\alpha = 0.05$.

Principal component analysis (PCA) was conducted using AlphaSoft (version 2021–7.2.8; AlphaMOS, Toulouse, France) to evaluate volatile profile differences among genotypes, storage

durations (0D and 14D), and harvests (1 and 2) (n = 24). Volatile peak data was downloaded from the AlphaSoft software in triplicate and combined in an excel sheet to compare all three replications. Replications were analyzed utilizing the Retention Index MXT-1701-FID2 (non-polar column) to properly categorize volatiles by functional group. Volatile peaks detected in only a single replication were considered non-reproducible and discarded. Peaks detected in two of three replicates were classified as reproducible, while those present across all three replicates were considered high-confidence volatile data.

3.3 Results and Discussion

3.3.1 Firmness Instrumental Measurements

No significant three-way interaction was detected for instrumental firmness ($P > 0.05$). The storage duration × harvest number and genotype × harvest number interactions were also not significant ($P > 0.05$). A significant genotype × storage duration interaction was observed ($P = 0.0023$). Instrumental firmness was affected by genotype ($P < 0.0001$) and storage duration ($P < 0.0001$), whereas harvest number did not influence firmness ($P = 0.5570$) (**Table 3.3**).

At harvest (0D), firmness ranged from 155.39 g·mm^{-1} in 'Vernon' to 251.30 g·mm^{-1} in UGA.RE.9. After 14D of storage firmness generally increased for most genotypes with changes ranging from 1.59 to 63.93 g·mm^{-1}.'The largest increases during storage were observed in 'Vernon' (63.93 g·mm^{-1}) and 'Legacy' (58.89 g·mm^{-1}), whereas MS1110 remained relatively stable (1.59 g·mm^{-1} increase). Intermediate increases were observed in 'Brightwell' and 'Optimus' at 5.10 g·mm^{-1} and 12.46 g·mm^{-1}, respectively.

The increases in firmness observed for most genotypes after 14D of cold storage are consistent with previous findings in blueberry texture behavior throughout storage where moisture loss, cuticle tightening and cell wall changes can impact firmness throughout storage (Carsorzo et al. 2024). Similar genotype specific firmness patterns have been documented in both RE and SHB blueberries showing the importance of genotype selection for maintaining desirable texture throughout storage (Farneti et al. 2022; Giongo et al. 2013). These findings reinforce the need for breeders and growers to consider firmness goals and not just at harvest when evaluating fruit for marketability.

3.3.2 General Composition

No significant three-way interaction was detected for SSC, Tacid, or SSC:Tacid ratio ($P > 0.05$). The storage duration × harvest number and genotype × storage duration interactions were also not significant. In contrast, the genotype × harvest number interaction was significant for SSC ($P < .0001$), Tacid ($P = 0.0002$), and SSC:Tacid ratio ($P = < .0001$). Genotype significantly affected SSC ($P < .0001$), Tacid ($P = 0.0041$), and SSC:Tacid ratio ($P < .0001$). Storage duration affected only SSC:Tacid ratio ($P = 0.0303$), whereas harvest number did not influence any compositional traits ($P > 0.05$) (**Table 3.3**).

Across the genotype × harvest number interaction, SSC was highest in 'Brightwell' during both Harvest 1 (13.70 ± 0.43 °Brix) and Harvest 2 (13.17 ± 0.16 °Brix), whereas MS1110 (9.77 ± 0.23 °Brix) and 'Optimus' (9.67 ± 0.34 °Brix) exhibited the lowest SSC values during Harvest 1. 'Vernon' was relatively unstable with harvest 1 having 11.33 ± 0.49 °Brix compared to harvest 2 at 9.78 ± 0.63 °Brix, respectively (**Table 3.4**). The significant genotype × harvest number interaction

indicated genotypes responded differently between harvests. Despite these differences, ‘Brightwell’ consistently maintained high SSC across both harvests, suggesting stable sugar accumulation. These findings support previous reports that SSC is strongly influenced by genotype, while environmental conditions during fruit development may contribute to harvest-to-harvest variation in sugar accumulation (Cappai et al. 2018; Gilbert et al. 2015; Oh et al. 2025). With the knowledge that SSC can be tied to perceived sweetness it is important to select genotypes that will retain that quality throughout storage by selecting genotypes with high initial SSC (Canales et al. 2024; Saftner et al. 2008)

Tacid exhibited a significant genotype × harvest number interaction, with MS1110 highest during harvest 1 (0.61 ± 0.07%) and ‘Vernon’ highest during harvest 2 (0.52 ± 0.04%). ‘Optimus’ had the lowest Tacid during harvest 1 (0.22 ± 0.01%) but was unavailable for harvest 2 due to insufficient yield. MS1110 had the lowest Tacid during harvest 2 (0.38 ± 0.05%). Across genotypes, ‘Vernon’ (0.51 ± 0.02) had the highest Tacid while ‘Optimus’ had the lowest Tacid (0.22 ± 0.01) (**Fig. 3.2a**). Tacid decreased as storage duration length increased where 0D averaged at 0.48% and 14D averaged at 0.39%, respectively (**Fig. 3.2b**).

The decrease in Tacid throughout storage is consistent with trend reported within the literature for general composition traits (Brizzolara et al. 2020; Maksimović et al. 2022; Yan et al. 2020). Although the rate of change varied based on genotype suggesting differences in metabolic activity throughout storage. Genotypes with higher acidity such as ‘Vernon’, MS1110 and UGA.RE.9 may experience more intense shifts in flavor after storage while lower acidity genotypes such as ‘Optimus’ contained more mild flavor profiles overall. These results are

contingent with previous studies where acidity was genotype dependent with known significant shifts throughout storage (Saftner et al. 2008; Oh et al. 2025; Yan et al. 2020).

The SSC:Tacid ratio exhibited a significant genotype × harvest number interaction, with ‘Optimus’ highest (45.53 ± 2.60) and MS1110 lowest (17.24 ± 2.25) during harvest 1. Conversely, during harvest 2, MS1110 had the highest ratio (37.25 ± 4.26), whereas ‘Vernon’ had the lowest (19.43 ± 2.17). Genotypes also significantly differed with ‘Optimus’ (45.53 ± 2.60) having the highest SSC:Tacid ratio l while ‘Vernon’ (21.55 ± 1.55) was lowest. Storage duration was also found to be significant and SSC:Tacid increased from 27.68 (0D) to 32.19 on 14D storage.

These findings align with previous consumer preference studies reporting optimal SSC:Tacid ratios between 35 and 45 (Canales et al. 2024). Among the genotypes evaluated, only ‘Optimus’ consistently fell within this preferred range and maintained a relatively stable SSC:Tacid ratio across storage. In contrast, MS1110 exhibited substantial variation between harvests, and may reflect subtle differences in fruit maturity, or source-sink dynamics occurring during the production season. Such variation suggests that sweetness-acidity balance in MS1110 may be more sensitive to harvest timing than in other genotypes (Zapien-Macias et al. 2025). Genotypes therefore differed not only in sweetness-acidity balance but also in how that balance changed across harvests and storage. Nevertheless, SSC:Tacid ratio alone does not fully reflect perceived flavor, as similar ratios can result from different concentrations of sugars and organic acids (Schwieterman et al. 2014; Ikegaya, 2024).

3.3.3 Trained Panel Differentiates Sensory Traits

Sensory attributes showed minimal response to genotype, storage duration, and their interaction (**Tables 3.5, 3.6 and 3.7**). Due to incomplete data for UGA.RE.9 (missing data for storage at 14D), inferential analyses were conducted using the five genotypes with complete datasets ('Brightwell', 'Vernon', MS1110, 'Legacy', and 'Optimus'). Descriptive statistics (means ± standard error), however, are reported for all six genotypes in **Table 3.8, 3.9 and 3.10**.

3.3.4 Aroma Intensities

Sensory aroma attributes showed minimal significant differences in genotype, storage timepoint, and their interaction. Aroma responses were largely limited across genotypes, harvest numbers and storage conditions (**Table 3.5**). No differences were seen among the three way or genotype x harvest number and storage duration x harvest number interactions for any aroma attributes ($P > 0.05$). Sweet ($P = 0.0327$) and overripe ($P = 0.0066$) aroma were affected by the genotype x storage duration interaction. Fruity and floral aroma were significantly affected by genotype (fruity $P < 0.0001$ and floral $P = 0.0438$) and storage duration (fruity $P = 0.0014$ and floral $P = 0.0081$). Green aroma was significantly affected by genotype ($P = 0.0096$) and harvest number ($P = 0.0004$), respectively.

Significant aroma differences for genotype and storage duration are reported in **Table 3.8**. Fruity aroma exhibited the greatest variation among aroma attributes, with the highest intensity observed in 'Optimus' (6.1 ± 0.4) and MS1110 (5.0 ± 0.5) at 0D, whereas UGA.RE.9 was lowest (2.9 ± 0.4). By 14D, fruity aroma generally decreased across genotypes, consistent with the significant storage duration effect**.** Floral aroma was highest in 'Optimus' (4.5 ± 0.6) and 'Legacy' (3.0 ± 0.5) and decreased during storage across most genotypes, declining to values between 2.2

and 3.2 after 14D. Sweet aroma was highest at 0D in ‘Optimus’ (3.3 ± 0.2) and MS1110 (3.1 ± 0.3) whereas UGA.RE.9 had the lowest (1.6 ± 0.2). Following storage, sweet aroma declined in ‘Optimus’ (2.1 ± 0.2) but remained relatively stable or increased slightly in the remaining genotypes. Green aroma was lowest in ‘Legacy’ after 14D storage at 1.3 ± 0.1 in ‘Legacy’ but was highest in UGA.RE.9 on 0D (3.1 ± 0.5). Overripe aroma was highest in ‘Optimus’ at 0D (4.6 ± 0.6) whereas UGA.RE.9 (2.6 ± 0.4) and ‘Legacy’ (2.7 ± 0.6) were lowest. After 14D storage, overripe aroma increased in ‘Vernon’ (4.5 ± 0.6) and MS1110 (4.0 ± 0.6), while declining in ‘Optimus’ from 4.6 ± 0.6 to 2.7 ± 0.5, respectively.

Fruity aroma exhibited the greatest variation among genotypes, with ‘Optimus’ and MS1110 consistently receiving the highest ratings, suggesting a greater abundance of aroma-active volatiles associated with fresh fruit character. In contrast, UGA.RE.9 and ‘Vernon’ exhibited lower fruity aroma intensities highlighting genotype-dependent differences in aroma perception. Fruity aroma also declined during storage, likely reflecting reductions in volatile compounds associated with fresh blueberry aroma (Farneti et al. 2020; Yan et al. 2020). Floral, sweet, green, and overripe aromas remained relatively consistent across storage, indicating that short-term cold storage had limited effects on overall aroma perception. Similar genotype-dependent differences in blueberry aroma and volatile composition have been reported previously, with cultivar genetics often exerting a greater influence on sensory aroma characteristics than storage duration (Beaulieu et al. 2014; Yan et al. 2020; Moazzem et al. 2024).

3.3.5 Taste and Flavor Intensities

Sensory flavor attributes showed limited responses to genotype, storage duration, harvest number, and their interactions as reported in Table 3.6. No significant effects were detected for musty/earthy flavor across any main effect or interaction ($P > 0.05$). Overripe flavor was unaffected by all main effects and two-way interactions, with only the three-way interaction being significant ($P = 0.0369$). Likewise, honey flavor was unaffected by genotype, storage duration, harvest number, and all two-way interactions, but was significant for the three-way interaction ($P = 0.0369$). Among interaction effects, significant three-way interactions were observed for sweet taste ($P = 0.0015$), sour taste ($P < 0.0001$), astringent flavor ($P = 0.0004$), malty flavor ($P = 0.0300$), and honey flavor ($P = 0.0369$). While genotype × storage duration interactions were observed for sour taste ($P < 0.0001$), astringent flavor ($P = 0.0123$), and green flavor ($P = 0.0157$). Similarly, genotype × harvest number interactions were detected for sweet taste ($P = 0.0096$), sour taste ($P = 0.0085$), and malty flavor ($P = 0.0005$). Storage duration × harvest number interactions were significant for sweet taste ($P = 0.0403$), sour taste ($P < 0.0001$), and astringent flavor ($P = 0.0003$). Sweet taste and sour taste were significantly affected by genotype ($P < 0.0001$ for both). Sweet taste was also affected by storage duration ($P = 0.0237$), whereas sour taste was affected by harvest number ($P = 0.0015$).

Sour taste was highest at 0D in ‘Optimus’ (5.3 ± 0.7) and UGA.RE.9 (5.0 ± 0.5) while ‘Brightwell’ had the lowest (3.1 ± 0.4). After 14D, genotypic responses differed substantially, with MS1110 increasing from 3.9 ± 0.6 to 7.3 ± 0.5, whereas ‘Optimus’ declined from 5.3 ± 0.7 to 2.0 ± 0.2. Astringent flavor increased during storage in ‘Brightwell’ (2.7 ± 0.3 to 3.1 ± 0.4), ‘Vernon’ (3.2 ± 0.5 to 3.6 ± 0.5), and MS1110 (2.9 ± 0.5 to 3.8 ± 0.5), while declining in ‘Legacy’ (3.2 ± 0.5 to 2.6 ±

0.5) and ‘Optimus’ (3.1 ± 0.5 to 2.0 ± 0.4). Green flavor was highest at 0D in ‘Optimus’ (3.6 ± 0.5), whereas ‘Legacy’ and UGA.RE.9 had the lowest intensities (1.9 ± 0.3). During storage, green flavor increased slightly in MS1110 (2.2 ± 0.4 to 2.6 ± 0.5) and ‘Vernon’ (2.2 ± 0.4 to 2.3 ± 0.4), but declined in ‘Optimus’ from 3.6 ± 0.5 to 2.0 ± 0.4, respectively (**Table 3.9**).

A significant storage duration x harvest number interaction was seen for sweet, sour and astringent taste (**Table 3.11**). During harvest 1, sweet taste declined from 3.91 ± 0.25 at 0D to 3.12 ± 0.27 after 14D storage, whereas harvest 2 remained relatively stable. During harvest 1, sour taste increased from 3.57 ± 0.29 at 0D to 4.23 ± 0.43 after 14D storage. In contrast, harvest 2 exhibited higher sourness at 0D (5.06 ± 0.46) and decreased slightly to 4.17 ± 0.38 following storage. While astringent taste during harvest 1 increased from 2.73 ± 0.26 at 0D to 3.33 ± 0.32 after 14D storage, whereas harvest 2 decreased from 3.65 ± 0.37 to 2.84 ± 0.32 over the same storage period.

SSC and Tacid are primary drivers of sweetness and sourness perception in blueberries (Gilbert et al. 2015). The increase in sourness observed in MS1110 during storage while ‘Optimus’ declined in sourness, astringency, and green flavor, indicated contrasting flavor stability among genotypes. Conversely, ‘Brightwell’ and ‘Vernon’ generally maintained more consistent sensory profiles throughout storage. These findings agree with previous reports demonstrating that blueberry flavor perception is strongly influenced by overall taste balance and genotype-dependent changes in sugar and acid composition during storage (Gilbert et al. 2015; Saftner et al. 2008). Moreover, the significant harvest number × storage duration interactions for sweet, sour, and astringent taste further suggest fruit harvested at different times within the season may

respond differently during storage and can be attributed to phenolic content as well as general fruit composition (Zapien-Macieas et al. 2025; Forney et al. 2012)

In contrast, musty/earthy, malty, honey, and overripe flavor attributes remained low in intensity and were largely unaffected by genotype or storage duration. The stability of these flavor characteristics suggests that short-term cold storage had minimal influence on sensory attributes associated with off-flavors or advanced fruit maturation. Similar stability of flavor attributes during refrigerated storage has been reported in blueberries and other berry crops, even when instrumental analyses detect changes in volatile composition (Lippi et al. 2023; Moazzem et al. 2024). In this study, flavor attributes remained low in intensity, and blueberry flavor quality was largely maintained during 14 d of cold storage. Sensory changes were generally subtle and driven primarily by sweetness, sourness, and genotype-specific responses, which were unlikely to substantially affect overall consumer acceptance.

3.3.6 Sensorial Texture Intensities

Mixed model analysis of sensory texture attributes is presented in **Table 3.7**. Significant three-way interactions were observed for seediness ($P = 0.0004$) and firmness ($P = 0.0309$). Among the two-way interactions, storage duration × harvest number ($P = 0.0096$) and genotype × harvest number ($P = 0.0224$) significantly affected seediness. Genotype × storage duration interactions were also significant for firmness ($P = 0.0044$) and seediness ($P = 0.0146$). For the main effects, genotype influenced both firmness ($P = 0.0158$) and seediness ($P = 0.0006$). Firmness was additionally affected by storage duration ($P = 0.0200$), whereas seediness differed by harvest number ($P = 0.0200$).

Sensorial firmness was highest in 'Optimus' at 0D during harvest 2 (3.81 ± 0.50) and was lowest in 'Brightwell' at harvest (0D) during harvest 2 (2.28 ± 0.28). At 14D firmness intensity was highest in 'Legacy' (4.00 ± 0.47) during the first harvest and was lowest in 'Optimus' (2.58 ± 0.36) during the second harvest (**Fig. 3.3a**). UGA.RE.9 and 'Legacy' were omitted due to a lack of fruit material during the second harvest. UGA.RE.9 was additionally not served to panelists during 14D for the first harvest due to extreme diminished quality. Fibrous intensity evaluation was not significant ($P < 0.05$) for all effects tested. All genotypes ranged between 4.4 ± 1.0 to 4.9 ± 1.0 at harvest (0D) of storage while 14D ranged from 5.0 ± 1.0 to 4.4 ± 1.0. The genotype that did change the least throughout storage was 'Legacy' with a 0.2 point intensity increase from 0D to 14D. Seedy intensity at 0D was highest in UGA.RE.9 (7.00 ± 1.04) during harvest 1 and was lowest in MS1110 during harvest 1 (3.03 ± 0.90). After 14D of storage, MS1110 during the harvest 2 (7.19 ± 1.09) was perceived as the seediest while 'Optimus' during harvest 2 (2.85 ± 0.49) was perceived to be the least seedy genotype. UGA.RE.9 and 'Legacy' were again omitted due to a lack of fruit material during the harvest 2. UGA.RE.9 was additionally not served to panelists during 14D for the first harvest due to extreme diminished quality.

Sensory texture responses were influenced primarily by genotype and genotype-dependent changes during storage. Although instrumental firmness increased for several genotypes during storage, corresponding changes in sensory firmness were less consistent, suggesting that trained panelists were less sensitive to subtle texture changes detected instrumentally. Similar discrepancies between instrumental and sensory texture evaluations have been reported in blueberries and other fruit crops, where mechanical measurements detected texture differences that were not always perceived by sensory panels (Saftner et al. 2008; Blaker

et al. 2014). Consequently, instrumental assessments may capture structural changes in berry tissues that do not necessarily translate into meaningful differences in eating quality.

Genotypic differences in sensory firmness indicated texture perception is strongly influenced by inherent fruit characteristics, including skin strength, flesh structure, and berry anatomy. Similar cultivar-dependent differences in blueberry firmness and texture have been reported previously, demonstrating how firmness is highly heritable and an important breeding target for fresh-market quality and postharvest performance (Giongo et al. 2013; Farneti et al. 2022). 'Legacy' and 'Optimus' generally exhibited greater firmness intensity, whereas 'Brightwell' tended to be perceived as less firm. Since firmness contributes to consumer perceptions of freshness and overall fruit quality, these genotype-dependent differences may influence market acceptance even when fruit remain within commercially acceptable ranges (Saftner et al. 2008; Gilbert et al. 2014).

Seediness was also affected by genotype and several interaction effects, demonstrating that perceptions of seed texture vary among blueberry genotypes. However, no consistent increase or decrease in seediness was observed throughout storage indicating seed-related texture traits remained relatively stable. This stability is expected because seed characteristics are largely genetically determined and are not substantially altered by short-term refrigerated storage. Overall, results from this study these findings indicated genotype had the greatest influence on sensory texture attributes, while overall texture quality remained relatively stable throughout storage. Similar conclusions have been reported in blueberry texture studies, where genotype explained more variation in sensory texture than postharvest storage conditions (Saftner et al. 2008; Farneti et al. 2022).

3.3.7 Electronic Nose

At harvest (0D), alcohols represented the most abundant volatile functional group across all blueberry genotypes, followed by hydrocarbons, aldehydes, ethers, and ketones (**Fig. 3.4a**). Total volatile abundance varied among genotypes, with UGA.RE.9 and MS1110 exhibiting the lowest overall volatile signals at 11.5% and 11.8%, respectively. 'Optimus' (25.9%) and 'Vernon' (21.2%) had the highest volatile abundance. Specifically, 'Legacy' at harvest indicated higher abundances of aldehydes, carboxylic acids, hydrocarbons, and terpenes while 'Vernon' at harvest indicated increases in organochlorines, monoterpenes and hydrocarbons compared to all other genotypes. Conversely, MS1110 showed a larger representation for ketones and lactones. Minor contributions were observed from esters, lactones, amines, aromatic hydrocarbons, monoterpenes, nitriles and sulfur-containing compounds, and other functional groups (**Fig. 3.4a**).

After 14D of storage, UGA.RE.9 was excluded from volatile analysis because insufficient fruit was available for evaluation (**Fig. 3.4b**). Storage altered the relative abundance of multiple volatile functional groups, with alcohols, hydrocarbons, and carboxylic acids generally increasing across genotypes, while aldehydes decreased in all genotypes except MS1110. The RE advanced selection MS1110 exhibited the greatest increase in total volatile abundance, rising from 11.8% at harvest to 33.6% after 14D of storage. This increase was primarily driven by greater abundance of alcohols, aldehydes, hydrocarbons, ketones, and organosulfur compounds. Similarly, total volatile abundance in 'Brightwell' increased from 16.5% at harvest to 21.9% after storage, largely due to increases in alcohols, hydrocarbons, and thiols. In contrast, 'Optimus' (25.9% at harvest to 12.9% on 14D) and 'Vernon' (21.2% at harvest to 15.4% on 14D), accompanied by reductions in

alcohols, hydrocarbons, and aldehydes. ‘Legacy’ remained relatively stable throughout storage, exhibiting only minor changes in volatile composition (**Fig. 3.4b**).

These results for volatile profiling align with previous studies showing differences between genotypes and storage durations (Farneti et al. 2025). Alcohols were the most dominant functional group across all genotypes followed by hydrocarbons and aldehydes (Farneti et al. 2017; Li et al. 2025). Storage for 14 days did impact volatile profiles with an increase in alcohols, hydrocarbons and carboxylic acids with a slight decrease in aldehydes (Farneti et al. 2025). The overall increase in volatiles suggests increased postharvest metabolic activity, where decreased volatile profile percentages suggest reduced volatile retention (Xu et al. 2021; Yan et al. 2020). Both genotype and storage duration did strongly influence volatile profile abundance and overall composition highlighting the importance of observing postharvest volatile profile behavior into fruit quality evaluation postharvest (Demir et al. 2011; Farneti et al. 2025).

PCA of 0 d volatile profiles revealed clear separation among genotypes and harvest numbers (Fig. 3.5). PC1 and PC2 accounted for 74.22% and 15.72% of the total variation, respectively, explaining 89.94% of the variability in volatile composition at harvest. ‘Brightwell’ (Harvests 1 and 2), ‘Vernon’ (Harvest 1), and UGA.RE.9 (Harvest 1) formed distinct clusters, with all three biological replicates exhibiting high within-group consistency. ‘Optimus’ (Harvest 2) and ‘Legacy’ (Harvest 1) clustered closely together, indicating similar volatile profiles. In contrast, ‘Vernon’ (Harvest 2) and MS1110 (Harvests 1 and 2) were clearly separated from the remaining genotypes, suggesting distinct volatile compositions.

PCA of 14D volatile profiles revealed shifts in clustering patterns relative to harvest samples (**Fig. 3.6**). UGA.RE.9 was excluded from the 14D analysis because insufficient fruit was

available for evaluation. PC1 and PC2 explained 72.15% and 22.90% of the total variation, respectively, accounting for 95.05% of the variability in volatile composition. ‘Brightwell’ (harvest 1 and 2) and ‘Optimus’ (harvest 1 and 2) clustered in close proximity on the positive side of PC1 indicating similar volatile profiles after storage. MS1110 (harvest 1) clustered closely with ‘Legacy’ (harvest 1) in the upper-right quadrant, whereas MS1110 (harvest 2) separated from both samples, suggesting a distinct postharvest volatile profile. ‘Vernon’ harvests 1 and 2 were clearly separated from each other and from all remaining genotypes, with ‘Vernon’ harvest 2 exhibiting the greatest isolation along the negative side of PC1.

The e-nose PCA results demonstrated volatile profiles are strongly genotype dependent with storage taking secondary effect. The clear clustering of genotypes indicated each genotype did have its own unique volatile profile consistent with findings that blueberry volatile composition is genetic based (Beaulieu et al. 2014; Farneti et al. 2020; Jin et al. 2025). The e-nose successfully discriminated against genotypes and detected storage related changes that show its value as an instrument for rapid assessment of volatile profiles (Wilson 2013; Moazzem 2023). These results accommodate the sensory findings suggesting that while panelists perceived minimal aroma differences the volatiles were influenced heavily by genotype.

3.5 Conclusion

Across the six blueberry genotypes evaluated, postharvest quality outcomes differed primarily as a function of genotype, with storage timepoint and harvest number exerting a secondary influence. Instrumental compositional measurements showed consistent declines in SSC and Tacid during storage, with genotype-dependent magnitudes of change. Consequently,

SSC:Tacid ratios increased across all genotypes, though the extent of increase varied, reflecting differential compositional stability. Descriptive sensory analysis indicated genotype significantly influenced key attributes including fruity aroma, sweetness, and sourness, while storage timepoint primarily affected sweetness. Most other sensory attributes, including floral, green, and overripe aromas, as well as flavor and texture traits, were not significantly differentiated among genotypes or across storage. These results suggest trained panelists detected differences in a limited subset of attributes despite measurable compositional changes. In contrast, e-nose volatile profiling provided greater discrimination among genotypes, with PCA explaining 88.87% of total variability and separating samples into distinct clusters primarily driven by genotype. Storage related shifts in volatile profiles were present but less pronounced than genotypic differences, reinforcing the dominant role of genetic background in shaping aroma characteristics. These findings demonstrated genotype is the primary determinant of postharvest sensory and compositional quality in blueberries, while storage duration induces consistent but smaller changes. The limited sensitivity of descriptive sensory analysis relative to instrumental and volatile profiling indicates that integrating multiple analytical approaches provides a more comprehensive assessment of fruit quality. For breeders and growers, these results highlight the importance of genotype selection for maintaining desirable flavor and compositional traits during storage and support the use of volatile profiling tools to enhance detection of subtle quality differences not captured by sensory evaluation alone.

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3.6 Tables

Table 3.4. Blueberry genotypes and advanced selections used for postharvest quality and shelf-life evaluations. All genotypes were planted and harvested at the E.V. Smith Research Center in central Alabama (Tallassee, AL).

Type	Genotype	Breeding Program	Planting Date	Year of Evaluation
SHB Cultivars	'Optimus'	Gainesville, Florida[1]	4/3/2022	2025
	'Legacy'	USDA, New Jersey[2]	4/3/2023	2025
RE Cultivars	'Vernon'	UGA[3]	3/29/2022	2025
	'Brightwell'	UGA	4/14/2023	2025
RE Selections	MS1110	USDA, Poplarville[4]	4/3/2023	2025
	UGA.RE.9	UGA	3/29/2022	2025

Cultivars are those that are released to growers, while advanced selections are not yet released to stakeholders. [1]University of Florida (UF) blueberry breeding program, Gainesville, Florida, [2]USDA; New Jersey: United States Department of Agriculture, Agriculture Research Service, New Jersey Agricultural Experimental Station, [4]Fall Creek: Fall Creek Farm and Nursery Inc; UGA: University of Georgia. SHB = Southern highbush; RE = Rabbiteye.

Table 3.2. Descriptive sensory attributes of blueberry with definitions, reference standards and perceived intensity.

Attributes	Definition	Scale Intensity	Reference Standard
Aroma Attributes			
Fruity	A sweet, floral, aromatic blend, reminiscent of variety of ripe fruits such as apricots, peaches	8.5	Blackberry WONF 3RA654 (McCormick & Wild Inc., Hunt Valley, MA); Place one drop of chemical on a cotton ball in a solo cup. Cover.
Floral	The somewhat sweet aromatics generally associated with fruit and flowers	9	Geraniol Pure; Put 1 drop geraniol in 1 L of distilled water in a solo cup. Cover.
Sweet	Aromatics associated with the impression of sweet substances such as fruit or flowers	4	Fisher Scientific Vanillin; Place 0.5 g of Fisher Scientific Vanillin in 500 mL of water in a solo cup. Cover.
Green	Sharp, slightly pungent aromatics associated with green plant/vegetable matter, such as asparagus, Brussels sprouts, celery, green beans, parsley, spinach, etc.	9	Fresh parsley water; 25 g of fresh parsley, rinse, chop, and add 300 mL of water. Let it sit for 15 min. Filter and serve liquid part.

Overripe	Aromatics associated with fruit that have passed beyond maturity, or ripeness toward decay	8.5	Overripe blueberry left at 25°C overnight
Flavor Attributes			
Sweet	Degree of sweetness upon chewing (Gunness et al., 2009)	2	2% Sucrose solution in water
		5	5% Sucrose solution in water
		7.5	7% Sucrose solution in water
		10	10% Sucrose solution in water
Sour	Degree of sourness upon chewing (Gunness et al., 2009)	2	0.05% Citric acid solution in water
		5	0.08% Citric acid solution in water
		7.5	0.11% Citric acid solution in water
		10	0.15% Citric acid solution in water

Astringent	Feelings associated with dry sensation associated with green tea or immature persimmons. The shrinking or puckering of the tongue surface caused by substances such as tannins or alum. Puckering sensation typically perceived when tasting alum, tannin in water. A feeling that shrivels the tongue associated with tannins	7	Alum 0.125%
Musty/Earthy	Somewhat sweet, heavy aromatics associated with decaying vegetation and damp black soil (Jaffe et al., 2017)	4	Methyl isoborneol (50 ppb)
Malty	Flavor tends to be roasted and earthy	8	Turbinado sugar 50 g/L
Honey	Flavor tends to be subtle with floral notes	8.5	Honey solution 75 g/L

Green	Sharp, slightly pungent flavor note associated with green plant/vegetable matter, such as asparagus, brussel sprouts, celery, green beans, parsley, spinach, etc. (Descriptive Analysis Book p336)	9	(Z)-3-hexenal in water, 0.5–3.0 ppm
Overripe	Degree of off-flavor upon chewing (gunness et al., 2009)	10	Puree of overripe blueberry left at 25°C overnight
Texture Attributes			
Firmness	Force required to with the front of teeth	3	Olive (soft pitted canned)
Fibrous	Stringy texture upon chewing	1	Almond (raw)
		15	Pineapple (canned pieces)
Seedy	Grainy texture upon chewing	1	Yogurt (natural, served at room temperature)
		15	Dry fig

Table 3.3. Mixed model *P*-Values for all dependent variables of blueberry general composition fruit quality traits.

	Dependent Variable			
Independent Variable	SSC	Tacid	SSC:Tacid	Firmness
Genotype	<.0001	0.0002	<.0001	<.0001
Storage Duration	0.1779	0.0055	0.0303	<.0001
Harvest Number	0.2114	0.058	0.0923	0.557
Genotype × Storage Duration	0.9394	0.9275	0.9911	0.0023
Genotype × Harvest Number	<.0001	0.0041	0.0002	0.0554
Storage Duration × Harvest Number	0.7019	0.0554	0.9627	0.407
Genotype × Storage Duration × Harvest Number	0.7002	0.9893	0.6634	NA

P-values were obtained from mixed-model ANOVA performed in RStudio, (Version 2026.07.0, Posit team) and R (R Core Team) evaluating the main effects of genotype, storage duration, harvest number and their interaction. Significance was defined as $P < 0.05$ ($\alpha = 0.05$). SSC = soluble solids content; Tacid = titratable acidity; SSC = soluble solids content-to-titratable acidity ratio.

Table 3.4. General composition traits for soluble solids content (SSC), titratable acidity (Tacid) and SSC:Tacid throughout harvest.

	Dependent Variable		
	SSC	Tacid	SSC:Tacid
		Harvest 1	
Brightwell	13.70 ± 0.43	0.40 ± 0.03	35.60 ± 3.84
Vernon	11.33 ± 0.49	0.49 ± 0.03	23.67 ± 2.03
MS1110	9.77 ± 0.23	0.61 ± 0.07	17.24 ± 2.25
UGA.RE.9	11.82 ± 0.32	0.48 ± 0.04	25.75 ± 2.22
Legacy	12.50 ± 0.35	0.41 ± 0.05	32.70 ± 3.47
Optimus	9.67 ± 0.34	0.22 ± 0.01	45.53 ± 2.60
		Harvest 2	
Brightwell	13.17 ± 0.16	0.41 ± 0.02	32.25 ± 1.74
Vernon	9.78 ± 0.63	0.52 ± 0.04	19.43 ± 2.17
MS1110	12.95 ± 0.21	0.38 ± 0.05	37.25 ± 4.26
UGA.RE.9	NA	NA	NA
Legacy	NA	NA	NA
Optimus	NA	NA	NA

Data shows the mean values ± standard error. UGA.RE.9, 'Legacy' and 'Optimus' were excluded from Tukey Honestly Significant Difference post-hoc test due to missing data during the second harvest. SSC = soluble solids content; Tacid = titratable acidity; SSC = soluble solids content-to-titratable acidity ratio.

Table 3.5. Mixed effect model *P*-Values for only the significant dependent aroma intensity variables

Independent Variable	**Dependent Variable**				
	Fruity Aroma	Floral Aroma	Sweet Aroma	Green Aroma	Overripe Aroma
Genotype	<.0001	0.0438	0.0166	0.0096	0.4652
Storage Duration	0.0014	0.0081	0.0621	0.5531	0.6055
Harvest Number	0.9157	0.411	0.2816	0.0004	0.1427
Genotype × Storage Duration	0.059	0.0852	0.0327	0.7455	0.0066
Genotype × Harvest Number	0.5392	0.278	0.291	0.1844	0.1116
Timepoint × Harvest Number	0.5441	0.524	0.6084	0.205	0.7851
Genotype × Storage Duration × Harvest Number	0.6413	0.8408	0.1088	0.8243	0.0237

P-values were obtained from mixed-model ANOVA performed in RStudio, (Version 2026.07.0, Posit team) and R (R Core Team) evaluating the main effects of genotype, storage duration, harvest number and their two and three-way interactions. Significance was defined as $P < 0.05$ ($\alpha = 0.05$).

Table 3.6. Mixed effect model P-Values for all dependent variables of taste and flavor sensory intensity traits.

Independent Variable	**Dependent Variable**							
	Sweet Taste	Sour Taste	Astringent Flavor	Musty/Earthy Flavor	Malty Flavor	Honey Flavor	Green Flavor	Overripe Flavor
Genotype	<.0001	<.0001	0.096	0.3105	0.0778	0.3814	0.0996	0.9393
Storage Duration	0.0237	0.7628	0.696	0.9574	0.2018	0.9026	0.1805	0.4136
Harvest Number	0.0553	0.0015	0.2667	0.4085	0.091	0.0796	0.7265	0.124
Genotype × Storage Duration	0.0569	<.0001	0.0123	0.8457	0.1367	0.6517	0.0157	0.7786
Genotype × Harvest Number	0.0096	0.0085	0.2787	0.2772	0.0005	0.1325	0.7198	0.0847
Storage Duration × Harvest Number	0.0403	<.0001	0.0003	0.8614	0.3027	0.0966	0.7993	0.6572
Genotype × Storage Duration × Harvest Number	0.0015	<.0001	0.0004	0.16	0.03	0.0369	0.097	0.4841

P-values were obtained from mixed-model ANOVA performed in RStudio, (Version 2026.07.0, Posit team) and R (R Core Team) evaluating the main effects of genotype, storage duration, harvest number and their two and three-way interactions. Significance was defined as $P < 0.05$ ($\alpha = 0.05$).

Table 3.7. Mixed effect model *P*-Values for all dependent variables of textural sensory intensity traits.

	Dependent Variable		
Independent Variable	Firmness	Fibrous	Seedy
Genotype	0.0158	0.957	0.0006
Storage Duration	0.02	0.905	0.9659
Harvest Number	0.9583	0.8402	0.02
Genotype ×Storage Duration	0.0044	0.6365	0.0146
Genotype × Harvest Number	0.684	0.3923	0.0224
Timepoint × Harvest Number	0.7184	0.3596	0.0096
Genotype × Storage Duration × Harvest Number	0.0309	0.0781	0.0004

P-values were obtained from mixed-model ANOVA performed in RStudio, (Version 2026.07.0, Posit team) and R (R Core Team) evaluating the main effects of genotype, storage duration, harvest number and their two and three-way interactions. Significance was defined as $P < 0.05$ ($\alpha = 0.05$).

Table 3.8. Sensory panel ratings (mean ± SE) of aroma intensities (fruity, floral, sweet, green, and overripe) across six blueberry genotypes at harvest (0D) and after 14D of storage.

	Aroma Intensities									
	Fruity		Floral		Sweet		Green		Overripe	
Genotype	0D	14D	0D	14D	0D	14D	0D	14D	0D	14D
Brightwell	4.1 ± 0.5ab	3.6 ± 0.3ab	2.7 ± 0.5a	2.2 ± 0.4a	2.5 ± 0.2a	2.2 ± 0.2a	1.6 ± 0.3a	1.8 ± 0.2a	3.8 ± 0.4a	3.3 ± 0.5a
Vernon	3.3 ± 0.4b	3.4 ± 0.3ab	2.9 ± 0.5a	2.6 ± 0.4a	2.2 ± 0.2a	2.3 ± 0.2a	1.6 ± 0.3a	1.9 ± 0.4a	3.2 ± 0.5a	4.5 ± 0.6a
MS1110	5.0 ± 0.5a	4.5 ± 0.4ab	2.9 ± 0.4a	2.3 ± 0.4a	3.1 ± 0.3a	2.7 ± 0.3a	2.1 ± 0.4a	1.9 ± 0.4a	3.9 ± 0.5a	4.0 ± 0.6a
Legacy	4.7 ± 0.5ab	4.2 ± 0.4ab	3.0 ± 0.4a	3.0 ± 0.5a	2.1 ± 0.2a	2.5 ± 0.2a	2.2 ± 0.4a	1.3 ± 0.1a	2.7 ± 0.6a	3.1 ± 0.6a
Optimus	6.1 ± 0.4a	4.1 ± 0.4ab	4.5 ± 0.6a	2.6 ± 0.4a	3.3 ± 0.2a	2.1 ± 0.2a	2.8 ± 0.6a	2.5 ± 0.5a	4.6 ± 0.6a	2.7 ± 0.5a
UGA.RE.9*	2.9 ± 0.4	NA	2.8 ± 0.4	NA	1.6 ± 0.2	NA	3.1 ± 0.5	NA	2.6 ± 0.4	NA

*UGA.RE.9 was not analyzed alongside the rest of the 5 cultivars for mean separation due to an incomplete dataset. Data shows the mean values ± standard error and different letter groups indicate significant differences according to Tukey's Honest Significant difference test, α = 0.05.

Table 3.9. All taste and flavor intensities including sweet, sour, astringent, musty, malty, honey, green and overripe across all genotypes and timepoints reported from the trained descriptive panelists (n = 9).

	Taste Intensities				**Flavor Intensities**											
	Sweet		Sour		Astringent		Musty		Malty		Honey		Green		Overripe	
Genotype	0D	14D	0D	14D	0D	14D	0D	14D	0D	14D	0D	14D	0D	14D	0D	14D
'Brightwell'	4.6 ± 0.4a	3.7 ± 0.3ab	3.1 ± 0.4bc	3.3 ± 0.3bc	2.7 ± 0.3a	3.1 ± 0.4a	1.3 ± 0.2a	1.1 ± 0.2a	2.4 ± 0.4a	2.7 ± 0.4a	1.4 ± 0.3a	1.7 ± 0.4a	2.2 ± 0.3a	1.9 ± 0.3a	2.8 ± 0.5a	2.6 ± 0.4a
'Vernon'	2.9 ± 0.5ab	2.7 ± 0.2ab	3.6 ± 0.3bc	4.5 ± 0.5b	3.2 ± 0.5a	3.6 ± 0.5a	1.3 ± 0.3a	1.5 ± 0.2a	2.7 ± 0.5a	1.9 ± 0.4a	1.6 ± 0.3a	1.4 ± 0.4a	2.2 ± 0.4a	2.3 ± 0.4a	2.6 ± 0.5a	3.0 ± 0.4a
MS1110	3.4 ± 0.4ab	2.0 ± 0.4b	3.9 ± 0.6bc	7.3 ± 0.5a	2.9 ± 0.5a	3.8 ± 0.5a	1.3 ± 0.2a	1.2 ± 0.2a	2.3 ± 0.5a	1.4 ± 0.4a	1.5 ± 0.4a	1.2 ± 0.3a	2.2 ± 0.4a	2.6 ± 0.5a	2.7 ± 0.5a	2.6 ± 0.4a
'Legacy'	4.6 ± 0.5a	2.9 ± 0.3ab	4.5 ± 0.5b	3.2 ± 0.3bc	3.2 ± 0.5a	2.6 ± 0.5a	1.2 ± 0.3a	1.2 ± 0.2a	1.9 ± 0.4a	2.2 ± 0.5a	1.6 ± 0.3a	1.5 ± 0.3a	1.9 ± 0.3a	2.1 ± 0.4a	2.6 ± 0.6a	3.1 ± 0.5a
'Optimus'	3.5 ± 0.7ab	4.1 ± 0.4ab	5.3 ± 0.7ab	2.0 ± 0.2c	3.1 ± 0.5a	2.0 ± 0.4a	1.0 ± 0.2a	1.0 ± 0.2a	1.9 ± 0.3a	1.7 ± 0.4a	1.7 ± 0.4a	1.7 ± 0.3a	3.6 ± 0.5a	2.0 ± 0.4a	2.2 ± 0.5a	2.9 ± 0.4a
UGA.RE.9*	2.5 ± 0.2	NA	5.0 ± 0.5	NA	3.3 ± 0.3	NA	0.8 ± 0.3	NA	1.8 ± 0.3	NA	1.3 ± 0.4	NA	1.9 ± 0.3	NA	2.5 ± 0.5	NA

*UGA.RE.9 was not analyzed alongside the rest of the 5 cultivars for mean separation due to an incomplete dataset. Data shows the mean values ± standard error and different letter groups indicate significant differences according to Tukey's Honest Significant difference test, α = 0.05.

Table 3.10. All texture intensities across all genotypes and timepoints reported from the trained descriptive panelists (n = 9).

	Texture Traits					
	Firmness		Fibrousness		Seediness	
Genotype	0D	14D	0D	14D	0D	14D
Brightwell	2.5 ± 0.2a	2.4 ± 0.2a	4.8 ± 1.0a	4.4 ± 1.0a	5.1 ± 0.5a	4.6 ± 0.5a
Vernon	2.5 ± 0.2a	2.9 ± 0.2a	4.4 ± 1.0a	4.8 ± 0.9a	5.1 ± 0.7a	5.8 ± 0.4a
MS1110	2.4 ± 0.2a	3.3 ± 0.2a	4.6 ± 1.0a	5.0 ± 1.0a	4.4 ± 0.7a	6.1 ± 0.7a
Legacy	2.7 ± 0.2a	4.0 ± 0.3a	4.8 ± 1.0a	5.0 ± 1.0a	4.0 ± 0.4a	3.6 ± 0.5a
Optimus	3.3 ± 0.3a	2.8 ± 0.2a	4.9 ± 1.0a	4.6 ± 1.0a	5.0 ± 0.7a	3.8 ± 0.5a
UGA.RE.9*	2.8 ± 0.2	NA	4.7 ± 1.0	NA	7.0 ± 0.7	NA

*UGA.RE.9 was not analyzed alongside the rest of the 5 cultivars for mean separation due to an incomplete dataset. Data shows the mean values ± standard error and different letter groups indicate significant differences according to Tukey's Honest Significant Difference test, α = 0.05.

Table 3.11. Taste, flavor and texture attributes by harvest number and storage duration among the six blueberry genotypes.

	Dependent Variable							
	Sweet Taste		Sour Taste		Astringent Taste		Seedy Texture	
	0D	14D	0D	14D	0D	14D	0D	14D
Harvest 1	3.91 ± 0.25	3.12 ± 0.27	3.57 ± 0.29	4.23 ± 0.43	2.73 ± 0.26	3.33 ± 0.32	4.56 ± 0.39	4.86 ± 0.37
Harvest 2	3.11 ± 0.41	3.20 ± 0.29	5.06 ± 0.46	4.17 ± 0.38	3.65 ± 0.37	2.84 ± 0.32	5.78 ± 0.47	5.07 ± 0.46

Data shows the mean sensory intensity values ± standard error.

3.7 Figures

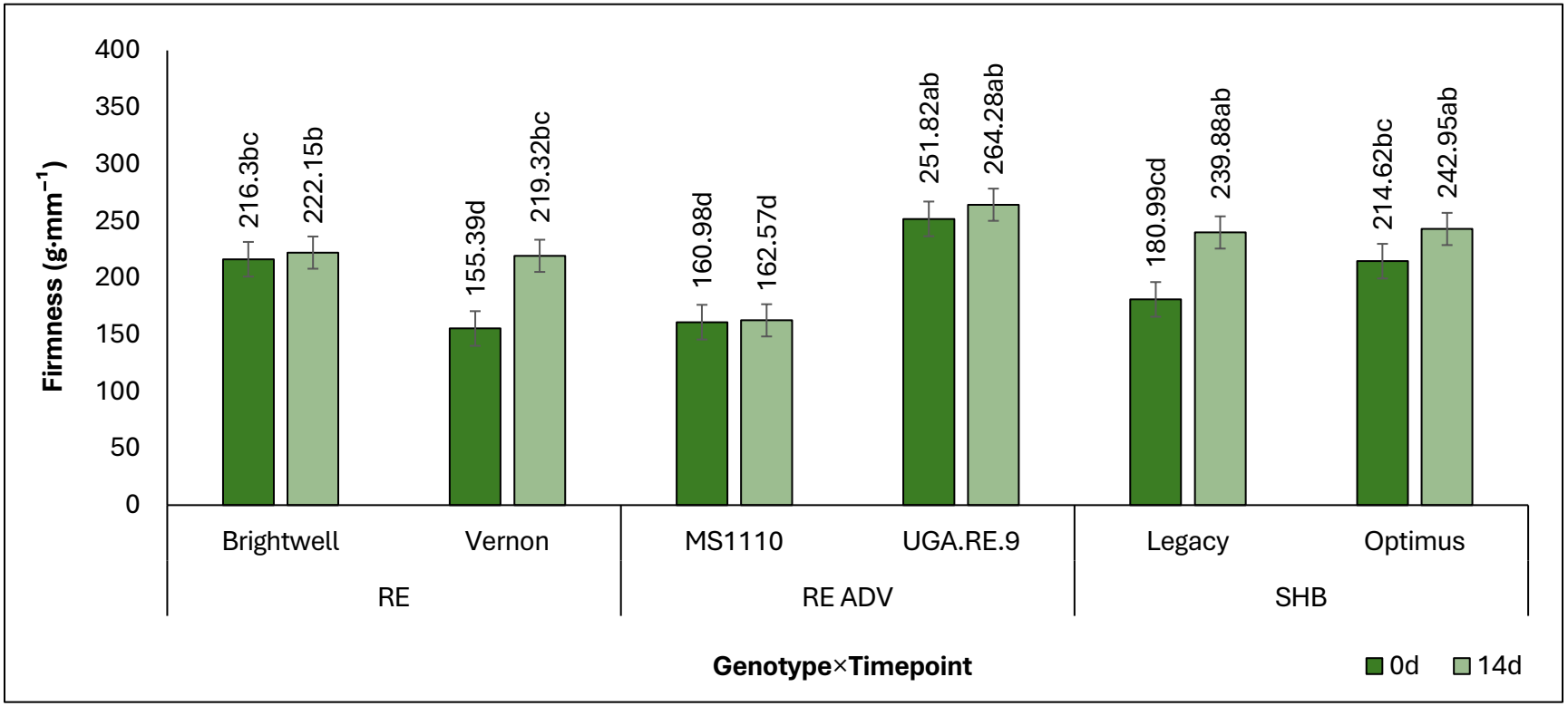

Figure 3.1. Instrumental firmness (g·mm^{-1}) of six blueberry genotypes at harvest (0D) and after 14D of storage. Error bars represent ± standard error of the mean. Bars represent mean values, and different letters indicate significant differences among genotype × timepoint combinations according to Tukey's honest significant difference (HSD) test (α = 0.05).

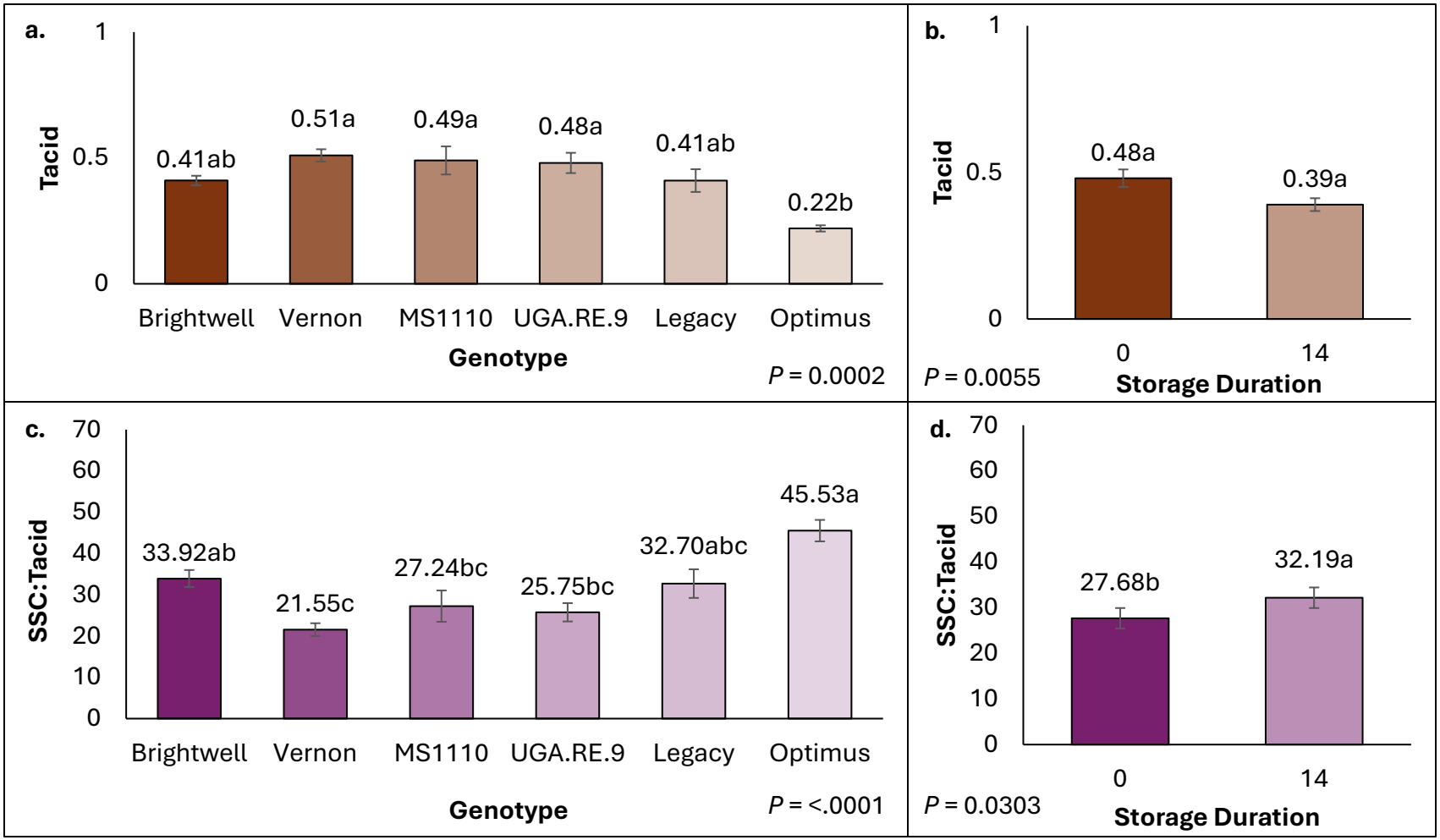

Figure 3.2. Titratable acidity (Tacid) for genotype (a) and across storage duration (b), and soluble solids content to titratable acidity ratio (SSC:Tacid) measured across genotype (c) and mean SSC:Tacid across storage duration (d). Error bars represent ± standard error of the mean. Data labels show the mean values ± standard error, and different letters indicate significant differences according to Tukey's Honest Significant difference test, α = 0.05, for genotype. Different letters indicating significant differences according to Students T Test for storage duration, α = 0.05.

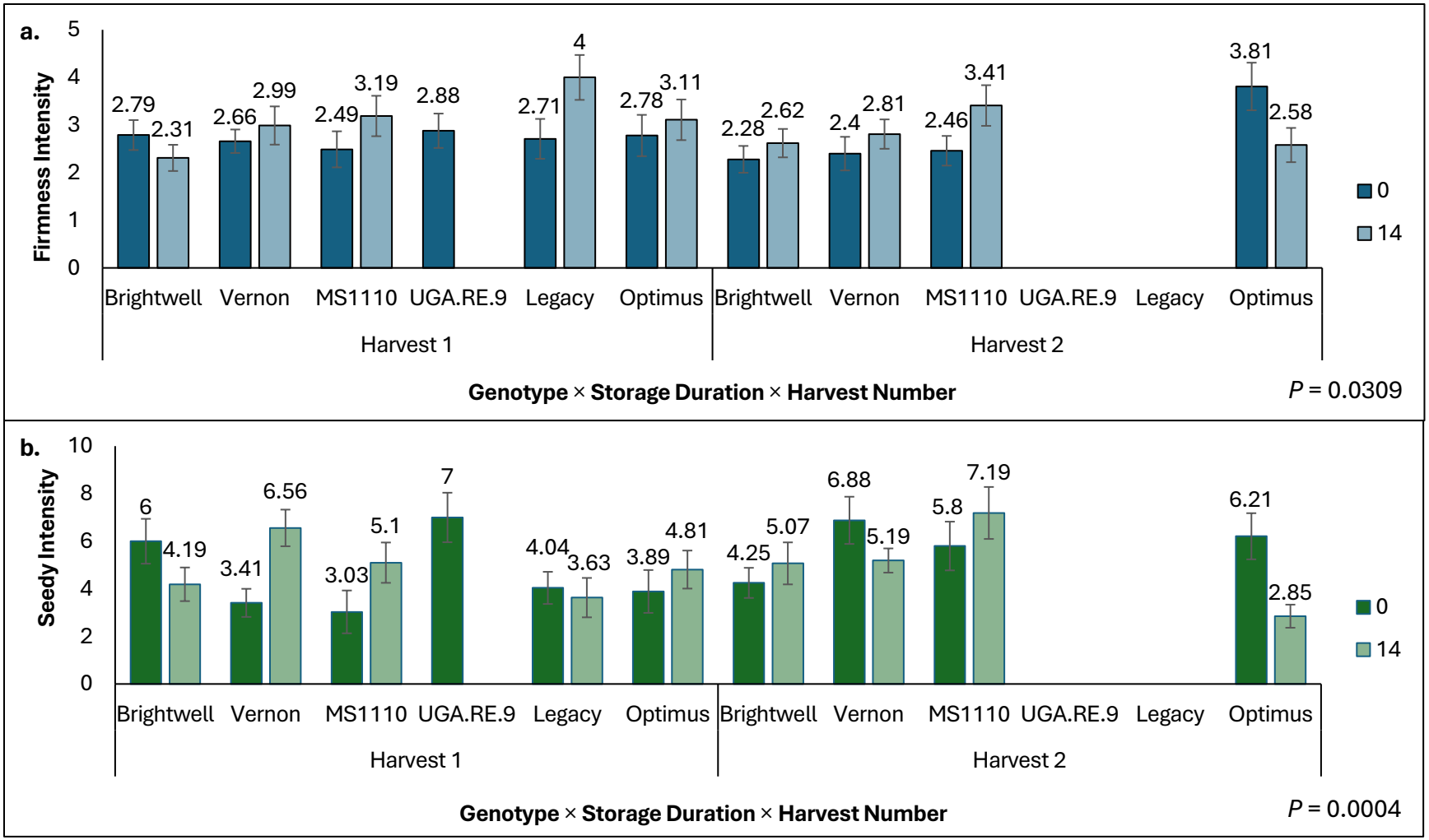

Figure 3.3. Firmness intensity for genotype × storage duration × harvest number (a) and seedy intensity measured across genotype × storage duration × harvest number (b). Error bars represent ± standard error of the mean. Data labels show the mean values, and different letters indicate significant differences according to Tukey's Honest Significant difference test, α = 0.05.

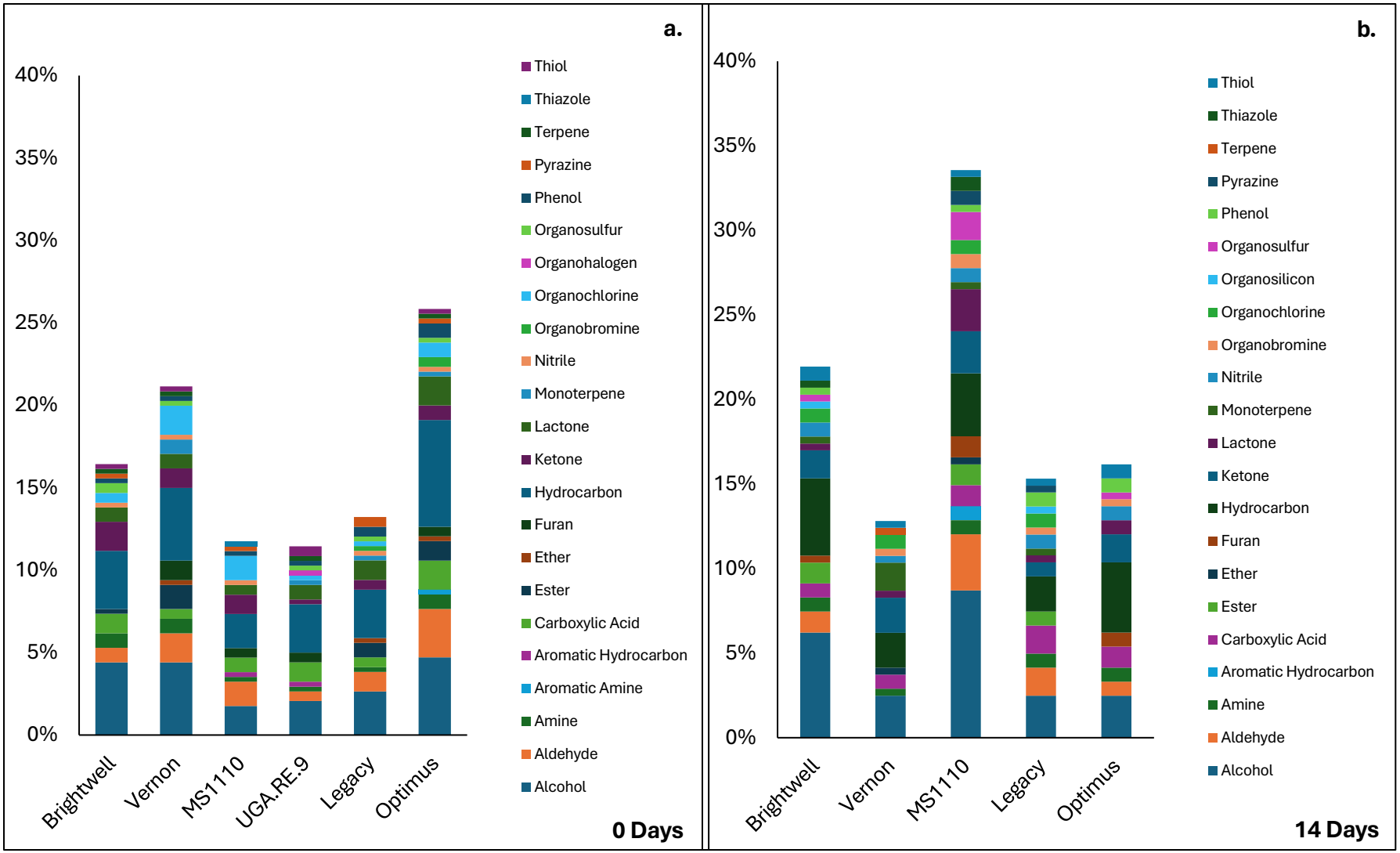

Figure 3.4. Stacked bar graphs showing the distribution of relative abudnaces (%) of volatile chemical classes in all genotypes at 0 Days (harvest) (a) and after 14 Days (b) of storage. Volatile profiles were characterized using the Heracles NEO electronic-nose. UGA.RE.9 was excluded from the 14D analysis because insufficient fruit material was available during the 2025 season.

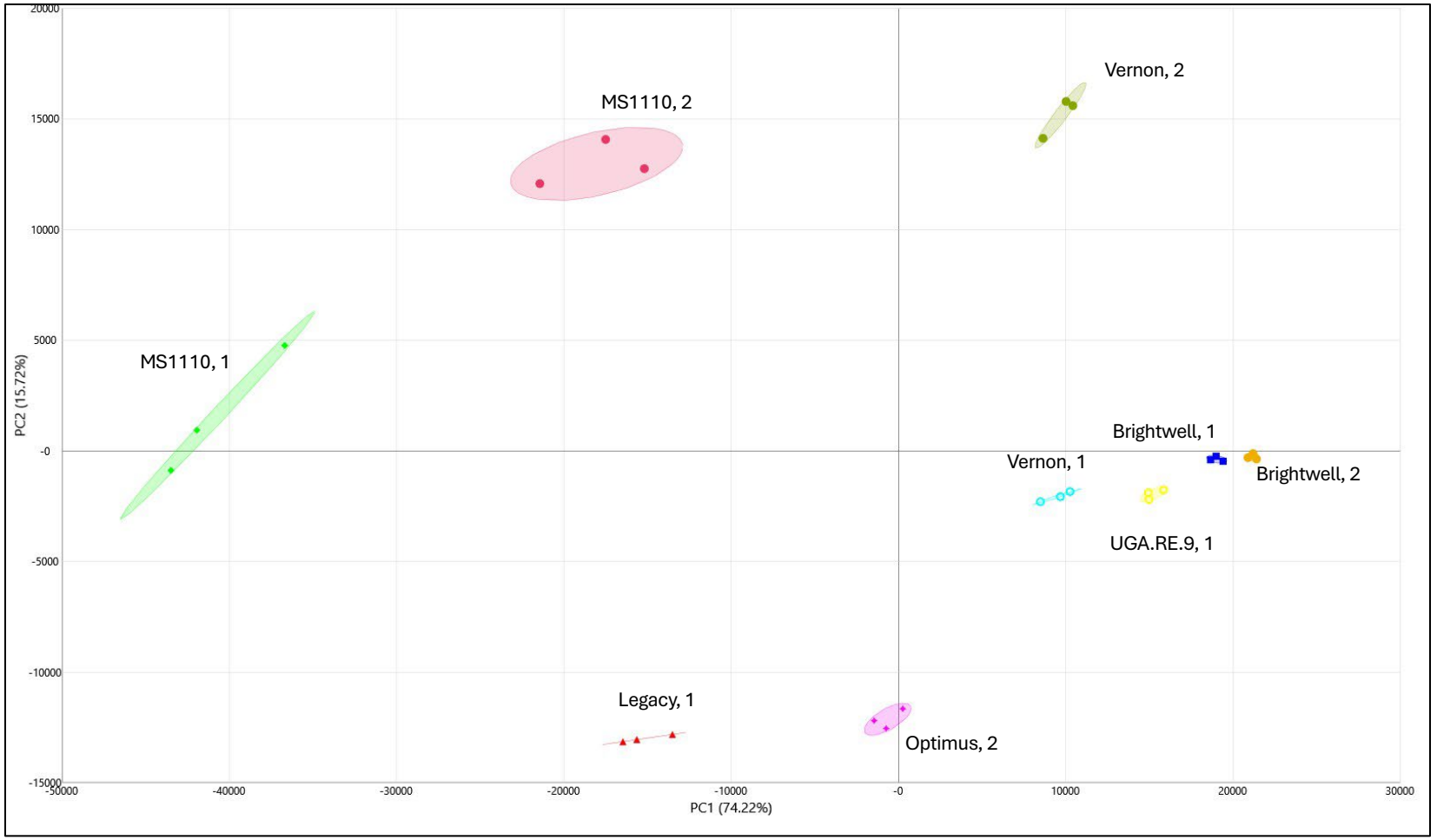

Figure 3.5. Principal component analysis (PCA) of volatile profiles of blueberry genotypes at harvest (0D) generated using the AlphaSoft multivariate analysis function from Heracles NEO electronic-nose data. Samples represent two harvests conducted 7 days apart. The numeral following each genotype designation indicates harvest number (1 = Harvest 1; 2 = Harvest 2). Principal component 1 (PC1) and principal component 2 (PC2) explained 74.22% and 15.72% of the total variation, respectively.

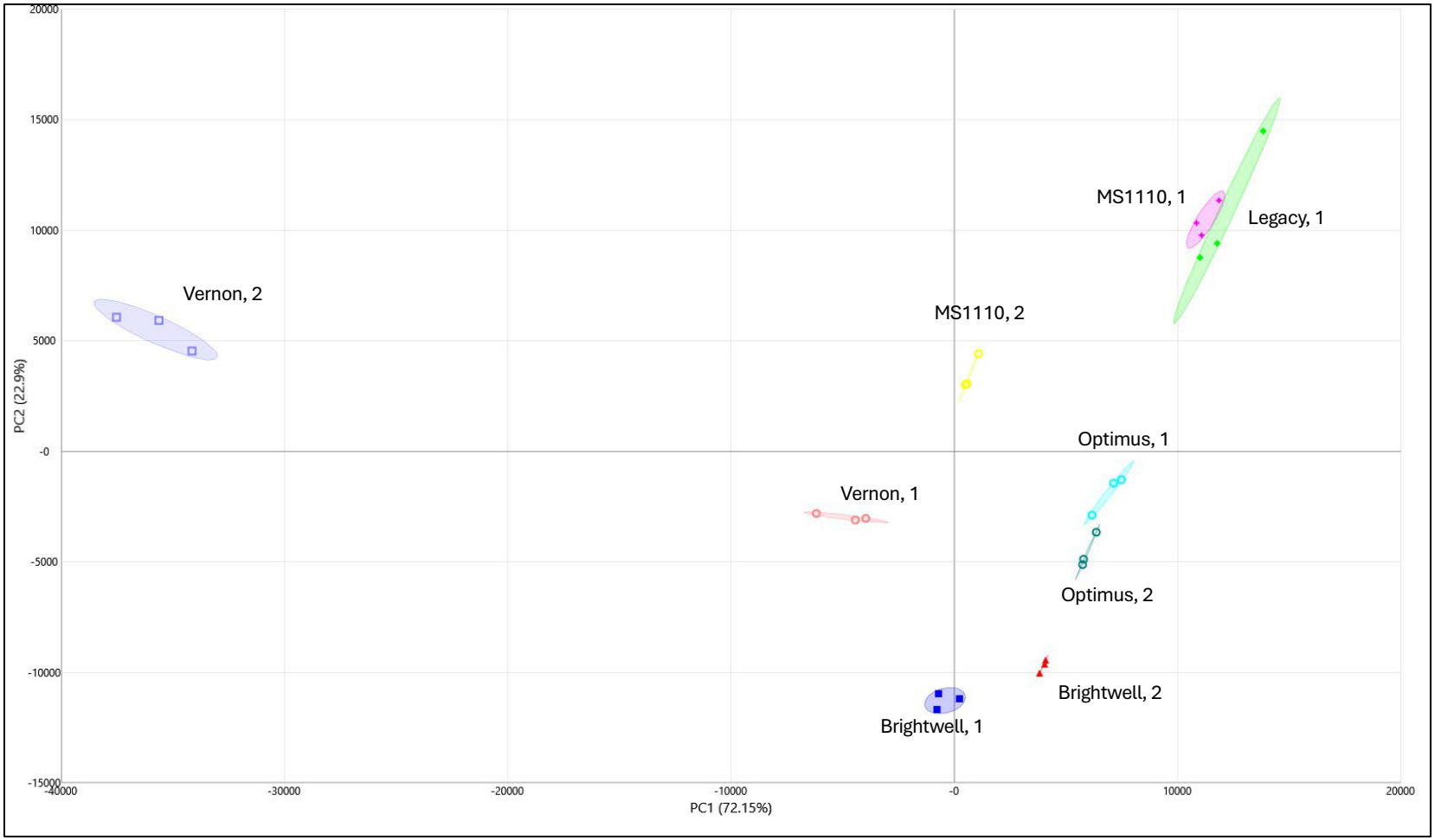

Figure 3.6. Principal component analysis (PCA) of volatile profiles of blueberry genotypes after 14 days of cold storage generated using the AlphaSoft multivariate analysis function from Heracles NEO electronic-nose data. Samples represent two harvests conducted 7 days apart. The numeral following each genotype designation indicates harvest number (1 = Harvest 1; 2 = Harvest 2). Principal component 1 (PC1) and principal component 2 (PC2) explained 74.22% and 15.72% of the total variation, respectively.