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Characterization of Aroma-Active Compounds in Northern Highbush Blueberries "Bluecrop" (*Vaccinium corymbosum* "Bluecrop") and "Elliott" (*Vaccinium corymbosum* "Elliott") by Gas Chromatography−Olfactometry Dilution Analysis and Odor Activity Value

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ABSTRACT: Aroma-active compounds in northern highbush blueberries "Bluecrop" (*Vaccinium corymbosum* "Bluecrop") and "Elliott" (*Vaccinium corymbosum* "Elliott") were isolated using solvent extraction followed by solvent-assisted flavor evaporation. The aroma-active compounds were compared by aroma extract dilution analysis (AEDA) on a polar and a chiral column and odor activity values (OAVs). The results showed that both cultivars had similar aroma-active compounds, but the flavor dilution (FD) factors and OAV values varied, responsible for the subtle aroma differences. AEDA (FD ≥ 64) suggested that the important aroma-active compounds in both cultivars could be ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, (Z)-3-hexenal, 1-octen-3-one, (Z)-3-hexen-1-ol, methional, linalool, (E,Z)-2,6-nonadienal, 2-methylbutanoic acid, α-terpineol, (E,E)-2,4-nonadienal, β-damascenone, geraniol, geranyl acetone, o-cresol, eugenol, and vanillin. Different isomers can have different sensory thresholds and attributes. Enantiomer analysis revealed that although S-(+)-linalool was dominant in blueberry, R-(−)-linalool had a higher FD value and OAVs than that of the (S)-isomer. The S-(−)-α-terpineol was slightly elevated than R-(+)-α-terpineol in both cultivars.

KEYWORDS: "Bluecrop" blueberry, "Elliott" blueberry, AEDA, OAV, R-(−)-linalool, S-(+)-linalool, S-(−)-α-terpineol, R-(+)-α-terpineol

■ INTRODUCTION

Blueberries are native to North America and were first cultivated in the early 20th century. Blueberry cultivars can be classified as northern and southern highbush (*Vaccinium corymbosum*) blueberry, lowbush (*Vaccinium angustifolium*) blueberry, and rabbiteye (*Vaccinium ashei*) blueberry. Northern highbush blueberry is the most widely planted cultivar in the USA, which grows 5−9 feet tall. Blueberries are rich in anthocyanin and other phytochemicals. Extensive research has demonstrated the health benefits of consuming blueberry fruits.[1−3] The antioxidant-rich blueberry is regarded as a "superfruit."

Global blueberry production has grown significantly over the past decades and continues to grow. The United States is the world's largest blueberry producer. Over 670 million pounds of blueberries were produced and utilized in 2019, leaping 21% from 2018.[4] Although berry firmness, texture, size, color, flavor, sweetness, and juiciness are typically used in the field to assess blueberry eating quality, consumer preference studies illustrated that blueberry's fruit quality is best correlated with flavor scores.[5−7]

The fresh blueberry fruit flavor is primarily determined by volatile compounds formed during ripening.[8−12] A wide range of volatiles has been identified, but the literature on blueberry's aroma composition is inconclusive due to the genetic and biological variability.[8,12,13] An earlier study by Parliment and Scarpellino[14] found that a combination of linalool and (Z)-3-hexen-1-ol gave a blueberry-like aroma, whereas Horvat and Senter[8] showed that volatile recombination of (Z)-3-hexen-1-ol, (E)-2-hexen-1-ol, (E)-2-hexenal, linalool, and geraniol gave a sensory perception similar to the extract isolated from blueberries. Fruit maturity and genetic make-up strongly affect blueberry's volatile profile.[9,12,13,15] Highbush and rabbiteye blueberry are primarily characterized by the high level of "C6 compounds", such as (Z)-2-hexenal, (Z)-2-hexen-1-ol, and terpene alcohols.[8,10,12,13,16] In contrast, wild lowbush blueberry is typically characterized by high concentrations of branch-chain esters.[16−18] Depending on volatile composition, blueberry fruit flavor can be complex.[19]

Chirality also plays a vital role in the aroma and odor. There is a growing interest in understanding enantiomer's contribution to food flavor because different isomers can have different sensory thresholds and attributes.[20−23] It is well known that the odor threshold of (R)-(−)-linalool (woody, lavender-like) is nearly 9 times lower than (S)-(+)-linalool (sweet, floral-like) (0.8 ppb vs 7.4 ppb).[21] Linalool enantiomer distribution has been studied in blackberry,[24] raspberry,[25] passion fruit,[26]

wine,[21] hard iced tea,[23] tea,[20] and beer.[27] In nature, many chiral compounds have a dominant enantiomer form. Enantiomeric ratios of chiral compounds have been used as indicators for adulteration by adding synthetic compounds or natural components from different origins.[22,28]

Although blueberry volatile composition research can be dated back to the 1970s,[14] blueberry aroma study is minimal, and the results were not conclusive. With the advances in analytical technology, the knowledge on blueberry flavor quality has accumulated rapidly in the past decades. Using headspace solid-phase microextraction coupled to gas chromatography−mass spectrometry (HS-SPME-GC−MS) and proton transfer reaction-time of flight-mass spectrometry (PTR-ToF-MS), over a hundred volatile compounds from commercial blueberry cultivars have been identified.[9] Still, the aroma contributing compounds have not been well characterized in blueberry. The gas chromatography−olfactometry technique, an approach using the human nose as a detector to monitor the presence of an odorant in the effluent of a gas chromatograph, is crucial to assess aroma-active compounds contributing to the overall blueberry flavor.[29]

The objectives of this study were to identify aroma-active compounds and chiral isomers in two northern highbush blueberries "Bluecrop" (*V. corymbosum* "Bluecrop") and "Elliott" (*V. corymbosum* "Elliott") by gas chromatography−mass spectrometry/olfactometry (GC/O) with aroma extract dilution analysis (AEDA) on a polar and a chiral column and the odor active values (OAV). Two cultivars were chosen because of their popularity and different fruiting seasons. Bluecrop is the most widely planted mid-season cultivar (ripens in July in Pacific Northwest in the USA). The berries are medium to large size, light blue, firm, and have a good flavor. Elliott is a high yield, late-season cultivar, fruiting through September. The berries are of medium size and have a mild taste. Both cultivars are appropriate for most commercial operations. The improved understanding of blueberry flavor at the molecular level will enhance multidisciplinary collaboration among horticulture, genetic breeding, food science, and flavor chemistry to produce high-quality agricultural products.

■ MATERIALS AND METHODS

Chemicals. All chemical standards used for identification and quantitation in this study were of analytical reagent grade from Millipore Sigma (St. Louis, MO) unless otherwise stated in the parentheses: eugenol, 2-heptanone, 1-nonanol, and (E,E)-2,4-nonadienal (TCI America, OR); benzaldehyde, geranyl acetate, 2-hexenoic acid, and 2-nonanone (Alfa Aesar, MA); and decanoic acid (Spectrum Chemicals, CA). Dichloromethane (HPLC grade, Burdick & Jackson, Muskegon, MI) was freshly distilled. Methanol (HPLC grade) was purchased from EM Science (Gibbstown, NJ). Ethanol was purchased from Aaper Alcohol and Chemical Co. (Shelbyville, KY). Anhydrous sodium sulfate and sodium chloride (99.9%, ACS certified) were supplied by Avantor Performance Materials (Center Valley, PA). Water was obtained from the Milli-Q purification system (Millipore, Bedford, MA).

Isotope-labeled compounds were purchased from CDN Isotopes (Pointe-Claire, Quebec, Canada). Their mass spectra are listed: ethyl butanoate-4,4,4-d_3 (99.8%): mass spectrometry ion, m/z (%), 74 (100), 89 (89), 101 (20), 119 (molecular weight (MW)); ethyl 2-methyl bututanoate-d_9 (99.1%): m/z (%), 66 (100), 94 (42), 107 (64), 139 (MW); ethyl 3-methylbutanoate-d_9 (>98%): m/z (%), 66 (72), 72 (10), 91 (100), 112 (8), 121 (12), 139 (MW); hexanal-d_{12} (99.0%): m/z (%), 50 (68), 64 (100), 74 (14), 80 (41), 84 (14), 92 (20), 112 (MW); nonanal-d_{18} (99.1%): m/z (%), 50 (74), 62 (100), 92 (38), 108 (38), 112 (49), 128 (23), 140 (7), 160 (MW); 2-

phenyl-d_5-ethan-1,1,2,2-d_4-ol (99.2%): m/z (%), 70 (16), 98 (100), 131 (MW); linalool-d_3 (99%): m/z (%), 55 (39), 69 (45), 74 (100), 83 (44), 96 (89), 109 (15), 124 (39), 139 (15), 157 (MW); α-terpineol-d_3 (98.5%): m/z (%), 62 (100), 72 (6), 81 (46), 93 (76), 124 (43), 139 (74), 157 (MW); vanillin-(methoxy-d_3) (99%): m/z (%), 154 (100), 81 (17), 109 (14), 126 (12), 137 (4), 155 (87) (MW).

Blueberry Samples. Two northern highbush blueberries, Bluecrop (*V. corymbosum* Bluecrop) and Elliott (*V. corymbosum* Elliott), were obtained from the North Willamette Research and Extension Center (Aurora, OR; latitude 45°16′47″N, longitude 122°45′23″W) of Oregon State University. Bluecrop and Elliott blueberries were planted on raised beds with a water irrigation system and mulched with a 5 cm deep layer of sawdust. A permanent grass cover crop was grown in the row aisles and mowed during the growing season, as required. The soil is classified as a Willamette silt loam (a fine-silty, mixed, superactive mesic Pachic Ultic Argixeroll). Fruits from both varieties were hand-picked, transported to the laboratory in a cooler, and stored at −20 °C until analysis.

Volatile Isolation by Solvent-Assisted Flavor Evaporation. Frozen blueberries (about 600 g) of each cultivar were blended into powder with liquid nitrogen. Five hundred gram powder was weighed into a 1-L Mason jar, and 400 mL of freshly distilled dichloromethane was added into the Mason jar and capped. The mixture was shaken for 20 h at 180 rpm in an Orbital shaker (model: E-class, Barnstead lab-line, Dubuque, IA). The mixture was poured into a separator funnel and settled, and the lower portion was collected. The puree was extracted two more times with 200 mL of dichloromethane each. The extracts were combined and went through a solvent-assisted flavor evaporation (SAFE)[30] device at 50 °C under a vacuum of 3.7 Pa to remove the nonvolatile constituents. Anhydrous sodium sulfate was added to the distillate to remove water. The dried distillate was concentrated in a Kuderna−Danish concentrator in a 45 °C water bath with a Vigreux column to approximately 10 mL and further concentrated to 100 μL with a gentle stream of nitrogen.

GC−MS/Olfactometry Analysis Using a ZB-Wax Column and a Chiral Column. GC−MS/Olfactometry (GC/O) analysis was conducted using an Agilent 6890 gas chromatograph equipped with an Agilent 5973 mass selective detector (Agilent Technologies, Inc., Santa Clara, CA) and a sniffing port (ODP 2, Gerstel, Inc., Germany). Sample separation was performed with the ZB-WAX*plus* column (30 m × 0.25 mm i.d., 0.5 μm firm thickness, Phenomenex, Inc., Torrance, CA) and a CycloSil-B column (30 m × 0.25 mm i.d., 0.25 μm film thickness, Agilent Technologies, Inc.), respectively. Helium was used as the carrier gas at 2.5 mL/min. The column effluent was split into 0.7 mL/min into the detector and the rest to the sniffing port. The sample (1 μL) was injected into the injector in a spitless mode. The injector and ion source temperatures were 250 and 230 °C, respectively. The initial oven temperature was 40 °C and held for 4 min and then increased to 230 °C at a rate of 4 °C/min with a 10 min holding. Electron ionization mass spectrometric data from m/z 35 to 350 were collected using a scan rate of 5.27 s^{-1}, with an ionization voltage of 70 eV.

Aroma Extract Dilution Analysis. Four experienced panelists conducted the aroma extract dilution analysis (AEDA) to screen the aroma-active compounds. The volatile extracts of Bluecrop and Elliott were mixed stepwise with dichloromethane at a ratio of 1:1 (v/v) until no odor was perceived. Each mixture was analyzed as previously described for GC/O analysis by a ZB-WAX*plus* and a CycloSil-B column, respectively. The flavor dilution (FD) factor of each volatile compound was reported as the maximum dilution number at which the odorant could be perceived. A standard mixture of *n*-alkane (C_5−C_{30}) was analyzed under the same GC condition as AEDA. Retention indices (RI) were calculated according to a modified Kováts method[31] based on a particular retention time. Odorants were identified by sensory attributes, mass spectra from the NIST 05a and Wiley 275 databases (Agilent Technologies Inc.), and RI obtained in the laboratory using reference compounds. The individual chemical standard was injected and evaluated as needed under the same conditions.

Table 1. Chemical Standards, Target Ions, and Calibration Curves Used for Quantitation of Volatiles[a]

compounds	m/z	equations	range (mg/L)	R^2
ethyl butanoate-4,4,4-d₃ (IS)	74			
methyl 2-methylbutanoate[b]	88	$y = 0.3219x - 0.001206$	0.1–1000	0.9987
methyl 3-methylbutanoate[b]	85	$y = 0.2569x + 0.001117$	0.1–1000	0.9999
ethyl 2-methylbutanoate-d₉ (IS)	107			
ethyl 2-methylbutanoate[b]	57	$y = 0.7827x + 0.0004079$	0.5–1000	0.999
ethyl 3-methylbutanoate-d₉ (IS)	91			
ethyl 3-methylbutanoate[b]	88	$y = 0.08604x + 0.00006139$	0.5–1000	0.9998
nonanal-d₁₈ (IS)	62			
octanal[b]	84	$y = 0.7199x + 0.002167$	1.0–1000	0.9987
decanal[b]	57	$y = 0.7753x - 0.0008139$	1.0–1000	0.9999
€-2-nonenal[b]	55	$y = 1.948x + 0.003281$	0.1–500	0.9998
α-terpineol-d₃ (IS)	139			
α-terpineol[b]	136	$y = 1.278x + 0.07603$	0.5–1000	0.9989
hexanal-d₁₂ (IS)	64			
hexanal	56	$y = 4.101x + 0.815$	1–400	0.9947
€-2-pentenal	55	$y = 2.904x + 0.0123$	1–400	0.9765
(Z)-3-hexenal	69	$y = 3.83x + 0.193$	1–400	0.9983
€-2-hexenal	83	$y = 3.681x + 0.07138$	1–400	0.9958
nonanal-d₁₈ (IS)	62			
nonanal	57	$y = 0.949x - 0.0019$	1–400	0.9976
(E,Z)-2,6-nonadienal	70	$y = 1.821x + 0.1056$	1–400	0.9960
phenylacetaldehyde	91	$y = 1.418x - 0.001$	1–400	0.9948
(E,E)-2,4-nonadienal	81	$y = 1.488x + 0.1311$	1–400	0.9862
linalool-d₃	74			
2-heptanone	58	$y = 1.822x - 0.01176$	1–400	0.9982
eucalyptol	81	$y = 1.389x + 0.02019$	1–400	0.9971
1-octen-3-one	70	$y = 1.511x - 0.00965$	1–400	0.9988
€-3-hexen-1-ol	67	$y = 1.139x - 0.01303$	1–400	0.9977
(Z)-3-hexen-1-ol	67	$y = 1.486x - 0.0264$	1–400	0.9988
linalool	71	$y = 1.678x - 0.0012$	1–400	0.9972
β-damascenone	121	$y = 1.972x - 0.0645$	1–400	0.9999
geraniol	69	$y = 0.589x + 0.0126$	1–400	0.9984
2-phenyl-d₅-ethan-1,1,2,2-d₄-ol (IS)	98			
2-phenylethyl alcohol	91	$y = 2.53x - 0.0278$	1–400	0.9984
eugenol	164	$y = 1.549x - 0.014$	1–400	0.9909
vanillin	151	$y = 12.494x - 0.0669$	10–500	0.9996

[a]IS, stable isotope-labeled internal standards used to quantitate compounds below them. [b]Compounds quantitated by SPME-GC−MS.

Quantitation of Volatile Compounds. *Blueberry Sample Preparation.* Frozen blueberry samples were blended into powder with liquid nitrogen, and 200 g of powder was weighed into a 1-L glass Mason jar with a screw cap. Three hundred milliliters of redistilled dichloromethane were added to extract volatile compounds. After shaking at 180 rpm for 2 h, the dichloromethane layer was collected. The extraction step was repeated two more times with 200 mL of redistilled dichloromethane. An aliquot of 100 μL mixture of stable isotope-labeled internal standards was added to the combined extracts. Volatiles were then isolated by the SAFE and concentrated to 100 μL as described previously. Each sample was prepared in triplicates.

Standard Curve Preparation. The volatile compounds with high FD factors were quantitated using standard curves. The standard curves were built from the mixed stock solutions containing the compounds listed in Table 1. Eight data points with an incremental concentration of the standards (0.1−1000 mg/L) were prepared. A mixture of stable isotope-labeled chemicals was used as internal standards to quantitate compounds with the same or similar chemical structure (Table 1). For each data point, a defined amount of stock solution and 100 μL mixture of internal standards were spiked into 500 mL of redistilled dichloromethane, and the mixture was then subjected to SAFE distillation. The distilled standard solution was concentrated to 100 μL as prepared for the sample and subjected to

GC−MS analysis. The actual data points used to calculate individual volatile compounds depended on their concentration.

Volatile Quantitation by Gas Chromatography−Mass Spectrometry. Quantitation of aroma compounds was performed on Agilent 6890 GC equipped with an Agilent 5973 MSD. An aliquot of 1 μL of concentrated extract (standards and samples) was injected spitless into the GC−MS system on a ZB-Wax column (30 m length, 0.25 mm inner diameter, 0.5 μm film thickness, Phenomenex). Helium was used as the carrier gas at a constant flow rate of 2.5 mL/min. The GC injector temperature was set at 250 °C. The initial oven temperature was 40 °C and held for 4 min, and then was ramped up to 230 °C at 4 °C/min and held for 10 min. The MS transfer line and ion source temperatures were 250 and 230 °C, respectively. The ionization voltage was 70 eV. The solvent-delay time was set as 5 min. The selected mass ion was used for quantitation. The unique quantitation mass ion was carefully chosen to ensure the highest response and lowest interference (Table 1). The results were calculated using Enhanced Chemstation software E.02 (Agilent Technologies, Inc.).

Methyl 2-methylbutanoate, methyl 3-methylbutanoate, ethyl 2-methylbutanoate, and ethyl 3-methylbutanoate were quantitated by HS-SPME-GC−MS with the corresponding isotope-labeled internal standards due to their coelution with solvent dichloromethane. Octanal, decanal, (E)-2-nonenal, and α-terpineol were also quantitated by the SPME method for better sensitivity. Approximately

Table 2. Odorants and Their FD Factors on a WAX Column in Bluecrop and Elliott Blueberries[a]

compounds	RI$_{WAX}$	identification	description	FD value Bluecrop	FD value Elliott
methylbutanoate	983	RI, Std, A	fruity	32	4
2,3-butanedione (diacetyl)	992	MS, RI, Std, A	buttery, cheesy	32	64
methyl 2-methylbutanoate	1017	MS, RI, Std, A	fruity	128	32
methyl 3-methylbutanoate	1026	MS, RI, Std, A	fruity	8	ND
ethyl butanoate	1040	MS, RI, Std, A	fruity	8	ND
ethyl 2-methylbutanoate	1057	MS, RI, Std, A	fruity, apple	256	128
ethyl 3-methylbutanoate	1075	MS, RI, Std, A	fruity	512	128
hexanal	1086	MS, RI, Std, A	green, grassy	32	128
unknown	1111		green, gasoline	32	64
(E)-2-pentenal	1144	RI, Std, A	green, grassy	16	16
(Z)-3-hexenal	1149	MS, RI, Std, A	green, leafy	256	128
2-heptanone	1190	MS, RI, Std, A	fruity	16	8
eucalyptol	1212	MS, RI, Std, A	mint, camphor	256	2
(E)-2-hexenal	1223	MS, RI, Std, A	green	8	32
unknown	1247		potato, moldy	64	64
octanal	1295	MS, RI, Std, A	citrus	16	32
1-octen-3-one	1307	MS, RI, Std, A	mushroom	128	64
(Z)-3-hexenyl acetate	1328	MS, RI, Std, A	fruity, floral	16	2
6-methyl-5-hepten-2-one	1344	MS, RI, Std, A	fruity	8	2
(E)-3-hexen-1-ol	1376	MS, RI, Std, A	green	32	8
2-nonanone	1385	MS, RI, Std, A	fruity, floral	32	ND
(Z)-3-hexen-1-ol	1394	MS, RI, Std, A	green, grassy	128	256
nonanal	1396	MS, RI, Std, A	floral, citrusy	16	4
(E)-2-hexen-1-ol	1417	MS, RI, Std, A	green, berry	16	8
(E)-2-octenal	1432	MS, RI, Std, A	oily, fatty	8	2
acetic acid	1458	MS, RI, Std, A	vinegar	4	ND
methional/1-octen-3-ol	1462	MS, RI, Std, A	mashed potato/green	256	128
2-methyl-3-buten-2-ol	1470	MS, RI, Std, A	floral	4	4
decanal	1507	MS, RI, Std, A	green, oily	16	64
benzaldehyde	1523	MS, RI, Std, A	nutty, cherry	8	2
(E)-2-nonenal	1540	MS, RI, Std, A	green, fatty	16	32
linalool	1560	MS, RI, Std, A	floral	256	256
(E,Z)-2,6-nonadienal	1591	Std, RI, Std, A	cucumber	256	128
butanoic acid	1610	MS, RI, Std, A	cheesy	8	8
γ-butyrolactone	1631	MS, RI, Std, A	nutty	2	2
benzeneacetaldehyde	1646	MS, RI, Std, A	floral, sweet	64	16
1-nonanol	1665	MS, RI, Std, A	spicy, floral	4	4
2-methylbutanoic acid	1681	MS, RI, Std, A	sweaty, dirty sock	64	64
α-terpineol	1707	MS, RI, Std, A	oily, citrusy	128	128
(E,E)-2,4-nonadienal	1716	MS, RI, Std, A	green, fatty	256	128
geranyl acetate	1737	MS, RI, Std, A	floral	32	64
citronellol	1780	MS, RI, Std, A	citrus, green	32	64
(Z)-geraniol (nerol)	1815	MS, RI, Std, A	sweet, floral	8	32
β-damascenone	1824	MS, RI, Std, A	honey, tobacco	256	64
hexanoic acid	1857	MS, RI, Std, A	rancid	8	16
geraniol	1863	MS, RI, Std, A	floral, citrus	256	128
geranyl acetone	1870	MS, RI, Std, A	floral	128	64
benzyl alcohol	1887	MS, RI, Std, A	floral, mocha	8	2
2-phenylethyl alcohol	1921	MS, RI, Std, A	floral, fruity	32	16
2-hexenoic acid	1977	MS, RI, Std, A	sour, animal	1	1
caryophyllene oxide	1988	MS, RI, Std, A	woody, herbal	16	8
o-cresol	2016	MS, RI, Std, A	phenolic, metallic	64	128
cinnamaldehyde	2037	MS, RI, Std, A	creamy, sweet	4	2
p-cresol	2087	MS, RI, Std, A	animal, horse barn	16	16
m-cresol	2107	MS, RI, Std, A	smoky, hospital	4	2
γ-decalactone	2162	MS, RI, Std, A	sweet, fruity	8	8
eugenol	2177	MS, RI, Std, A	clove	64	128
unknown	2209	MS, RI, Std, A	spicy, smoky	16	16
unknown	2235	MS, RI, Std, A	spicy, smoky	32	64

Table 2. continued

compounds	RI$_{WAX}$	identification	description	FD value Bluecrop	FD value Elliott
decanoic acid	2288	MS, RI, Std, A	sweaty, soapy	2	8
3-phenyl-2-propen-1-ol (cinnamyl alcohol)	2295	MS, RI, Std, A	floral, woody	8	4
vanillin	2585	MS, RI, Std, A	vanilla	128	128

^{a}RI, retention index; MS, mass spectrum agreed with authentic compound; A, aroma descriptions by panelists; Std, standard run in the laboratory; and ND, not detected.

30 g of frozen blueberries was blended into powder with liquid nitrogen. Two grams of blueberry powder were weighed into a 20 mL vial and mixed with 5 mL of citrate buffer (0.2 M, pH 3.2, 1% NaF, saturated with NaCl). Then, 20 μL of isotope-labeled internal standard mixture containing ethyl butanoate-4,4,4-d$_3$ (25 mg/L), ethyl 2-methylbutanoate-d$_9$ (25 mg/L), ethyl 3-methylbutanoate-d$_9$ (7.5 mg/L), nonanal-d$_{18}$ (50 mg/L), and α-terpineol-d$_3$ (10 mg/L) were added. Triplicate analyses were performed on each sample. The analytes' standard calibration curves were prepared by spiking incremental amounts of chemical standards and 20 μL isotope-labeled internal standards into 5 mL of citrate buffer (0.2 M, pH 3.2, 1% NaF, saturated with NaCl).

Sample vials were equilibrated at 45 °C for 10 min in an incubator. After equilibration, a three-phase divinylbenzene/carboxen/poly-(dimethylsiloxane) (DVB/CAR/PDMS) fiber (2 cm × 50/30 μm, Supelco, Inc., Bellefonte, PA) was inserted into the sample vial. Headspace volatiles were collected for 50 min. The volatiles were then desorbed into the GC injection port in a splitless mode at 250 °C for 6 min. All of the steps were controlled by an autosampler (Gerstel, Inc., Linthicum, MD).

Compounds were separated on a ZB-WAX column (30 m × 0.25 mm i.d., 0.5 μm film thickness, Phenomenex, Inc.) as previously described. The results were calculated using Chemstation software (E.02.01, Agilent Technologies, Inc.). Chemical standards, target ions, and calibration curves used for quantification of volatiles in blueberry are presented in Table 1.

Chiral Isomer Analysis with a CycloSil-B Chiral Column. Two grams of blueberry powder was weighed into a 20 mL autosampler vial. Then, 8 mL phosphate buffer (pH 6.5, saturated with NaCl), 20 μL internal standard solution (5 mg/L 4-octanol), and a 1 cm Teflon stir bar were added into the vial. The vial was tightly capped, and the sample was equilibrated at 45 °C in a thermostatic agitator for 5 min. A DVB/CAR/PDMS fiber (Supelco, Inc) was inserted into the vial to extract volatiles at 45 °C for 30 min with stirring (250 rpm). After extraction, the fiber was inserted into the injection port (250 °C) to desorb the analytes. The extraction and injection were achieved by an autosampler (Gerstel, Inc.).

An Agilent 6890 gas chromatograph equipped with an Agilent 5973 mass selective detector (Agilent Technologies, Inc.) was used for analysis. MS transfer line and ion source temperatures were 280 and 230 °C, respectively. The compounds were separated using a CycloSil-B GC column coated with 30% hepatkis (2,3-di-O-methyl-6-O-tert-butyl-dimethylsilyl)-β-cyclodextrin in DB-1701 (30 m × 0.25 mm i.d., 0.25 μm film thickness, Agilent Technologies, Inc.). The oven temperature was started at 60 °C with a 3 min hold and then increased to 230 °C at 3 °C/min with a 10 min holding. Helium was used as the carrier gas with a 2.5 mL/min flow rate. Quantitation was achieved using the selected mass ion. Each sample was analyzed in triplicates. The enantiomer ratio was calculated based on the peak area. Standards were run to confirm the R- and S-enantiomers. The concentration of individual enantiomers was calculated by multiplying the total concentration with the isomer percentage.

■ RESULTS AND DISCUSSION

The GC–MS/Olfactometry technique, which couples the GC with human olfaction and mass spectrometry, can simultaneously detect odor characteristics and chemical identity chromatographically separated volatile compounds in blue-

berries.[10] To further evaluate the potential sensory importance of odorants to the overall blueberry aroma, a sequential dilution of concentrated aroma extract approach based on sensory thresholds in air was used in this study. However, the GC/O results need to be interpreted with care because of the difference in the sense of smell among panelists, potential loss of highly volatile components during extract concentration, and solvent interference during GC analysis. An intensive review of GC/O has been published by Delahunty et al.[29]

The effectiveness of volatile isolation from samples is essential. Static headspace solid-phase microextraction is frequently used, although a more advanced technique, stir-bar sorptive extraction (SBSE), can be used as an alternative with more absorption capacity. However, there is a lack of reproducibility among the different SPME fibers. Also, there are competitions among volatiles for each SPME fiber.[32] Traditional liquid–liquid extraction followed by a solvent-assisted flavor evaporation technique to isolate and concentrate volatiles more likely reflect blueberry's aroma profile.

Aroma-Active Volatiles Recognized by GC–MS/Olfactometry with a ZB-WAXplus Column. Aroma-active compounds and their FD values in two northern highbush blueberry cultivars detected by the ZB-WAX*plus* column are shown in Table 2. The results showed that both cultivars had a similar aroma profile, but the FD factors varied. The predominant aroma-active compounds were esters, alcohols, aldehydes, and terpenes, which agree with the findings in four southern highbush blueberries ("Jewel," "Prima Dona," "Snow Chaser," and "Kestrel") determined by GC/O–GC–MS.[10] Overall, blueberries are less aromatic and contain fewer volatile compounds than other small fruits.[24,33,34]

According to the FD factors, the major aroma contributors to the two cultivars (FD ≥ 64) could be ethyl 2-methylbutanoate (FD = 512, in Bluecrop, 128 in Elliott, fruity note), ethyl 3-methylbutanoate (FD = 512, 128, fruity), (Z)-3-hexenal (FD = 256, 128, green), 1-octen-3-one (FD = 128, 64, mushroom), (Z)-3-hexen-1-ol (FD = 128, 256, green), methional (FD = 256, 128, mushed potato), linalool (FD = 256, 256, floral), (E,Z)-2,6-nonadienal (FD = 256, 128, cucumber), 2-methylbutanoic acid (FD = 64, 64, sweaty, cheesy), α-terpineol (FD = 128, 128, oily, citrusy), (E,E)-2, 4-nonadienal (FD = 256, 128, green), β-damascenone (FD = 256, 64, honey), geraniol (FD = 256, 128, floral), geranyl acetone (FD = 128, 64, floral), o-cresol (FD = 64, 128, phenolic), eugenol (FD = 64, 128, clove), and vanillin (FD = 128, 128, vanilla).

Esters. Unsurprisingly, esters play a vital role in blueberry aroma, especially for their "fruity" perception.[10] Esters are synthesized during ripening from carboxylic acids and alcohols derived from amino acids or lipids in the fruit. Eight esters including methylbutanoate, methyl 2-methylbutanoate, methyl 3-methylbutanoate, ethyl 2-methylbutanoate, ethyl 3-methyl-butanoate, and (Z)-3-hexenyl acetate were identified in the

two cultivars. Bluecrop had higher FD factors than that of Elliott. Both cultivars had high FD factors of ethyl 2-methylbutanoate (FD = 512, 128) and ethyl 3-methylbutanoate (FD = 512, 128), giving fruity notes. Methyl 2-methylbutanoate was detected in Bluecrop with a high FD factor of 128, compared to an FD factor of 32 in Elliott, also contributing to the fruity odor. Methyl 3-methylbutanoate showed a low FD of 8 in Bluecrop but was not detected in Elliott. However, methyl 3-methylbutanoate was reported as an important aroma compound in wild blueberry (*V. angustifolium*).[18] The GC/O study conducted by Du et al.[10] also showed that methyl 2/3-methylbutanoate and ethyl 2/3-methylbutanoate had high aroma intensity in Jewel and Prima Dona, but low or not detected in Snow Chaser and Kestrel cultivars, although more than 2 dozen esters were detected in blueberries by SPME-GC−MS and PTR-ToF-MS.[9] It is expected that panelists may not perceive all of the esters due to their low concentration and high sensory thresholds. Some highly volatile esters might be lost during the sample concentration. Esters were mainly present in fully ripened fruits, although (Z)-3-hexenyl acetate, (Z)-3-hexenyl butanoate, and (E)-2-hexenyl acetate have been detected at a high level in green fruit.[9]

C₆ Compounds and Green Aldehydes. C$_6$ compounds, also called "green-leaf volatiles," derived from linoleic and α-linolenic acids (primarily from berry membrane lipids) through a cascade of enzymatic reactions. The C$_6$ aldehydes can be reduced to the corresponding alcohols by aldehyde reductases or oxidized to C$_6$ acids. They were predominantly detected in unripe fruits, imparting a fresh fruity note. C$_6$-alcohols have been used as varietal markers to assess wine origin.[35] In the current study, hexanal, (Z)-3-hexenal, (E)-2-hexenal, (E)-3-hexen-1-ol, (Z)-3-hexen-1-ol, and (E)-2-hexen-1-ol were identified, giving typical "fresh grassy, earthy, and green" notes to blueberry. Both cultivars had high FD factors of (Z)-3-hexenal (FD = 256, 128) and (Z)-3-hexen-1-ol (FD = 128, 256), suggesting that they could be contributing aroma compounds. Hexanal also showed a high FD factor in Elliott (FD = 128) but a relative low factor in Bluecrop (FD = 32). Paré[36] and Rothstein found that the oxidation of linolenic acid by lipoxygenase could lead to the formation of (Z)-3-hexenal. In turn, it can be transformed into (E)-2-hexenal by isomerase. The GC/O study showed that the (E)-2-hexenal had aroma intensity greater than 7.5 (scale 0−10) in all four southern highbush blueberry cultivars.[10] C$_6$ compounds, especially C$_6$ aldehydes, thought to be a strong inducer, involved in biological defense responses in plants.[37,38] It was suggested that jasmonic acid, a product released from linolenic acid hydrolysis, is a key regulator. Jasmonic acid can stimulate physiological and defensive processes or trigger the synthesis and release of green-leaf volatiles. In general, C$_6$ compounds decrease during fruit ripening with variability between cultivars. The research data revealed that the concentrations of (E)-2-hexenal and (E)-2-hexen-1-ol decreased with ripening in three different cultivars of Rabbiteye blueberries.[8] However, this is not always the case. Separate research[9] with five highbush blueberry cultivars showed that (Z)-3-hexenal concentration decreased quickly during ripening, while (E)-2-hexenal continue to increase until reaching an utmost level at the pink stage and last to fully mature stage.

Besides C$_6$ aldehydes and alcohols, several other aldehydes imparting green aroma attributes were also identified in Bluecrop and Elliott blueberries. Major ones with high FD factors were (E,Z)-2,6-nonadienal (FD = 256, 128, cucumber) and (E,E)-2,4-nonadienal (FD = 256, 128, green). The rest of the aldehydes were (E)-2-pentenal, octanal, (E)-2-octenal, nonanal, (E)-2-nonenal, and decanal. Like the C$_6$ aldehydes, these aliphatic aldehydes are generated from polyunsaturated carboxylic acids.[36]

Terpenoids. Terpenoids are enzymatically synthesized from acetyl-CoA and pyruvate from carbohydrate catabolism. This biosynthetic pathway is also called the mevalonate pathway because mevalonic acid is an intermediate. In the current study, the terpenoids with a high FD factor were linalool (FD = 256, 256, floral), α-terpineol (FD = 128, 128, oily, citrusy), geraniol (FD = 256, 128, floral), geranyl acetone (FD = 128, 64, floral), and β-damascenone (FD = 256, 64, honey-like). Other terpenoids, including citronellol (citrus), nerol (sweet), and caryophyllene oxide (woody), could also contribute to the blueberry aroma. Linalool has been cited as one of the most important aroma-active compounds in blueberry.[10,12,15,16,19] Linalool showed an aroma intensity greater than 7.5 (0−10 scale) in a GC/O study with four southern highbush blueberry cultivars.[10] Research with three Rabbiteye blueberry cultivars illustrated that linalool is primarily produced in fruit at the pink stage and preserved during the last fruit ripening phases with different end levels. In contrast, geraniol is mainly formed between the pink and the ripe stage.[8]

β-Damascenone, a terpenic ketone, is a vital C$_{13}$-norisoprenoid that resulted from carotenoid degradation.[39] It is believed that the glycoconjugates such as carotenoid neoxanthin are stored in fruits as intermediate compounds. They can be hydrolyzed to β-damascenone during food and drink processing.[40] As it is characterized by an extremely low odor threshold in water (0.02−0.09 ng/g),[41] β-damascenone has been widely identified as a key aroma-active compound in fruits, vegetables, and beverages, including beer, liquor, and wine, imparting a pleasant honey-like odor.[40] Eucalyptol, which has a minty scent, was also identified with much higher FD factors in Bluecrop than Elliott. Eucalyptol and caryophyllene oxide were typically produced in the green stage and decreased with fruit ripening.[9]

Terpenoids widely exist in citrus fruits and other berries such as raspberries and blackberries.[13,24,34] Terpenoids are essential aroma compounds, and they are also involved in the plants' defense system.[42] It has been found that with leaf damage from insect feeding, some terpenes, including linalool, are released at a much higher level, together with green-leaf volatiles and (Z)-3-hexenyl acetate. In general, plants release more terpenes at high temperatures and maximal photosynthesis during the day.

Benzenoids. Several benzenoids were identified in the two cultivars. Eugenol is a cinnamate derivative found initially in clove oil. Bluecrop and Elliott had FD factors of eugenol 64 and 128, respectively, impacting typical clove notes. Vanillin is the primary component of the extract of the vanilla bean. Both cultivars had high FD factors of vanillin, contributing to the vanilla smell. Both eugenol and vanillin had been reported in highbush and wild blueberries and other fruits.[17,24]

Benzyl alcohol, 2-phenylethyl alcohol, and cinnamyl alcohol were identified in blueberries, but the FD factors were relatively low. 2-Phenylethyl alcohol had FD factors of 32 in both Bluecrop and Elliott, contributing a pleasant rosy odor. *o*-Cresol (FD = 64, 128) and *p*-cresol (FD = 16, 16) were also identified, giving phenolic and horsy smells. All above five compounds had been previously studied in wild and highbush

Table 3. Odorants and Their FD Factors on a CycloSil-B Column in Bluecrop and Elliott Blueberries[a]

compounds	RI	identification	description	FD value Bluecrop	FD value Elliott
methylbutanoate	807	RI, Std, A	fruity	32	4
methyl 3-methylbutanoate	829	MS, RI, Std, A	fruity	8	ND
methyl 2-methylbutanoate	840	MS, RI, Std, A	fruity	32	8
(E)-3-penten-2-ol	862	MS, RI, Std, A	green, grassy	8	ND
(E)-2-pentenal	883	MS, RI, Std, A	green	4	8
hexanal	889	MS, RI, Std, A	green	32	64
ethyl 2-methylbutanoate/ethyl 3-methylbutanoate	892	MS, RI, Std, A	fruity	128	32
(Z)-3-hexenal	902	MS, RI, Std, A	green	64	32
1-pentanol	955	MS, RI, Std, A	green	2	4
(E)-2-hexenal	965	MS, RI, Std, A	green, apple	4	8
unknown	996		potato	16	4
1-octen-3-one/(Z)-3-hexen-1-ol	1052	MS, RI, Std, A	mushroom/green	32	32
(E)-2-hexen-1-ol/methional	1061	MS, RI, Std, A	potato	64	32
eucalyptol	1074	MS, RI, Std, A	minty	64	ND
(Z)-2-heptenal	1078	MS, RI, Std, A	metallic, green	32	64
r-butyrolactone	1095	MS, RI, Std, A	creamy, oily	1	1
unknown	1158		fruity, floral	8	8
3-methylbutanoic acid	1162	MS, RI, Std, A	cheesy, sweaty	32	16
2-methylbutanoic acid	1177	MS, RI, Std, A	cheesy, sweaty	8	8
nonanal	1185	MS, RI, Std, A	fragrancy	4	8
benzeneacetaldehyde	1192	MS, RI, Std, A	floral	64	16
1-octanol	1217	MS, RI, Std, A	waxy, fatty	1	4
R-(−)-linalool	1226	MS, RI, Std, A	lavender	128	128
S-(+)-linalool	1228	MS, RI, Std, A	floral, citrus	16	16
(E)-2-nonenal	1261	MS, RI, Std, A	stinky bug, grassy	16	16
(E)-2-dodecenal	1274	MS, RI, Std, A	waxy, woody	16	8
(E,Z)-2,6-nonadienal	1278	MS, RI, Std, A	cucumber	64	16
decanal	1288	MS, RI, Std, A	floral	4	4
hexanoic acid	1291	MS, RI, Std, A	rancid	2	4
benzyl alcohol	1301	MS, RI, Std, A	woody	2	2
(E)-2-hexenoic acid	1332	MS, RI, Std, A	herbal	4	2
2-phenylethyl alcohol	1340	MS, RI, Std, A	floral, rosy	16	4
S-(−)-α-terpineol	1352	MS, RI, Std, A	piney, floral	32	32
R-(+)-α-terpineol	1365	MS, RI, Std, A	oily, rubber	32	16
unknown	1386		woody, grassy	4	8
geraniol	1400	MS, RI, Std, A	floral, citrus	128	64
β-damascenone	1428	MS, RI, Std, A	honey	64	32
cinnamaldehyde	1473	MS, RI, Std, A	woody, spicy	8	4
eugenol	1506	MS, RI, Std, A	clove	32	64
o-cresol	1560	MS, RI, Std, A	metallic	32	32
vanillin	1653	MS, RI, Std, A	vanilla	64	32
caryophyllene oxide	1673	MS, RI, Std, A	creamy	16	64

[a]RI, retention index; MS, mass spectrum agreed with authentic compound; A, aroma descriptions by panelists; Std, standard run in the laboratory; and ND, not detected.

blueberries.[9,17] Benzaldehyde, benzeneacetaldehyde, and cinnamaldehyde with FD ranged from 2 to 64, have been found in wild and lowbush blueberries.[17]

Other Compounds. The sulfur-containing aldehyde, methional, contributing mushed potato odor, had high FD factors in both blueberry cultivars. They are derived from the degradation of sulfur-containing amino acids. Methional has not been reported in blueberry but was characterized as an aroma contributor to blackberry flavor.[24] Several ketones and lactones, including 2,3-butanedione (diacetyl), 2-heptanone, 1-octen-3-one, 6-methyl-5-hepten-2-one, 2-nonanone, γ-butyrolactone, and γ-decalactone were recognized in this study. They can be originated from bacterial metabolism, oxidation/degradation of polyunsaturated fatty acids, and amino acid

degradation. 1-Octen-3-one showed a high FD of 128 and 64 in Bluecrop and Elliott, respectively, giving a typical mushroom aroma. 2-Heptanone was identified, contributing a fruity note. Diacetyl showed an FD of 32 and 64 in Bluecrop and Elliott, respectively, giving buttery and cheesy notes. 2-Nonanone was only detected in Bluecrop with an FD of 32, impacting fruity note. The rest of the ketones with lower FD values had less impact on blueberry flavor. Acetic, 2-methylbutanoic, hexanoic, 2-hexenoic, and decanoic acids were identified by GC/O, in agreement with previous reports.[9,17] Among the acids, 2-methylbutanoic acid showed an FD factor of 64 in both cultivars, giving sweaty and cheesy odors. Other acids had low FD factors in the two cultivars. Volatile compounds in berry fruits are very complex and dependent on cultivar, fruit

Table 4. Concentrations (μg/L) and OAV of Selected Odorants in Bluecrop and Elliott Blueberries

compounds	threshold[a,b] (μg/L)	concentration (μg/kg)		OAV	
		Bluecrop	Elliott	Bluecrop	Elliott
		Esters			
methyl 2-methylbutanoate	0.25	1.6 ± 0.0	1.0 ± 0.1	6	4
methyl 3-methylbutanoate	4.4	6.9 ± 0.9	ND	2	ND
ethyl 2-methylbutanoate	0.063	1.8 ± 0.3	0.34 ± 0.05	29	5
ethyl 3-methylbutanoate	0.110	2.3 ± 0.3	1.5 ± 0.1	21	14
		Aldehydes			
(E)-2-pentenal	1.5	9.4 ± 3.3	27.6 ± 5.3	6	18
hexanal	10	209.3 ± 3	247.9 ± 7.6	21	25
(Z)-3-hexenal	0.21	16 ± 1.7	14.5 ± 1.4	76	69
(E)-2-hexenal	190	210.3 ± 5.1	235.7 ± 9.8	1	1
octanal	0.7	3.8 ± 0.1	4.1 ± 1	5	6
nonanal	8	19.9 ± 2.9	4.6 ± 0.7	2	1
(E)-2-nonenal	0.19	0.33 ± 0.01	0.97 ± 0.09	2	5
(E,Z)-2,6-nonadienal	0.01	20.5 ± 2.9	29.0 ± 6.0	2050	2900
(E,E)-2,4-nonadienal	0.19	8.8 ± 1.1	6.7 ± 0.1	46	35
decanal	0.4	2.8 ± 0.2	3.5 ± 0.2	7	9
		Terpenoids			
geraniol	2.5	206.9 ± 6.4	47.1 ± 2.9	83	19
linalool	6	124.6 ± 2.7	116.7 ± 0.5	21	19
α-terpineol	330	45.8 ± 2	157.9 ± 5.4	<1	<1
eucalyptol	1.1	19.4 ± 5.2	2.7 ± 0.2	15	2
β-damascenone	0.056	0.59 ± 0.01	0.56 ± 0.04	11	10
		Benzenoids			
eugenol	1.3	92.4 ± 5.3	58.7 ± 4.4	15	10
vanillin	25	42.3 ± 3.7	38.5 ± 0.2	2	2
		Alcohols			
(E)-3-hexen-1-ol	110	433.8 ± 34.3	116.7 ± 3.8	4	1
(Z)-3-hexen-1-ol	13	371.3 ± 37.7	166.8 ± 3.4	28	13
2-phenylethyl alcohol	140	327.5 ± 13.7	88 ± 2.4	2	<1
		Ketones			
1-octen-3-one	0.036	1.8 ± 0.3	1.7 ± 0.3	50	47
2-nonanone	5	15.7 ± 0.9	3.8 ± 0.1	3	<1

[a]Reference 46. [b]Reference 47.

maturity, growing environment, harvest and storage conditions, and analysis methods.[12,13,28]

Aroma-Active Volatiles Recognized by GC/O with a CycloSil-B Column. Because different optical isomers can persist distinct aroma and aroma intensities, enantiomer-specific evaluation of aroma-active compounds is necessary to unveil their aroma contribution. A modified β-cyclodextrin stationary phase in CycloSil-B column (30% heptakis (2,3-di-O-methyl-6-O-t-butyl-dimethylsilyl)-β-cyclodextrin in DB-1701) was used to resolve the enantiomers. The mid-polarity nature of the DB-1701 phase ((14% cyanopropyl-phenyl)-methylpolysiloxane) can separate other compounds based on their boiling point and interaction with the phase.

The results of GC/O with the chiral CycloSil-B column for Bluecrop and Elliott blueberries are presented in Table 3. Overall, less volatile compounds were detected by the CycloSil-B column with lower FD values compared to the ZB-WAXplus column. The decreased responses could be due to the nonspecific binding of aroma compounds with the cyclodextrin phase. The modified cyclodextrin phase is probably not as inert as the DB-5 or DB-wax phase. In addition, inclusion-dominated chiral separation generally has lower analyte capacity due to fewer interaction sites than a surface interaction mechanism. However, the trends of FD

factors of the aroma-active compounds identified were in good agreement with the wax column.

Two isomers of linalool and α-terpineol, R-(−)-, S-(+)-linalool, S-(−)-, and R-(+)-α-terpineol, were recognized by the four panelists. Other isomers might not be aroma contributors, or their concentrations were below the odor thresholds. The R-(−)-linalool exhibited a lavender-like aroma, whereas the S-(+)-linalool had floral and citrus notes. In both cultivars, the R-(−)-linalool isomer had much higher FD factors than the S-(+)-isomer. Both cultivars had the same FD factors of R-(−)-linalool (FD = 128) and (S)-(+)-linalool (FD = 16), which was in agreement with the results obtained on the wax column. The volatile qualities of S-(−)-α-terpineol and R-(+)-α-terpineol were also distinct. S-(−)-α-terpineol had a piney, floral note, and the R-(+)-α-terpineol had an oily odor. Both and S-(−) and R-(+) isomers had close FD factors, and there was no difference between the two cultivars for the isomer aroma contribution. Other aroma-active compounds identified with high FD (≥64) factors in Bluecrop cultivar were ethyl 2/3-methylbutanoate, (Z)-3-hexenal, methional, eucalyptol, benzeneacetaldehyde, (E,Z)-2, 6-nonadienal, geraniol, β-damascenone, and vanillin. For Elliott cultivar, hexanal, (E)-2-heptenal, R-(−)-linalool, geraniol, eugenol, and caryophyllene oxide could be essential odorants (FD ≥ 64). To our

Table 5. Quantitation Results of Enantiomers Identified in Blueberries

compounds	threshold (μg/L)[a]	enantiomeric ratio[b] (%)		concentration (μg/L)[c]		OAV	
		Bluecrop	Elliott	Bluecrop	Elliott	Bluecrop	Elliott
R-(−)-linalool	0.8	16	13	19.9	15.2	25	19
S-(+)-linalool	7.4	84	87	104.7	101.5	14	14
R-(+)-α-terpineol	6800	44	42	20.2	66.3	<1	<1
S-(−)-α-terpineol	9180	56	58	25.6	91.6	<1	<1

[a]Reference 44. [b]Based on the peak area from the isomeric chromatogram. [c]Calculated by multiplying the total concentration (Table 4) with the peak area percentage.

knowledge, using a chiral column for AEDA to evaluate the relative aroma contribution of chiral isomers is the first time.

Odor Activity Value of Aroma-Active Compounds in Blueberry. Although the consensus is that the higher the FD factor it has, the more potent the volatile compounds could be to the overall blueberry aroma, the FD factor is not a direct measure of a volatile's odor contribution. A calculation of OAVs (ratio of the concentration to odor threshold) is frequently used to evaluate volatile compound potency to the overall aroma. The OAVs of the aroma-active compounds are summarized in Table 4.

In blueberries, not only fewer aroma compounds have been identified than strawberry, blackberry, and raspberries but also the quantity of volatiles is less,[24,33,34] reflecting less aromatic for blueberry. Quantitative results showed that all of the compounds quantitated had concentrations higher than their threshold, except for α-terpineol, indicating that they are blueberry aroma contributors. The data of OAVs were in good agreement with AEDA-GC/O results. Volatiles with OAVs greater than 10, including ethyl 3-methylbutanoate, hexanal, (Z)-3-hexenal, (E,Z)-2,6-nonadienal, (E,E)-2,4-nonadienal, linalool, β-damascenone, geraniol, (Z)-3-hexan-1-ol, eugenol, and 1-octen-3-one, also had high FD factors.

Two oxygenated monoterpenes, linalool, and α-terpineol were further studied for their enantiomer composition (Table 5). Based on peak areas on the CycloSil-B column, S-(+)-linalool was dominant in both blueberry cultivars. Bluecrop with a linalool (R/S) isomeric ratio of 16/84, and Elliott had a linalool (R/S) isomeric ratio of 13/87. Linalool enantiomer distribution in blueberry agrees that in blackberry,[24] orange juice,[43] and citrus oils[44] with the S-(+)-form dominating. Although S-(+)-linalool was dominant in blueberry, R-(−)-linalool had a higher FD value and OAVs than that of the (S)-isomer. It suggested that the (R)-(−)-linalool could be more important than the (S)-(+)-linalool because the S-(+)-linalool has a 9 times higher odor threshold than R-(−)-linalool. The S-(−)-α-terpineol was slightly elevated than R-(+)-α-terpineol in both cultivars. Bluecrop had an α-terpineol (R/S) isomeric ratio of 44/56. Elliott had a similar α-terpineol (R/S) isomeric ratio of 42/58. The same trend was reported in lemon-flavored hard iced tea,[23] while the S/R-α-terpineol ratio varied depending on varieties in blackberry.[24] α-Terpineol could impart a pine, citrus, woody, or floral odor to the product. There were no reliable thresholds for S-(−)-α-terpineol and R-(+)-α-terpineol in a water-based matrix. But their thresholds in *Citrus sudachi* peel oils were reported to be 9.18 and 6.80 ppm, respectively.[44] Although the calculated OAV values of α-terpineol and its enantiomers were less than 1, they may have a synergistic effect and still contribute to the blueberry aroma; besides, the GC/O study suggested that they were aroma contributors. This discrepancy of OAVs with GC/O results is probably because the AEDA was performed based

on the GC column's effluent. In contrast, the OAV value was calculated based on sensory thresholds in the matrix. Also, GC/O is a "sensomics" approach that uses the human nose as a detection device. The olfactometric screening inherits the bias of human behavior. Thus, GC/O may yield inconsistent results, as reviewed by Delahunty et al.[29] Overall, both GC/O and OAVs assign relative importance to each aroma-active compound. FD factors and OAV values are not linear relationships.

Terpenes are not stable and can be easily transformed into other compounds. Clark and Chamblee[45] showed that limonene could be hydrolyzed to α-terpineol without stereo preference with a mixture of the *cis*-isomer, *trans*-isomer, and 4-terpineol under acidic conditions. Terpene alcohols can then be transformed from one to another.

The chiral enantiomers can undergo racemization, especially under acidic conditions.[23] In the hard-tea aging study,[23] an increased concentration of R-(+)-α-terpineol and decreased S-(−)-α-terpineol were observed with time. Kotter-Seel et al.[43] illustrated that the percentage distribution of (R)-(−)- and (S)-(+)-linalool in orange juice changed from an initial of 11 and 89%, respectively, to 38% (R)- and 62% (S)-enantiomer after 47 days at 35 °C. Similarly, racemization was observed during lemon-flavored hard-tea aging.[23] (R)-(−)-linalool was in greater abundance than its (S)-isomer in hard-tea samples at the beginning of aging. After 8 weeks of storage, the (R)/(S) ratio decreased with the conversion of (R)-(−)-linalool to (S)-(+)-linalool in a time and temperature-dependent model. Any change in the concentration of enantiomers or ratio of isomers in food may indicate the change of flavor quality.[21] The odor activity values of (R)-(−)- and (S)-(+)-linalool were also decreased with aging for hard tea, indicating the overall aroma loss with racemization.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.1c01044.

Sources and purities of chemicals and solvents used in this study and mass ion (*m/z*) of isotope-labeled compounds (PDF)

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Notes
The authors declare no competing financial interest.

◼ ABBREVIATIONS

GC−MS/O, gas chromatography−mass spectrometry/olfactometry; SBSE, stir-bar sorptive extraction; SPME, solid-phase microextraction; OAV, odor activity values; AEDA, aroma extract dilution analysis; FD, flavor dilution; RI, retention index; TDU, thermal desorption unit; SAFE, solvent-assisted flavor evaporation

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