Evaluating Impact of Harvest Date on Flavor and Volatile Attributes of Fresh-market Blackberries

Production Research and Special Needs Research

Principal Investigator:
Dr. Renee Threlfall, Research Scientist, Department of Food Science, University of Arkansas System Division of Agriculture, 2650 N. Young Ave., Fayetteville, AR 72704, Phone: 479-575-4677, Fax: 479-575-6936, rthrelf@uark.edu
Co-Investigators:
Dr. Margaret Worthington, Assistant Professor, Department of Horticulture, 316 Plant Science Building, University of Arkansas System Division of Agriculture, Fayetteville, AR 72701, Phone: 479-575-2122, Fax: 479-575-8619, mlworthi@uark.edu
Dr. John R. Clark, Distinguished Professor, Department of Horticulture, University of Arkansas System Division of Agriculture, 316 Plant Science Building, Fayetteville, AR 72701, Phone: 479-575-2810, Fax: 479-575-8619, jrclark@uark.edu.
Dr. Luke R. Howard, Professor, Department of Food Science, University of Arkansas System Division of Agriculture, 2650 N. Young Ave., Fayetteville, AR 72704, Phone: 479-575-2978, Fax: 479-575-6936, luke@uark.edu

Introduction to the Problem

Blackberry (*Rubus* subgenus *Rubus* Watson) plants are grown domestically and internationally with fresh-market blackberries harvested by hand and sold directly to the consumers. Fresh-market blackberry production in the Southeast has been a growing part of the United States market for the past decade (Fernandez et al., 2016). The University of Arkansas System Division of Agriculture (UA System) Blackberry Breeding Program located at the UA System Fruit Research Station, Clarksville, AR, has developed and patented 43 fresh-market blackberry cultivars. ‘Ouachita’ and ‘Osage’ are two of the most widely-grown cultivars released from the UA System (*personal communications, Dr. John Clark*). The most recent cultivars from the UA System are ‘Sweet-Ark® Caddo’ (Clark et al., 2019) released in 2018, ‘Sweet-Ark® Ponca’ released in 2019, and ‘Prime-Ark® Horizon’ released in 2020.

The blackberry season varies depending on location and cultivar, but fresh-market blackberries are typically harvested weekly for 3-4 weeks from plants as the fruit ripens. Previous studies have shown a relationship between harvest date and quality attributes but was dependent on cultivar (Cavender et al., 2019; Jacques et al., 2014). The aroma, size, flavor, and texture of blackberries also varies by cultivar. Although the basic tastes (sweetness, sourness, and bitterness) impact the flavor of blackberries, volatile aroma compounds (substances in fruit which vaporize at ambient temperature) are also responsible for aromas (smell) and aromatic (in the mouth) flavors of blackberries. Sugars (mostly glucose and fructose) are the major soluble solids in blackberries that impact the sweetness, and the acids, primarily citric, impact sourness. The composition of commercially acceptable fresh-market blackberry ranges from 8-11% soluble solids, 3.0-3.6 pH, and 0.7-1.4% titratable acidity (Threlfall et al., 2016). In addition, balance of sugars and acids are important attributes for fresh-market blackberries, especially to target consumer markets.

Volatile compounds also impact consumer perceptions and preferences of blackberry flavor. Unlike the basic tastes which are perceived by taste receptors on the tongue, volatiles are perceived through smell detected by olfactory receptors in the nose and mouth (Klee and Tieman, 2018). The olfactory system and odor thresholds of individuals vary, making olfactory perception a difficult trait to quantify (Hasin-Brumshtein et al., 2009). While many different volatiles affect blackberry flavor, specific compounds that drive consumer preferences vary (Klee and Tieman, 2018). Volatile compounds in blackberries include acids, esters, alcohols, aldehydes, ketones, lactones, and terpenoids. Early studies focused on the volatile constituents of blackberries and blackberry products with compounds, such as 2-heptanol, p-cymen-8-ol, 2-heptanone, 1-hexanol, a-terpineol, pulegone, 1-octanol, isoborneol, myrtenol, 4-terpineol, carvone, elemicine, and nonanal identified as major volatiles (Georgilopoulos and Gallois, 1987; Gulan et al., 1973; Scanlan et al., 1970). Blackberry aroma profiles are diverse, with different genotypes having unique aroma profiles. Jacques et al. (2014) identified 45 volatile compounds in ‘Tupy’, the predominant cultivar available commercially. The majority of volatiles in blackberries were comprised of terpenoids with limonene as the predominate compound (Du et al., 2010a and 2010b).

Wang et al. (2005) examined volatiles in ‘Chickasaw’ blackberries, a UA System cultivar, grown in Oregon and Arkansas and found that the flavor and aroma were strongly influenced by growing environment. The berries grown in Oregon had cut grass, green, fruity, citrus, and watermelon aromas, while the Arkansas berries had cinnamon, piney, floral, sweet, and caramel aromas. The most potent aroma compounds in Oregon-grown ‘Chickasaw’ were ethyl butanoate (fruity, apple-like), linalool (floral, perfume), methional (cooked potato), trans,cis2,6-nonadienal (green, cucumber), cis-1,5-octadien-3-one (green, grass), and 2,5-dimethyl-4-hydroxy-3(2H)-furanone (sweet, strawberry-like), while in Arkansas-grown ‘Chickasaw’ were ethyl butanoate, linalool, methional, ethyl 2-methylbutanoate (fruity), beta-damascenone (rose-like, berry), and geraniol (sweet, rose-like). In a similar study, Du et al. (2010a) investigated eight Oregon-grown blackberries and found a range of compounds, such as esters, terpenoids, aldehydes, ketones, alcohols, norisoprenoids, lactones, acids, and furanones.

Flavor dilution is the ratio of the concentration of the odorant in an initial extract to the concentration in the most dilute extract, but the odor is still detachable by Gas Chromatography-Olfactory analysis. The compounds with the most impactful aromas found by Wang et al. (2005) in Arkansas-grown ‘Chickasaw’ determined by their were ethyl butanoate, linalool, methional, ethyl 2-methylbutanoate, β-damascenone, geraniol, allo-ocimene, trans-2-hexenal, and 2,5-dimethyl-4-hydroxy-3(2H)-furanone; all with a flavor dilution = 512. Whereas, the odor activity value (OAV) estimates odor potency as a ratio of the volatile concentration to its odor detection threshold (Patton, 1957). Du et al., 2010a calculated OAVs and found furaneol, linalool, βionone, 2-heptanol, and carvone that contributed to the major aroma compounds in blackberries grown in the Pacific Northwest. In contrast to Wang et al. (2005), methional, β-damascenone, allo-ocimene, ethyl 2-methylbutanoate, and 2,5-dimethyl-4-hydroxy-3(2H)-furanone were not detected in Arkansas-grown blackberries (Meyers, 2021; Morin 2021; Morin et al., 2022).

Consumers want a fresh-market blackberry that is uniform in color, fresh, has a good shelf life, fair-priced, rich in nutraceuticals, and has unique flavors and aromas (Threlfall et al., 2020, 2021). A study conducted on blueberries showed lipid-derived volatiles explained 15% of overall liking scores in a sensory panel, and the carotenoid/terpene compound group explained 21% of the overall liking score (Colantonio et al., 2020). Threlfall et al. (2016) developed a fresh-market blackberry lexicon for descriptive sensory analysis to evaluate the appearance, aroma, basic tastes, aromatics, and feeling factors of UA System blackberries. Gilbert et al. (2015) identified breeding priorities for blueberry flavor using biochemical, sensory, and genotype by environment analyses and found many of the compounds affecting flavor including β-caryophyllene oxide and 2-heptanone were genetically controlled.

There is a critical need to determine the key volatile attributes that that impact the aroma and flavor of blackberries and can be used by blackberry breeders to help southern U.S. growers market blackberries. Since the UA System Blackberry Breeding Program contributes to the global blackberry industry, the objectives of the research were to evaluate the impact of harvest date on size, composition, and volatiles of Arkansas fresh-market blackberries.

Description of Experimental Design

For berry weight, composition, and volatiles, three cultivars (Natchez, Prime-Ark® Traveler, and Sweet-Ark® Ponca) and three harvest dates (early, middle, and late) were evaluated in triplicate in 2021.

Results

Berry weight attributes

The cultivar x harvest date interaction was not significant for berry weight (Table 1). Cultivar impacted berry weight with 'Natchez' (13.02 g) higher than 'Sweet-Ark® Ponca' (5.89 g) and 'Prime-Ark® Traveler' (5.39 g). Harvest date did not impact berry weight with an average berry weight of 8.1 g.

Composition attributes

The cultivar x harvest date interaction was significant for soluble solids, titratable acidity, and soluble solids/titratable acidity ratio (Table 1 and Fig. 1). Cultivar impacted the pH but not the harvest date. 'Sweet-Ark® Ponca' (3.71) had a higher pH than Natchez (3.35) and 'Prime-Ark® Traveler' (3.48) (Table 1). 'Sweet-Ark® Ponca' early harvest date (14.63%) had the highest soluble solids, and 'Prime-Ark® Traveler' late harvest date (9.33%) had the lowest (Fig. 1). Harvest date impacted the soluble solids of 'Natchez' and 'Sweet-Ark® Ponca' with the early harvest date higher in soluble solids than the late harvest date. 'Natchez' late harvest date had the highest titratable acidity and lowest soluble solids/titratable acidity ratio (1.14% and 8.83, respectively), and 'Prime-Ark® Traveler' middle harvest date had the lowest titratable acidity and highest soluble solids/titratable acidity ratio (0.44% and 24.03, respectively). Harvest date did not impact titratable acidity or soluble solids/titratable acidity ratio within any cultivar.

Volatile aroma attributes

In the three cultivars harvested on three dates, there were 139 volatile aroma compounds identified across 9 compound classes including 31 monoterpenes, 23 esters 23 alcohols, 23 aldehydes, 17 sesquiterpenes, 7 acids, 7 ketones, 5 aromatic hydrocarbons, 3 lactones (data not shown). Compound categories included chemical, floral, fruity, green/fat, roasted/caramelized, vegetal alcohols, floral, green/fat, vegetal, and roasted/caramelized aldehydes, fruity and vegetal aromatic hydrocarbons, fruity esters, vegetal and fruity ketones, vegetal, fruity, floral, green/fat monoterpenes, and green/fat, and fruity sesquiterpenes. 'Sweet-Ark® Ponca' Late harvest date had the lowest cumulative concentration of volatile compounds (1,369.27 µg/kg), and 'Prime-Ark® Traveler' middle harvest date had the highest (3,665.85 µg/kg).

Figure 2 shows the total volatile concentration for each cultivar and harvest date with different compound categories. For each cultivar, the middle harvest date had a higher overall volatile level than did the early harvest date. The four volatile aroma compounds with the highest levels found in Arkansas-grown blackberries were 2-butanol (alcohol with fruity apricot aromas), ethyl acetate (ester with ethereal grape-like notes), 2-hexen-1-ol (alcohol with fruity, green and banana aromas), and methyl octanoate (ester with waxy orange and vegetable notes) (Table 2).

The impactful volatile aroma compounds with the highest concentrations in 2021 were geraniol (0 - 107.47 µg/kg), a fruity monoterpene, and linalool (2.11 - 170.16 µg/kg), a floral monoterpene (Fig. 3). The next highest impactful compounds were 2-hexenal (1.28- 145.13 µg/kg), an aldehyde with fruity aromas, and ethyl 2-methylbutanoate, an ester with fruity aromas.

When a principle component analysis (PCA) was conducted on the compound class variables in 2021 (Fig. 4), two components explained (63.3%) of the variation in the data. PC1 (35.6%) had positive loadings for aromatic hydrocarbons, esters, aldehydes, alcohols, sesquiterpenes, monoterpenes, lactones, and acids. Cultivar/harvest date combinations positively loaded for PC1 included 'Sweet-Ark® Ponca' middle, 'Prime-Ark® Traveler' early, 'Natchez' late, 'Natchez' middle, and 'Prime-Ark® Traveler' middle. Ketones loaded negatively on PC1 along with cultivar/harvest date combinations 'Prime-Ark® Traveler' late, 'Natchez' early, ‘Sweet-Ark® Ponca’ early, and ‘Sweet-Ark® Ponca’ late. PC2 (27.7%) had positive loadings for sesquiterpenes, alcohols, acids, monoterpenes, ketones, and aromatic hydrocarbons. Cultivars/harvest date positively loaded for PC2 included ‘Natchez’ late, ‘Natchez’ middle, and ‘Natchez’ early. Aldehydes, lactones, and esters were all negatively loaded for PC2. Cultivars/harvest date negatively loaded for PC2 included ‘Sweet-Ark® Ponca’ middle and ‘Sweet-Ark® Ponca’ late. In addition, berry weight was positively correlated with aromatic hydrocarbons (r^2= 0.63, $p=0.0006$), and pH was positively correlated with esters (r^2= 0.69, $p=0.0411$).

Conclusion

The size, composition, and volatile attributes of Arkansas-grown fresh-market blackberries were evaluated. Three cultivars were harvested from UA System Fruit Research Station in Clarksville, AR on three harvest dates in 2021. The size of the blackberry was not impacted by harvest date. Harvest date did not impact titratable acidity or soluble solids/titratable acidity ratio within any cultivar but did impact soluble solids with ‘Natchez’ and ‘Sweet-Ark® Ponca’ early harvest date higher in soluble solids than the late harvest date. There were 139 volatiles identified in Arkansas-grown blackberry cultivars, mainly monoterpenes, esters, aldehydes, and alcohols. ‘Prime-Ark® Traveler’ (3,666.48 µg/kg) middle harvest date had highest total volatiles. Six impactful volatiles were identified in Arkansas-grown fresh-market blackberries including ethyl butanoate, linalool, ethyl 2-methylbutanoate, geraniol, allo-ocimene, and trans-2-hexenal. Generally, principal component analysis showed clustering around both harvest dates and cultivar, and the two primary components covered 63.3%. Berry weight was positively correlated with aromatic hydrocarbons, grassy-vegetal aromas. The combination of size, composition, and volatile attribute information can be a useful tool to steer breeding decisions, help southern U.S. growers market blackberries better, and determine commercial potential of Arkansas-grown, fresh-market blackberries.

Literature Cited

Cavender, G., M. Liu, J. Fernandez-Salvador, D. Hobbs, B. Strik, B. Frei, Y. Zhao, and M.D.M Contreras Gamez. 2019. Effect of different commercial fertilizers, harvest date, and storage time on two organically grown blackberry cultivars: Physicochemical properties, antioxidant properties, and sugar profiles. J. Food Qual. 2019:1-17. https://doi.org/10.1155/2019/1390358

Clark, J.R., M. Worthington, and T. Ernst. 2019. 'Caddo' thornless blackberry. HortScience 54:1632-1636. https://doi.org/10.21273/HORTSCI14119-19

Colantonio, V., L.V. Felipe Ferrão, D. Tieman, N. Bliznyuk, C. Sims, H. Klee, P.R. Munoz, and M.F.R. Resende. 2020. Metabolomic selection for enhanced fruit flavor. BioRxiv. https://www.biorxiv.org/content/10.1101/2020.09.17.302802v1

Du, X., C.E. Finn, and M.C. Qian. 2010a. Volatile composition and odour-activity value of thornless 'Black Diamond' and 'Marion' Blackberries. Food Chemistry. 119:1127–1134.

Du, X., A. Kurnianta, M. Mcdaniel, C. Finn, and M. Qian. 2010b. Flavour profiling of 'Marion' and thornless blackberries by instrumental and sensory analysis. Food Chemistry 121:1080–1088, https://doi.org/10.1016/j.foodchem.2010.01.053

Fernandez, G.E., E. Garcia, and D. Lockwood. 2016. Introduction. Fact-sheet, NC State Extension. https://content.ces.ncsu.edu/southeast-regional-caneberry-production-guide/introduction-1

Georgilopoulos, D.N., and A.N. Gallois. 1987. Volatile flavour compounds in heated blackberry juices. Zeitschrift für Lebensmittel-Untersuchung und -Forschung 185:299-306. https://doi.org/10.1007/BF01123035

Gilbert, J.F., M.J. Guthart, S.A. Gezan, M.P. de Carvalho, M.L. Schwieterman, T.A. Colquhoun, L.M. Bartoshuk, C.A. Sims, D.G. Clark, and J.W. Olmstead. 2015. Identifying breeding priorities for blueberry flavor using biochemical, sensory, and genotype by environment analyses. PLOSOne. 1-17. https://doi.org/10.1371/journal.pone.0138494

Gulan, M.P., M.H. Veek, R.A. Scanlan, and L.M. Libbey. 1973. Compounds identified in commercial blackberry essence. J. Agricult. Food Chem. 21:741-41. https://doi.org/10.1021/jf60188a016

Hasin-Brumshtein, Y., D. Lancet, and T. Olender. 2009. Human olfaction: from genomic variation to phenotypic diversity. Trends Genet. 25:178–184.

Jacques, A.C., F.C. Chaves, R.C Zambiazi, M.C. Brasil, and E.B. Caramão. 2014. Bioactive and volatile organic compounds in Southern Brazilian blackberry (*Rubus Fruticosus*) fruit cv. Tupy. Food Sci. Technol. (Campinas) 34:636-643. https://doi.org/10.1590/1678-457x.6243

Klee, H.J., and D.M. Tieman. 2018. The genetics of fruit flavour preferences. Nat. Rev. Genet. 19:347–356.

Meyers, A.L. 2022. Arkansas Fresh-market Blackberries: Identifying Unique Attributes and Harvest Practices that Impact Marketability. Thesis, University of Arkansas

Morin, P. 2020. Anti-inflammatory Properties of Blackberry Phenolic and Volatile Compounds. Masters' thesis. University of Arkansas, USA.

Morin, P., L.R. Howard, J. Tipton, C. Brownmiller, A. Mauromoustakos, R.T. Threlfall, J.R. Clark, and M. Worthington. 2022. Phenolics and volatiles in Arkansas Fresh-market blackberries (*Rubus* subgenus *Rubus* Watson). Food Sci. Tech. https://pubs.acs.org/doi/10.1021/acsfoodscitech.2c00219

Patton S, and D. Josephson. 1957. A method for determining significance of volatile flavor compounds in foods. Food Res 22(3):316–8

Scanlan, R.A., D.D. Bills, and L.M. Libbey, 1970. Blackberry Flavor Components of Commercial Essence. J. Agr. Food Chem. 18:744-744. https://doi.org/10.1021/jf60170a049

Threlfall, R.T., J.R. Clark, A.N. Dunteman, and M.L. Worthington. 2021. Identifying marketable attributes of fresh-market blackberries through consumer sensory evaluations. HortScience 56:30-35. https://doi.org/10.21273/HORTSCI15483-20

Threlfall, R.T., A.N. Dunteman, J.R. Clark, and M.L. Worthington. 2020. Using an online survey to determine consumer perceptions of fresh-market blackberries. Acta Horticulturae 1277:469-476. https://doi.org/10.17660/ActaHortic.2020.1277.67

Threlfall, R.T., O.S. Hines, J.R Clark, L.R. Howard, C.R. Brownmiller, D.M Segantini, and L.J.R. Lawless, 2016. Physiochemical and sensory attributes of fresh blackberries grown in the southeastern United States. HortScience 51:1351-1362. https://doi.org/10.21273/HORTSCI10678-16

Wang, Y., C. Finn, and M.C. Qian. 2005. Impact of growing environment on Chickasaw blackberry (Rubus L) aroma evaluated by gas chromatography olfactometry dilution analysis. J. Agricult. Food Chem. 53:3653-3571. https://doi.org/10.1021/jf048102m

Table 1. Main effect and interactions on berry weight and composition of fresh-market blackberry cultivars grown at the University of Arkansas System Division of Agriculture Fruit Research Station and harvested on three dates, Clarksville, AR (2021).

Effect [z]	**Berry weight (g)**	**Soluble solids (%)**	**pH**	**Titratable acidity (%)**[y]	**Soluble solids/titratable acidity ratio**
Cultivar					
Natchez	13.02 a	9.79 b	3.35 b	1.01 a	10.92 b
Prime-Ark® Traveler	5.39 b	12.72 a	3.48 b	0.78 b	19.37 a
Sweet-Ark® Ponca	5.89 b	10.84 c	3.71 a	0.56 c	17.22 a
P-value	*<0.0001*	*<0.0001*	*0.0004*	*<0.0001*	*0.0006*
Harvest date					
Early	8.27 a	12.05 a	3.54 a	0.80 ab	15.86 ab
Middle	8.26 a	11.27 b	3.58 a	0.68 b	18.24 a
Late	7.77 a	10.03 c	3.42 a	0.86 a	13.40 b
P-value	*0.3453*	*<0.0001*	*0.0997*	*0.0070*	*0.0499*
Cultivar x Harvest date (*P-value)*	*0.1316*	*<0.0001*	*0.1910*	*0.0076*	*0.0170*

[z] Cultivars and harvest dates were evaluated in triplicate. Means with different letters for each attribute are significantly different ($p<0.05$) using Tukey's Honestly Significant Difference test.
[y] Titratable acidity expressed as % citric acid.

Table 2. Four volatile aroma compounds (µg/kg) with the highest levels in fresh-market blackberry cultivars harvested on three dates from the University of Arkansas System Division of Agriculture Fruit Research Station, Clarksville, AR (2021)

	Compound			
	2-Butanol	Ethyl acetate	2-Hexen-1-ol	Methyl octanoate
	Compound class			
	Alcohol	Ester	Alcohol	Ester
	Aroma category			
	Fruity	Ethereal	Fruity	Waxy
	Aroma descriptors			
Cultivar [z]	Sweet apricot	Ethereal, fruity, sweet, grape and rum-like	Sharp, green, leafy, fruity, unripe banana	Waxy, green, orange, vegetable, herbal
Natchez				
Early	312.31±103.18	236.64±87.28	13.12±1.80	110.89±4.31
Middle	305.22±17.30	71.02±38.87	23.52±12.99	190.14±34.23
Late	759.72±353.72	3.58±3.04	274.08±102.06	156.95±5.17
Prime-Ark® Traveler				
Early	77.96±81.14	454.84±172.83	270.21±28.63	103.65±42.49
Middle	308.15±48.78	201.92±137.28	104.33±44.71	195.43±41.94
Late	370.8±94.06	477.42±55.26	126.46±85.83	150.25±11.09
Sweet-Ark® Ponca				
Early	98.58±22.14	161.92±49.96	256.9±49.07	91.67±19.01
Middle	90.36±12.55	156.71±44.55	179.26±59.01	48.97±5.69
Late	66.77±9.07	102.03±53.81	50.16±5.16	90.51±2.23

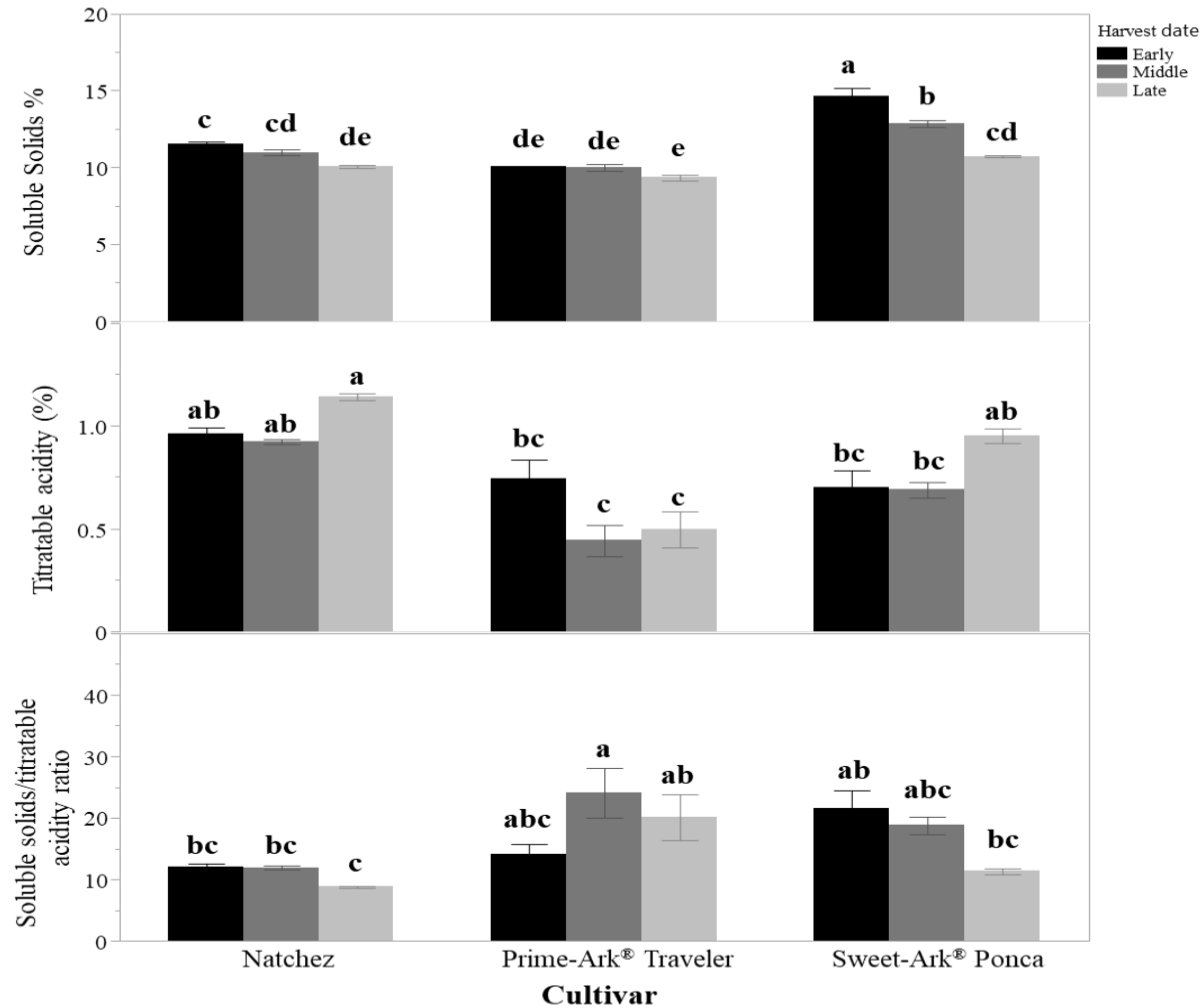

Fig. 1. Interaction of cultivar x harvest date on soluble solids, titratable acidity, and soluble solids/titratable acidity ratio of fresh-market blackberries harvested from the University of Arkansas System Division of Agriculture Fruit Research Station, Clarksville, AR (2021)

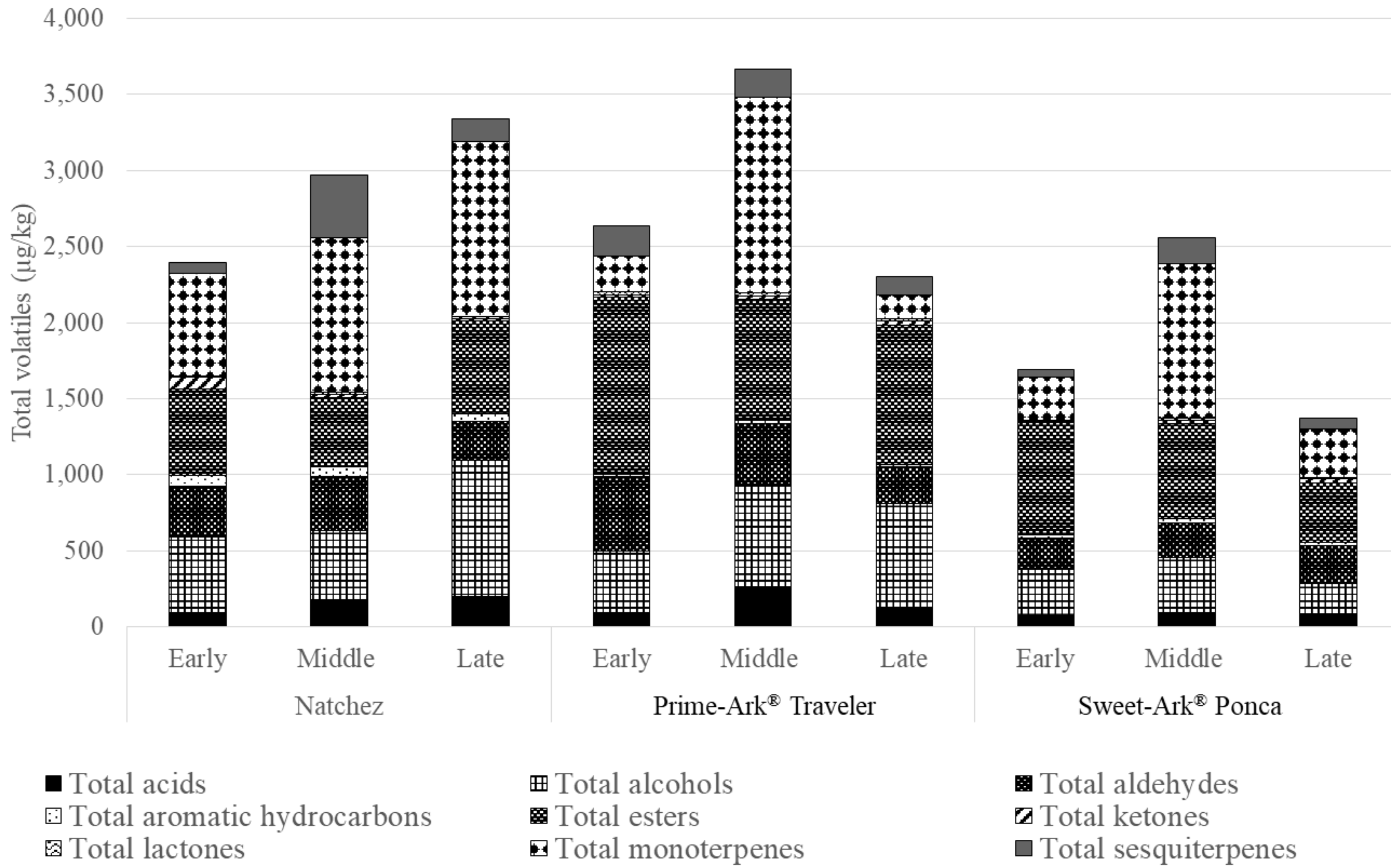

Fig. 2. Total concentrations of volatile aroma compounds identified in fresh-market blackberry cultivars grown at the University of Arkansas System Division of Agriculture Fruit Research Station and harvested on three dates, Clarksville, AR (2021).

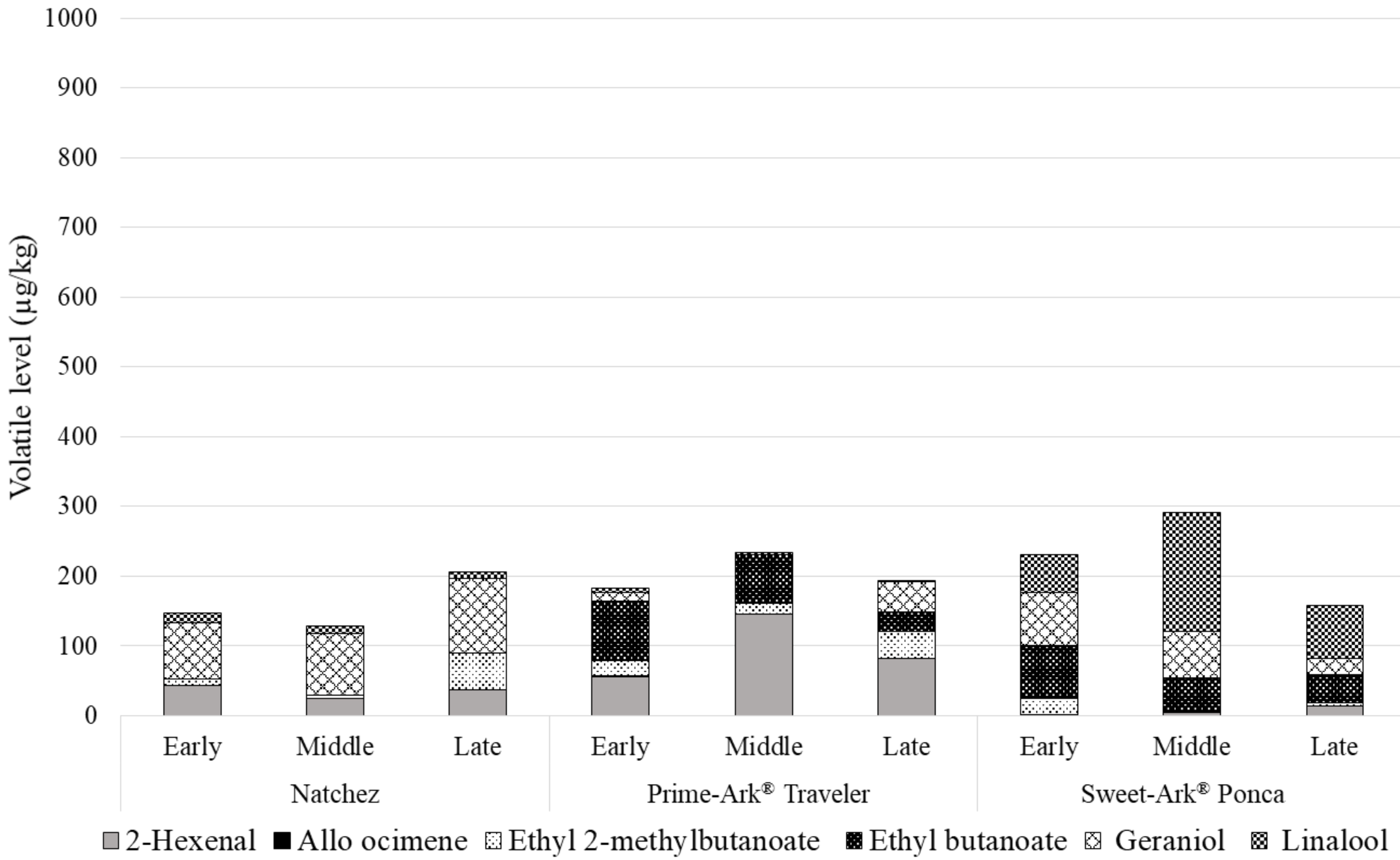

Fig. 3. Total concentrations of impactful volatile aroma compounds identified (µg/kg) in fresh-market blackberry cultivars grown at the University of Arkansas System Division of Agriculture Fruit Research Station and harvested on three dates, Clarksville, AR (2021).

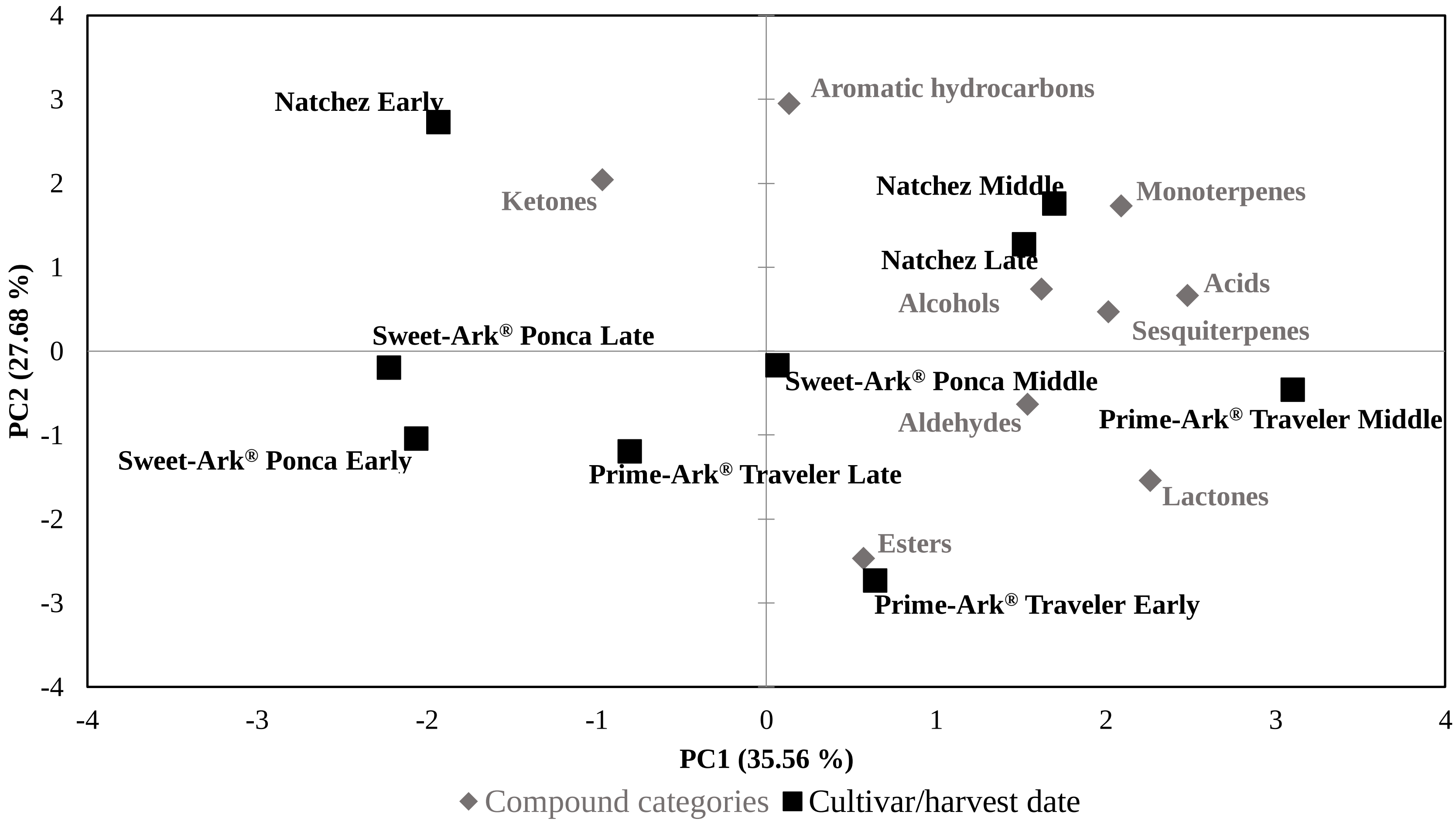

Fig. 4. Principal components (PC) analysis on volatile aroma compounds in fresh-market blackberry cultivars grown at the University of Arkansas System Division of Agriculture Fruit Research Station and harvested on three dates, Clarksville, AR (2021).
Percent of variation in data explained by each component.
Compound class variables represent the sum of the total ion chromatogram (TIC) relative peak areas (%) of positively identified compounds within each compound class.