Qualitative Analysis of Volatiles in Rabbiteye Blueberry Cultivars at Various Maturities Using Rapid Solid-phase Microextraction

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ABSTRACT. There are very few studies detailing the aroma, astringency, and flavor of rabbiteve blueberry [RAB (Vaccinium ashei)] fruit typically grown in the southeastern United States. The objectives were to investigate the rapid and qualitative solid-phase microextraction gas chromatographic-mass spectrometry volatile composition of several local RAB cultivars with an overall goal to build a database of possible flavor and aroma compounds. Volatile profiles were obtained in five Louisiana-grown RAB cultivars (Brightwell, Climax, Premier, Powder Blue, and Tifblue) assayed at four maturities. The method routinely captured 53 volatiles, including 12 aldehydes, six alcohols, 11 esters, four ketones, 17 terpenoids, one furan, and two aromatics. Of the 33 compounds considered important in blueberries, 17 were recovered in the RAB cultivars assessed. Herein, 10 compounds were recovered for the first time in blueberry (Vaccinium sp.) and five of those compounds were confirmed with standards [2-ethylfuran, (E)-2-pentenal, (Z)dehydroxylinalool oxide, (E)-dehydroxylinalool oxide and 1,4-cineole|. In general, terpenoids and their subclass linalools were the most significant volatiles followed closely by esters, aldehydes, and then alcohols. Terpenoids and linalools displayed the greatest significant differences in 'Powder Blue' and 'Premier'. Esters and aldehydes were the most significant compound classes based on cultivar effect per maturity in firm-ripe fruit. From the suite of 17 of the 33 important compounds in upright blueberry, 10 were recovered across the five cultivars at four maturities that displayed a high level of significance. These were linalool, methyl 3-methylbutanoate, 1,8-cineole, (E)-2-hexanal, (Z)-3hexenal, (Z)-3-hexenyl acetate, limonene, hexyl acetate, hexanal, and α -terpineol. These data will be useful to evaluate aroma volatiles in RAB and changes in processed and value-added byproducts.

Heightened consumer awareness of the health benefits of consuming phytonutrient-rich fruits and aggressive marketing have resulted in expanding markets for blueberries, fruit juices, a large number of nutritional supplements, and value-added foods containing berries. Diets rich in blueberries deliver anti-inflammatory, anticarcinogenic, and antimutagenic components that help protect the brain, cardiovascular and central nervous system as well as reduce cancer, obesity, and type 2 diabetes (Basu et al., 2010; Kalt et al., 2007; Miller and Shukitt-Hale, 2012; Seeram, 2008; Soto-Vaca et al., 2012). "Superfruits" such as blueberries have become popular as scientists, marketers, and consumers push forward knowledge and demand for high-antioxidant, healthier diets.

Over the last decade in the southeastern United States there has been an almost 4-fold increase in blueberry production and use from 12.5 million kilograms in 1999 to 96.5 million kilograms in 2009 [U.S. Department of Agriculture (USDA),

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2010a]. More important than production, however, during this same period, the national value of the used blueberry crop has increased over 3-fold from \$154.0 million to \$501.5 million (USDA, 2010b). In 2008, the southeastern U.S. blueberry industry had ≈9632 ha of rabbiteye blueberry and southern highbush blueberry [SHB (*Vaccinium darrowii* × *V. corymbosum*)] production with a market value of \$72.8 million (Braswell et al., 2009). North American blueberry production acreage has increased significantly over the past few years in response to recent high blueberry demand and prices. Through 2005, the most common RAB cultivars grown in Louisiana and Mississippi were Climax, Premier, and Tifblue (Marshall et al., 2006). 'Tifblue' has been considered the most productive of all RAB and is generally the standard to which RAB are compared.

Volatile Compounds in Rabbiteye Blueberries

An extensive literature search indicates that volatile and semivolatile compounds in RAB appear in only three papers from the same laboratory for three cultivars (Horvat et al., 1983, 1996; Horvat and Senter, 1985). The majority of cultivars currently in production and on the market have little to no volatile information reported. Studies remain sparse regarding flavor volatiles and higher molecular weight (MW) bitter/astringent compounds, processing, and sensory attributes related to RAB (Makus and Morris, 1993). The first report documenting volatile data in SHB was recently published, yet

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there were general volatile differences indicated among SHB, RAB, and northern highbush blueberry [NHB (*V. corymbosum*)] types (Du et al., 2011). Over several years, many reports have indicated that volatile composition varies markedly between locations, within season, years, maturities, and cultivars (Baloga et al., 1995; Du et al., 2011; Horvat and Senter, 1985; Rohloff et al., 2009). The objective of this study was to investigate the qualitative volatile composition of several local RAB cultivars because berries are often culled and/or comingled for juicing and value-added products. Thus, a rapid qualitative solid-phase microextraction (SPME) method was used with the overall goal to build a database of possible flavor and aroma compounds in local blueberries that are consumed fresh and as processed products.

Materials and Methods

Fruit harvest and maturity designation. Fresh handpicked RAB were harvested from Louisiana during Summer 2010. Cultivars Premier and Powder Blue were harvested from a local U-pick operation in Bush, LA, on 19 June 2010. Cultivars Climax, Brightwell, and Tifblue were harvested from the Louisiana State University, Bob R. Jones Idlewild Research Station in Clinton, LA, on 24 June 2010. A stalk separator/ blower with a conveyor belt (BEI Inc., South Haven, MI) was used to de-stalk and sort ripe 'Tifblue' fruit, and all other cultivars were manually sorted after harvest. Cultivars were analyzed as fresh fruit at four distinct maturities. Maturity was designated as mature green [MG (white/green)], light pink or "blue-red" (\approx 75% blue and \approx 25% red), firm-blue or firm-ripe [FR (100% blue)] (Eck, 1986), and soft-blue or soft-ripe (SR). A replication of each treatment was a bag of randomly harvested fruit coming from several bushes for the same cultivar all harvested on the same date. Bushes for a specific cultivar were interspersed in separate rows with other cultivars (as a result of self-incompatibility pollination issues) and three separate samples/rows were used to attain randomization within the field. All maturities came from the same harvest (divided into maturity groups based on color) and therefore date of harvest is not confounded with maturity and not part of the definition of the experimental unit.

JUICE SAMPLE PREPARATION. To derive a rapid headspace (HS) volatile juice sample, whole fresh berries were homogenized because MG and pink fruit would not readily juice as a result of low moisture content and firmness. Ten grams of berries of each color/firmness in each cultivar were placed in a 20-mL SPME vial and homogenized (Tissumizer SDT-1810; Tekmar, Cincinnati, OH) for \approx 1.5 min using an 8-mm-diameter probe. After homogenization, 2.2 g of NaCl was added, vials were sealed and vortexed for 30 s, and placed on an autosampling tray at 1 °C on gas chromatography-mass spectrometry (GC/MS), similar to previously described methods used in fruit juice (Beaulieu, 2006; Beaulieu and Grimm, 2001) and wine (Torrens et al., 2004).

HEADSPACE SOLID-PHASE MICROEXTRACTION GAS CHROMATOGRAPHY—MASS SPECTROMETRY. Volatile sample vials (20 mL) were equilibrated 10 min by oscillation in a 35 °C autosampler (MPS2 XL; Gerstel, Baltimore, MD); then the HS was exposed to 1-cm 50/30 μm triple-fiber (divinylbenzene carboxen polydimethylsiloxane) SPME (StableFlex; Supelco, Bellefonte, PA) for 15 min at 35 °C. Vials were continuously swirled at 100 rpm during SPME adsorption before injection

into GC/MS (HP6890/HP5973; Agilent Technologies, Santa Clara, CA) with a crosslinked 5% phenyl methyl silicone column with a 30 m × 0.25 mm × 0.25-µm film thickness (HP-5; Agilent Technologies). The injection port was operated in splitless mode and subjected to a pressure of 172.4 kPa of ultrahigh purity helium (99.9995%) for 1 min, and flow velocity was constant at 40 cm·s⁻¹ for the remainder of the GC run. Initial oven temperature was 50 °C, held 1 min, ramped 5 °C·min⁻¹ to 100 °C, then 10 °C·min⁻¹ to 250 °C and held 9 min. The HP5973 quadrupole MS was operated in the electron ionization mode at 70 electron volts at 200 °C with a continuous scan from 33 to 300 mass to charge ratio (m/z).

DATA ANALYSIS. MS data were collected with HP ChemStation software (A.03.00; Agilent Technologies) and searched against the Wiley7th/NIST02 registry of mass spectral data (McLafferty, 2000). Library identification was confirmed by standard comparisons, GC retention time (RT), MS ion spectra, and a retention index (RI), per Table 1. An RI was calculated from the averaged RT from triplicated series of straight chain alkanes $(C_6 - C_{18})$. Integrated target ion response was examined carefully by selected unique target ions per compound, qualifying ion (Q-ion) ratio, and GC/MS grade (greater than 95% to 99.9% purity) standards purchased from commercial sources {Aldrich Chemical Co. [Milwaukee, WI], Bedoukian Research [Danbury, CT], Fluka [Buchs, Switzerland (now Sigma-Aldrich, St. Louis, MO)], Fisher [Pittsburgh, PA], JT Baker [Phillipsburg, PA], Mallinckrodt [St. Louis, MO], Sigma [Sigma-Aldrich], and Sigma-Aldrich Fine Chemicals, Flavors and Fragrances Products [St. Louis, MO]}, according to Table 1. Compound classes were defined and comprised of aldehydes (ALD), alcohols (OLS), esters (EST), ketones (ONE), furans (FUR), terpenoids (OID), and aromatics (ARO) according to Table 1. OID were further subdivided into terpenes (ENE) and linalools (LOO).

STATISTICAL ANALYSIS. The experimental design was a completely randomized design with a factorial treatment structure for five cultivars at four maturities and three replicates per treatment. Volatile measurements were taken on 53 compounds and these were analyzed individually and as aforementioned compound classes (Table 1). The treatments had a factorial structure of five cultivars and four maturities resulting in 20 treatments. However, compounds were not detected in all treatment combinations. Omission of zeros for no detection from the data (Table 2) was performed because transformations of the responses including too many zeros is faulty (Sokal and Rohlf, 2012) and constructing a nonparametric test for treatment differences will not work with abundant zeros (Conover, 1971). Analysis of variance (ANOVA) was used to examine cultivar and harvest treatment effects for each compound individually and in groups with SAS (Version 9.1; SAS Institute, Cary, NC).

For any given compound, there were often small measurements and few with much larger measurements, resulting in a mean distribution that was not symmetric but skewed to the right. Volatile measurements did not meet the ANOVA assumptions required for the dependent variable because they were often not normally distributed. Using a single volatile measurement (integrated unique Q-ion MS response) as a percent of the total volatiles for a given sample was considered to correct for this problem. However, percentages were not used because not all compounds were measured in each treatment combination and therefore there was no way to consistently

obtain a denominator (i.e., total volatiles in all compounds) for every sample. Therefore, a log transformation was applied to raw volatile measurement to make it fit the assumption of normality required for ANOVA. This allowed distribution to approximate normality and balance the disproportionate high abundance vs. low abundance volatiles and to apply F-test inferences.

To measure how each volatile compound was affected by maturity and cultivar, three contrast statements (resulting in F

Table 1. Volatile compounds qualitatively recovered by headspace solid-phase microextraction using divinylbenzene carboxen polydimethylsiloxane fibers and gas chromatography—mass spectrometry in five rabbiteye blueberry cultivars.

Compound	Standard ^z	RI ^y	CAS no.x	Compound class ^w
Acetaldehyde	S	_	75-07-0	ALD
Ethyl acetate	M	607	141-78-6	EST
3-Methylbutanal	A	650	590-86-3	ALD
2-Methylbutanal	SAFC	660	96-17-3	ALD
1-Penten-3-ol	В	679	616-25-1	OLS
3-Pentanone	S	692	96-22-0	ONE
2-Ethylfuran ^v	SAFC	697	3208-16-0	FUR
Ethyl propanoate	A	709	105-37-3	EST
Methyl butyrate	F	722	623-42-7	EST
(E)-2-Pentenal ^v	A	763	1576-87-0	ALD
Methyl-2-methylpropanoate	u	765	547-63-7	EST
1-Pentanol	Fi	780	71-41-0	OLS
2-Methyl-4-pentenal	_	781	5187-71-3	ALD
(Z)-3-Hexenal	В	781	6789-80-6	ALD
Hexanal	A	783	66-25-1	ALD
(Z)-2-Pentenol	A	784	1576-95-0	OLS
Methyl 3-methylbutyrate	F	792	556-24-1	EST
(Z)-2-Hexenal	_	842		ALD
Ethyl 2-methylbutyrate	A	844	7452-79-1	EST
Ethyl 3-methylbutyrate	A	851	108-64-5	EST
(E)-2-Hexenal	A, B	852	6728-26-3	ALD
(Z)-3-Hexenol	A	853	928-97-2	OLS
(E)-2-Hexenol	A	862	928-95-0	OLS
1-Hexanol	F	867	111-27-3	OLS
2-Heptanone	S	888	110-43-0	ONE
Isopropyl 3-methylbutanoate	S	893	32665-23-9	EST
(E,E)-2,4-Hexadienal	S	916	142-83-6	ALD
(E)-2-Heptenal	SAFC	947	18829-55-5	ALD
Benzaldehyde	JT	954	100-52-7	ARO
6-Methyl-5-hepten-2-one	A	976	110-93-0	ONE
β-Myrcene	S	981	123-35-3	OID, ENE
(Z)-Dehydroxylinalool oxide ^{v,t}	В	982	13679-86-2	OID, LOO
(Z)-3-Hexenyl acetate	В	997	3681-71-8	EST
(E)-Dehydroxylinalool oxide ^v	В	997	13679-86-2	OID, LOO
Hexyl acetate	A	1004	142-92-7	EST
(E)-2-Hexenyl acetate	A	1006	10094-40-3	EST
Isocineole (1,4-cineole) ^v	F	1007	470-67-7	OID, ENE
p-Cymene	A	1018	99-87-6	OID, ENE
Limonene	Fi	1022	5989-27-5	OID, ENE
1,8-Cineole	A	1025	470-82-6	OID, ENE
Phenylacetaldehyde	A	1038	122-78-1	ARO
(Z)-Linalool oxide	A	1067	5989-33-3	OID, LOO
α-Terpinolene	F	1081	99-86-5	OID, ENE
(E)-Linalool oxide	A	1083	34995-77-2	OID, LOO
2-Nonanone	A	1086	821-55-6	ONÉ
Linalool	A	1097	78-70-6	OID, LOO
Nonanal	A	1101	124-19-6	ALD
α-Terpineol	A	1197	98-55-5	OID
δ-Elemene		1341	20307-84-0	OID, ENE
β-Bourbonene		1392	5208-59-3	OID, ENE
γ-Caryophellene ^t		1415	118-65-0	OID, ENE
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tests) were constructed from the ANOVA to address the factorial treatment structure with missing treatment combinations (Table 2). Overall F value measures the combined effect of both maturity and cultivar on the volatile's response (contrast H₀: all treatments are equal). Maturity within cultivar F value measures treatment effects resulting from maturity only (contrast H₀: all maturity treatments for cultivar Brightwell are equal + all maturity treatments for 'Climax' are equal + ...). Cultivar within maturity F value measures treatment effects resulting from cultivar only (contrast H₀: all cultivar treatments for MG maturity are equal + all cultivar treatments for pink maturity are equal + ...).

Results and Discussion

Using StableFlex triple-fiber SPME, the five cultivars (Brightwell, Climax, Premier, Powder Blue, and Tifblue) sampled at four maturities contained 53 volatiles (Table 1). Seven of the 53 compounds were only tentatively identified based on MS ion fragmentation patterns because standards were not obtained. With FR and SR fruit, a suite of 32 to 39 and 36 to 40 compounds, respectively, in a given cultivar were recovered. With pink and MG fruit, respectively, a suite of 28 to 33 and 31 to 34 compounds in a given cultivar was recovered. In the five RAB cultivars analyzed with this method, the volatiles recovered were principally OID (17), ALD (12), EST (11), OLS (six), and ONE (four).

Ten compounds not previously reported in blueberry were recovered (Table 1). These were: 2-ethylfuran, (E)-2-pentenal, 2-methyl-4-pentenal (tentative), isopropyl 3-methylbutanoate (tentative), (Z)-dehydroxylinalool oxide, (E)-dehydroxylinalool oxide, 1,4-cineole (isocineole), δ -elemene (tentative), α -caryophellene [also known as α -humulene (tentative)], and γ -caryophellene (tentative). Five were confirmed by standards and designated "first known observation" and the remainder are

Table 1. Continued.

Compound	Standard ^z	RI^y	CAS no.x	Compound class ^w
β-Caryophellene	F	1434	87-44-5	OID, ENE
α -Caryophellene (α -humulene)	F	1471	6753-98-6	OID, ENE

*Source of standards used for compound confirmation: A = Aldrich Chemical Co. (Milwaukee, WI); B = Bedoukian Research (Danbury, CT); F = Fluka [Buchs, Switzerland (now Sigma-Aldrich, St. Louis, MO)]; Fi = Fisher (Pittsburgh, PA); JT = JT Baker (Phillipsburg, PA); M = Mallinckrodt (St. Louis, MO); S = Sigma-Aldrich; SAFC = Sigma-Aldrich Fine Chemicals, Flavors and Fragrances Products (St. Louis, MO).

^tIsomer assumed based on other isomer standard confirmations, RT and mass ion fragmentation.

"tentative." (*Z*)-dehydroxylinalool oxide and (*E*)-dehydroxylinalool oxide (MS library hit "herboxide," MW 152.237), as confirmed by RT with standards and MS ion fragmentation patterns, were present (Table 1). These could possibly be the same two compounds labeled as (*Z*)-dihydrolinalool oxide and (*E*)-dihydrolinalool oxide (MW 156.27) as tentatively identified previously (Du et al., 2011). However, both oxidized forms of linalool may exist because there are slightly different ring unfolding structures that cause differing MWs, RTs, and RIs.

Over 3 years sampling blueberry volatiles, the composition varied markedly between locations, within season, years, harvest dates, and between maturities for the same cultivar (data not shown). Four of the five cultivars were repeated in a separate year harvested in the same two locations. Because locations varied and our methods varied as analytical approaches were improved (as more standards were acquired), data were not combined. During these and other blueberry evaluations of three SHB vs. three RAB harvested twice from the same plot in a single season, several additional volatiles were recovered including: 2-heptanol, γ-terpineol, hotrienol, β-damasenone, (Z)-geranylacetone, (E)-geranylacetone, geraniol, p-menth-1-en-9-al, and p-cymen-8-ol and the apparently "important" geraniol (data not shown). Regardless, within- and between-field variability yields incredible volatile differences, as documented in the literature (Du et al., 2011), and this is not easy to overcome. Hence, the use of other berries, like grapes (Vitis sp.), used to prepare juices to make wines, is considered "an art," not a science (Lund and Bohlmann, 2006).

Because dissimilarity in volatile trends has been reported in blueberries (Baloga et al., 1995; Du et al., 2011; Horvat and Senter, 1985; Rohloff et al., 2009), data were analyzed by omitting zeros for no detection. We purposely chose to highlight compounds detected, treating their presence, as opposed to considering their absence, as a zero. This effectively precluded occasional absence from negatively affecting compound distribution in the analysis. The typical two-way ANOVA with main effects and interactions could be misleading for compounds that had missing treatment combinations (Conover, 1971; Sokal and Rohlf, 2012). For example, performing the ANOVA with zeros included resulted in the highest overall F values generated in the FUR (one compound) and EST (11 compounds) classes (data not shown). Only 2-ethylfuran was recovered in very few treatments (Table 2), and

the esters tended to be either absent while immature or highly abundant when FR or SR, and individual esters also varied by maturity and slightly by cultivar (Table 2). ALD and OID ranked as the third and fourth most significant volatile classes, respectively (in the ANOVA with zeros included, data not shown). As a result of zeros, low df, and variability in individual compounds, these appraisals were not realistic. Subsequently, ANOVA F tests were obtained for: 1) overall treatment effect (including effects of both cultivar and maturity treatments) where df indicates number of treatments minus one (Tables 3 and 5); 2) effects of

maturity treatments for each cultivar group (Tables 4 and 6) and combined into one joint F test for maturity treatment effects within all cultivar groups (Tables 3 and 5); and 3) effect of cultivar treatments for each maturity group (Tables 4 and 6) combined into one joint F test for cultivar/treatment effects within all maturity groups (Tables 3 and 5).

Most volatiles displayed highly significant F values (P < 0.001) for all three contrast statement tests. This is similar to previously published quantitative volatile appraisal in SHB where an ANOVA was used (Du et al., 2011). However, herein, compounds were ranked to sensitivity to maturity and cultivar effects based on the magnitude of the F values. Table 2 is a summary of data used in the analysis by indicating all treatments and combinations, thereof reported as the percentage of observations delivering results (i.e., volatile measurements > 0 or y > 0). For example, in the ALD compound group, 'Tifblue' at the pink stage had 100% observations with y > 0 (based on 12 ALD × three replications = 36 observations) and 92% for 'Brightwell' at MG, where compounds were not recovered in some treatments and combinations (e.g., 33 of 36 with y > 0).

Variation by cultivar. When overall F values were sorted to display the greatest significance per compound class, OID and the subclass LOO were most significant followed closely by EST, ALD, and then OLS (Table 3). As expected based on previous maturity-related cantaloupe (Cucumis melo var. reticulatus) volatile data using a similar SPME method (Beaulieu, 2006), maturity differences delivered the most significance in compound classes generally believed to be aroma-important in blueberry such as OID, LOO, EST, OLS, and ARO. In general, blueberries have appreciable terpenoid content (Du et al., 2011; Hirvi and Honkanen, 1983a; Horvat et al., 1983; von Sydow and Anjou, 1969). Several compounds recovered and denoted in the literature are terpenoids and terpenoid alcohols (such as α-terpineol and linalool), which are common flavor and aroma compounds in blueberry (Du et al., 2011; Horvat and Senter, 1985; Parliment and Kolor, 1975; von Sydow and Anjou, 1969) and other berries such as grapes (Marais, 1983). As a result of diversity and range of terpenoid concentrations reported by cultivar in SHB, it has been speculated that terpenoids might be the group of compounds that help determine the characteristic

^yRetention index; no RI extrapolated for acetaldehyde as the retention time (RT) fell outside the RT range of the recovered alkanes.

^{*}Chemical Abstract Service number.

[&]quot;Volatile compound classes used for statistical grouping: ALD = total aldehydes; OLS = total alcohols; EST = total esters; ONE = total ketones; FUR = total furans; ARO = total aromatics; OID = total terpenoids (subclasses within OID include ENE = total terpenes and LOO = total linalools).

^vFirst known observation.

[&]quot;Standard not available.

Table 2. Percent of observations and total number of possible observations for volatiles by class, cultivar, and maturity for the 53 compounds comprising seven classes of aroma compounds recovered by headspace solid-phase microextraction gas chromatography—mass spectrometry in five rabbiteye blueberry cultivars at four maturities.

					n with y	$y > 0 \ (\%$				Observations (no.) ^x					
Cultivar	Maturityz	ALDw	ARO	EST	FUR	OID	OLS	ONE	ALD	ARO	EST	FUR	OID	OLS	ONE
									(36)	(6)	(33)	(3)	(51)	(18)	(12)
Brightwell	MG	92	50	27	100	47	100	75	33	3	9	3	24	18	9
	Pink	92	100	9	v	43	67	50	33	6	3	0	22	12	6
	FR	92	100	18	_	63	83	50	33	6	6	0	32	15	6
	SR	83	100	64	_	73	67	50	30	6	21	0	37	12	6
Climax	MG	92	50	27	_	49	72	25	33	3	9	0	25	13	3
	Pink	92	50	27	_	35	83	50	33	3	9	0	18	15	6
	FR	83	50	36		65	67	50	30	3	12	0	33	12	6
	SR	67	50	73		71	83	50	24	3	24	0	36	15	6
Powder Blue	MG	92	50	30	100	43	83	75	33	3	10	3	22	15	9
	Pink	92	50	39	100	35	72	50	33	3	13	3	18	13	6
	FR	83	100	67	_	65	72	50	30	6	22	0	33	13	6
	SR	75	100	82	_	65	67	50	27	6	27	0	33	12	6
Premier	MG	94	50	9	100	45	83	42	34	3	3	3	23	15	5
	Pink	92	50	39	100	33	67	75	33	3	13	3	17	12	9
	FR	81	83	48		65	67	58	29	5	16	0	33	12	7
	SR	83	100	73		65	72	50	30	6	24	0	33	13	6
Tifblue	MG	92	50	27	100	41	83	75	33	3	9	3	21	15	9
	Pink	100	50	36	100	41	83	75	36	3	12	3	21	15	9
	FR	100	100	64		59	83	75	36	6	21	0	30	15	9
	SR	83	100	82	_	61	83	75	30	6	27	0	31	15	9
Cultivar									(144)	(24)	(132)	(12)	(204)	(72)	(48)
Brightwell		90	88	30	25	56	79	56	130	21	40	3	114	57	27
Climax		83	50	41		55	76	44	120	12	54	0	112	55	21
Powder Blue		85	75	55	50	52	74	56	122	18	73	6	106	53	27
Premier		88	71	42	50	52	72	56	127	17	55	6	106	52	27
Tifblue		94	75	52	50	50	83	75	135	18	69	6	102	60	36
Maturity									(180)	(30)	(165)	(15)	(255)	(90)	(60)
MG		92	50	24	80	45	84	58	166	15	40	12	115	76	35
Pink		93	60	30	60	38	74	60	167	18	50	9	97	67	36
FR		88	87	47		63	74	57	158	26	78	0	161	67	34
SR		78	90	75		67	74	55	140	27	124	0	171	67	33

 $^{^{}z}MG$ = mature-green (white/green), pink, light pink or "blue-red" (\approx 75% blue and \approx 25% red); FR = firm-blue or firm-ripe (100% blue); SR = soft-blue or soft-ripe.

aroma per cultivar (Du et al., 2011). OID also yielded the second greatest significant difference based on cultivar, which was very close in ranking compared with ALD followed by EST (Table 3). Little emphasis is placed on FUR, because only one compound was recovered and only present in some cultivars in MG and pink stages (Table 2). ONE also had a more significant cultivar than maturity effect. Although

a subclass of OID, the ENE category (non-oxygenated terpenes) displayed more significance per cultivar (Table 3). This illustrates again the relative importance of OID, LOO, and one terpene alcohol (α -terpineol), which had the opposite and most pronounced significance related with maturity.

Aldehydes were reported and recovered long ago in lowbush blueberry [LOW (Vaccinium angustifolium and Vaccinium

 $^{^{}y}y > 0$ refers to the percentage of responses greater than 0 calculated based on number of compounds per class and replications, cultivars, and maturities observed divided by the total combinations possible.

^{*}Number of observations which includes compounds per cultivar and/or per maturity × three replications. Maximum number of observations is given in parentheses in italics atop each subsection.

[&]quot;Volatile compound classes used for statistical grouping: ALD = total aldehydes; OLS = total alcohols; EST = total esters; ONE = total ketones; FUR = total furans; ARO = total aromatics; OID = total terpenoids (subclasses within OID include ENE = total terpenes and LOO = total linalools).

v— Indicated the treatment combination did not exist to create an F value.

Table 3. Analysis of variance F values indicating differences in maturity/cultivar treatments in volatile compound classes recovered in five rabbiteye blueberry cultivars at four maturities using headspace solid-phase microextraction with divinylbenzene carboxen polydimethylsiloxane fibers and gas chromatography—mass spectrometry.

			F value					
Compound class ^z	Compounds (no.) ^y	df	Overall ^x	Maturity within cultivarw	Cultivar within maturity ^v			
OID	17	19	38.25	33.40	17.67			
LOO	5	19	23.48	26.74	5.42			
EST	11	19	21.21	22.36	17.24			
ALD	12	19	20.17	11.59	18.05			
OLS	6	19	20.09	18.47	9.97			
ARO	2	19	16.78	19.18	10.51			
FUR	1	6	12.16	21.72	1.52 NS			
ONE	4	19	12.12	4.19	13.73			
ENE	11	19	11.37	5.53	9.73			

²Compound classes are ranked by overall F value: ALD = total aldehydes; OLS = total alcohols; EST = total esters; ONE = total ketones; FUR = total furans; ARO = total aromatics; OID = total terpenoids (subclasses within OID include ENE = total terpenes and LOO = total linalools). ³Total number of possible compounds per class.

myrtillus)] and considered as important aroma components in highbush types (Hirvi and Honkanen, 1983a; Rohloff et al., 2009; von Sydow et al., 1970; von Sydow and Anjou, 1969). ALD displayed the most significance by cultivar effect. ALD also comprised the vast majority of volatiles (54% to 77%) in SHB, even using an extraction method that was not considered oxidative (Du et al., 2011). Although methods used herein and in previous RAB reports may favor oxidation (Horvat et al., 1983; Horvat and Senter, 1985), aldehydes dominated the volatile profiles. Unlike the methods of Du et al. (2011) where salt was added during sample preparation to reduce oxidation, we added salt only to drive volatiles into the HS, after blending. Many C₆ and C₉ unsaturated aldehydes are generally considered to be products of fatty acid oxidation. Nonetheless, appreciable and similar aldehydes in the cultivars of RAB tested here were recovered. Like in cucumber (Cucumis sativus) and tomato (Solanum lycopersicum), C₆ aldehydes and alcohols seem to be flavor-important (Parliment, 1977), regardless of whether they are generated by oxidation (Fleming et al., 1968; Parliment and Kolor, 1975; Riley and Thompson, 1998).

Additional F values were used to measure the specific effect of maturity within cultivar and presented separately for each cultivar as well as to measure cultivar effect within maturity (Table 4). The most differences in compound classes based on maturity effects per cultivar occurred in 'Climax' and 'Tifblue' compared against 'Brightwell', 'Powder Blue', and 'Premier' (Table 4). This could be because these former two cultivars do not share the same relative proportion of the original four land races as the latter three cultivars that share 25% background from 'Ethyl', 'Myers', 'Clara', and 'Black Giant'. This is logical as a result of common lineage tracing back to the same four parental land races (Aruna et al., 1995; Lyrene, 1983; Rowland et al., 2010). Diploid parental lineages from the putative progenitor species of cultivated LOW, NHB, and RAB contain many of the same aroma volatile patterns among species and the groupings of nine quantified flavor compounds correlated with the taxonomic allozyme groupings (Baloga et al., 1995). This coincides well with principle component analysis of the original seven progenitors of cultivated U.S. diploid blueberry (Bruederle and Vorsa, 1994). However,

Table 4. F values for additional analysis of variance tests to assess impact between compound classes recovered by headspace solid-phase microextraction gas chromatography—mass spectrometry in five rabbiteye blueberry cultivars at four maturities.

	F test for maturity effect per cultivar group						F test for cultivar effect per maturity					
Compound class ^z	Brightwell	Climax	Powder Blue	Premier	Tifblue	MG ^y	Pink	FR	SR			
OID	16.19	24.13	59.16	46.07	21.46	3.74*x	21.09	23.09	22.75			
LOO	16.46	22.12	45.32	31.22	18.59	2.25 ns	5.74	9.37	4.35**			
EST	26.54	26.41	18.16	28.78	11.91	14.89	4.68**	37.38	11.99			
ALD	10.03	13.30	2.37 NS	2.91*	29.36	5.51**	16.51	34.37	15.83			
OLS	15.78	23.46	9.01	8.24	35.87	5.50**	7.82	16.98	9.55			
ARO	4.67**	52.57	25.94	10.49	2.22 NS	11.49	10.62	15.39	4.55			
FUR	w	_	5.88*	29.02	30.26	1.87 ns	1.00 ns	_	_			
ONE	$0.21~\mathrm{NS}$	1.49 ns	1.22 NS	1.77 ns	16.27	11.26	22.89	8.84	11.91			
ENE	2.08 ns	8.53	5.21**	8.40	3.42*	5.63	17.81	6.49	8.99			

²Compound classes are ranked by overall F value: ALD = total aldehydes; OLS = total alcohols; EST = total esters; ONE = total ketones; FUR = total furans; ARO = total aromatics; OID = total terpenoids (subclasses within OID include ENE = total terpenes and LOO = total linalools).

^yMG is mature-green maturity, followed by pink, FR for firm-ripe, and SR for soft-ripe.

^{*}F value measure of significance of average treatment effect, which is the result of the combination of cultivar and maturity.

wF value measure of significance of average treatment effect, which is the result of maturity only.

 $^{^{}v}$ F value measure of significance of average treatment effect, which is the result of cultivar only. No superscript indicates significance at $P \le 0.001$ (NS = nonsignificant).

^{*}Ns, *, **Nonsignificant or significant at $P \le 0.05$, or 0.01, respectively. No superscript indicates significance at $P \le 0.001$.

w— Indicated the treatment combination did not exist to create an F value.

Table 5. Analysis of variance F values indicating differences in maturity/cultivar treatments for individual headspace solid-phase microextraction gas chromatography—mass spectrometry volatile compound recovered in five rabbiteye blueberry cultivars at four maturities.

Compound ^z	df	Overall F value ^y	Maturity within cultivarx	Cultivar within maturity ^w
β-Myrcene	9	548.20	299.41	475.50
Linalool	19	99.60	119.16	12.52
Methyl butyrate	8	64.66	11.77	73.44
Methyl 3-methylbutyrate	14	50.82	54.14	33.95
1,8-Cineole	19	40.31	12.17	45.24
2-Methyl-4-pentenal	19	39.87	34.24	18.59
(E)-2-Hexenal	19	38.79	36.95	26.81
2-Heptanone	18	38.44	7.44	45.84
(E,E)-2,4-Hexadienal	19	37.78	26.60	25.01
(Z)-3-Hexenal	19	37.34	32.29	17.78
(Z)-Linalool oxide	19	35.81	40.58	10.50
(Z)-3-Hexenyl acetate	9	33.46	52.34	7.57
Limonene	17	32.63	32.65	13.95
Hexyl acetate	7	32.51	38.69	5.32
Hexanal	19	31.18	15.61	31.04
α-Terpineol	19	30.91	8.15	31.43
(E)-Linalool oxide	19	27.43	30.33	14.59
(Z)-2-hexenal	19	26.42	22.03	14.11
Phenylacetaldehyde	18	24.13	28.12	6.41
Acetaldehyde	19	16.91	17.03	5.72
Isopropyl 3-methylbutanoate	12	14.75	17.24	14.21
Benzaldehyde	9	14.32	3.34* ^v	16.56
β-Caryophellene	5	14.28	27.75	4.32*
Nonanal	19	13.50	8.17	13.25
p-Cymene	7	13.28	4.20*	13.00
1-Hexanol	19	12.64	10.13	11.94
2-Ethylfuran	6	12.16	21.72	1.52 NS
2-Nonanone	2	11.57	11.57**	u
Isocineole (1,4-cineole)	5	11.27	3.35*	8.72**
Ethyl acetate	8	10.98	15.87	3.52*
(Z)-2-Pentenol	17	10.52	12.75	2.45*
(E)-2-Hexenol	19	10.10	10.53	7.54
(E)-2-Pentenal	16	8.45	10.21	2.82**
2-Methylbutanal	10	8.44	5.23**	6.17
(E)-2-Hexenyl acetate	13	8.44	6.17	4.06**
1-Pentanol	11	8.35	4.51**	5.94
1-Penten-3-ol	19	8.26	7.54	3.67
Ethyl propanoate	8	8.03	10.26	4.84**
3-Methylbutanal	13	6.35	4.13**	6.94
6-Methyl-5-hepten-2-one	19	4.95	4.01	5.12
(Z)-Dehydroxylinalool oxide	10	4.73**	3.51**	3.52**
(E)-Dehydroxylinalool oxide	9	3.93**	2.31 NS	3.81**
α-Terpinolene	6	3.43*	2.29 ns	3.70*

²Bold font indicates reported to be aroma impact or high abundance in the blueberry literature. Italicized font indicates terpenoid (OID) or linalool (LOO) compounds displaying significant F values that might be of future interest to study.

'Brightwell' and 'Powder Blue' are siblings (Aruna et al., 1995) and the volatile profile for compound classes based on maturity effects per cultivar in 'Brightwell' was rather dissimilar compared with 'Powder Blue' and 'Premier'. A rational explanation for this trend eludes interpretation without several seasons and/or locations compared.

F values again indicated the relative importance and significance of OID (Table 4). OID and LOO displayed the greatest

significant differences in maturity per cultivar in 'Powder Blue' and 'Premier'. Herein, ARO variation over maturity was most significant in the cultivar Powder Blue, yet the ARO weight or importance is likely not as strong because only two compounds (benzaldehyde and phenylacetaldehyde) comprised this class. At lower concentrations, these aromatic aldehydes are generally associated with positive aroma attributes such as almond, bilberry, floral, hyacinth, sweet, and fruity-like (Latrasse,

yF value measure significance of average treatment effect, which is the result of cultivar and maturity combination.

^xF value measure significance of average treatment effect, which is the result of maturity only.

wF value measure significance of average treatment effect, which is the result of cultivar only.

vs, *, **Nonsignificant or significant at $P \le 0.05$, or 0.01, respectively. No superscript indicates significance at $P \le 0.001$.

[&]quot;— Indicated the treatment combination did not exist to create an F value.

Table 6. F values for additional analysis of variance tests to assess impact between individual compounds recovered by headspace solid-phase microextraction gas chromatography—mass spectrometry in five rabbiteye blueberry cultivars at four maturities.

	F test for maturity effect per cultivar group						F test for cultivar effect per maturity group				
Compounds ^z	Brightwell	Climax	Powder Blue	Premier	Tifblue	MG	Pink	FR	SR		
β-Myrcene	922.67	125.09	85.91	322.91	40.48	у	_	484.85	466.15		
Linalool	71.05	137.89	169.43	153.59	63.85	3.06*x	7.04	24.75	15.22		
Methyl butyrate	_	32.19	11.98	0.81 ns	2.08 ns	_	_	65.15	79.66		
Methyl 3-methylbutyrate	12.69	35.63	71.46	58.62	62.93	_	23.01	51.19	24.90		
1,8-Cineole	$2.39^{\rm NS}$	17.65	16.07	5.32**	19.41	75.40	53.38	26.33	25.85		
2-Methyl-4-pentenal	35.65	34.68	20.48	18.00	62.37	17.02	16.97	18.02	22.33		
(E)-2-Hexenal	35.99	72.17	48.67	11.30	16.63	29.00	6.56	31.97	39.70		
2-Heptanone	12.43	8.43	4.49**	1.01 ns	11.19	1.27 NS	79.29	48.75	42.91		
(E,E)-2,4-Hexadienal	23.67	59.28	9.25	6.61	34.22	9.57	13.71	35.15	41.62		
(Z)-3-Hexenal	39.16	34.86	18.13	13.88	55.44	15.13**	12.58	18.99	24.43		
(Z)-Linalool oxide	19.52	25.38	77.29	69.48	11.25	4.56	6.49	13.63	17.30		
(Z)-3-Hexenyl acetate	81.09	60.09	25.03	16.09	79.43	13.31	1.83 NS	_	_		
Limonene	40.90	12.20	41.62	41.91	13.35	4.09*	7.40	20.93	18.43		
Hexyl acetate	_	_	1.72 ns		51.01	8.17**	1.25 NS	_	3.68 NS		
Hexanal	28.62	28.82	11.91	0.71 ns	7.99	11.45	14.28	50.03	48.40		
α-Terpineol	7.93	1.9 NS	9.30	9.41	12.23	23.54	32.32	40.17	29.67		
(E)-Linalool oxide	6.15**	3.24*	81.59	56.84	3.82*	6.32	1.17 ns	22.67	28.20		
(Z)-2-hexenal	17.61	47.09	9.77	7.76	27.93	6.23	6.11	26.74	17.37		
Phenylacetaldehyde	21.94	63.43	31.95	21.45	13.59	9.26	8.54	2.55 ns	4.92**		
Acetaldehyde	28.27	11.15	15.61	20.74	9.36	1.42 NS	5.13**	6.31	10.04		
Isopropyl	0.56 ns	0.29 ns	26.16	29.14	0.30 ns	_	4.21*	17.75	13.17		
3-methylbutanoate											
Benzaldehyde	7.75**		0.15 ns	$1.05~\mathrm{ns}$	$0.00~\mathrm{ns}$	_	_	12.67	19.47		
β-Caryophellene	_	_	_	_	27.75	4.32*	_	_	_		
Nonanal	6.2**	3.06*	$0.65~\mathrm{ns}$	25.34	5.57**	19.93	9.49	5.42	18.17		
p-Cymene	_		4.09 ns	5.39*	3.13 NS	_	_	18.11	10.45		
1-Hexanol	10.51	23.42	$0.87~\mathrm{ns}$	7.98	7.88	10.93	3.71*	20.63	12.49		
2-Ethylfuran	_	_	5.88*	29.02	30.26	1.87 ns	1.00 NS	_	_		
2-Nonanone	_		_		11.57**	_	_	_	_		
Isocineole (1,4-cineole)	4.36*	0.33 NS	_		_	_	_	13.59	3.85 NS		
Ethyl acetate	_		10.30	12.91	27.38	_	_	8.02*	1.27		
(Z)-2-Pentenol	15.95	15.25	6.48	10.28	17.70	1.68 NS	1.60 ns	4.66**	2.42 NS		
(E)-2-Hexenol	6.35	28.15	4.51	8.22	5.41**	11.14	1.05 NS	11.64	6.35		
(E)-2-Pentenal	10.48	1.99 ns	6.76	10.60	15.82	3.87*	2.46 NS	2.84 ns	1.41 ns		
2-Methylbutanal	0.31 NS	3.34 NS	11.18*	3.78 NS	6.38*	4.99*	7.35	_	_		
(E)-2-Hexenyl acetate		10.21**	6.08**	1.37 ns	8.12	8.98	1.22 NS	3.45*	1.56 NS		
1-Pentanol	4.74*	3.87			5.25	3.89	0.64	5.88	8.45		
1-Penten-3-ol	13.23	5.40**	3.22*	7.82	8.01	2.43 NS	2.07 ns	7.66	2.52 NS		
Ethyl propanoate	_	23.39	7.75*	4.5 NS	5.39*		_	$1.05~\mathrm{ns}$	7.69		
3-Methylbutanal	1.98 NS	4.24*	17.24	2.54 ns	1.47 ns	8.92	8.42	0.00 ns			
6-Methyl-5-hepten-2-one	0.38 Ns	2.25 NS	8.62	2.38 NS	6.44	2.90*	2.89*	6.25	8.46		
(Z)-Dehydroxylinalool oxide	8.01	4.25 NS	0.19 ns	0.54 ns	0.05 ns	_	_	5.46**	1.57 ns		
(E)-Dehydroxylinalool oxide	6.81**	3.87 ns	0.16 ns	0.31 ns	0.37 ns	_	_	5.88**	1.74 ns		
α-Terpinolene	1.72 ns	_	1.53 ns	3.63 ns	_	_		2.95 NS	4.20*		

^zBold font indicates reported to be aroma impact or high abundance in the blueberry literature. Italicized font indicates terpenoid (OID) or linalool (LOO) compounds displaying significant F values that might be of future interest to study.

1991). OLS and ALD variation over maturity was most pronounced in 'Tifblue'. Aside from the difference in FUR (2-ethylfuran), 'Powder Blue' and 'Premier' displayed rather similar trends.

VARIATION BY MATURITY. EST and ALD were the most significant classes of compounds based on cultivar effect per

maturity in FR fruit (Table 4). This is when berries are at their horticultural peak and just attaining optimal eating quality. These compound classes have specific compounds that are flavor-important in all blueberry types, and their peak at FR coincides nicely with the horticultural optimum stage for eating quality. Similarly, OID compounds were the next most

y—Indicated the treatment combination did not exist to create an F value.

 $^{^{}x}NS$, *, **Nonsignificant or significant at $P \le 0.05$, or 0.01, respectively. No superscript indicates significance at $P \le 0.001$.

significant across ripeness from pink through FR to SR. The significance of LOO compounds also peaked at the FR stage. This finding of high relative significance again lends credence to the likely importance of terpenoids and terpene alcohols within OID and LOO in RAB aroma, similar to other blueberry types.

Terpenes, C₆ unsaturated aldehydes, and unsaturated alcohols have also been reported to be the predominant compounds identified in the volatile extracts of RAB (Horvat et al., 1983, 1996; Horvat and Senter, 1985). Each cultivar displayed an increase in mid- to high MW volatiles (linalool, 154.25 MW and larger) with increasing maturity, whereas the lower MW compounds like hexanol (102.17 and lower MW) decreased (Horvat and Senter, 1985). Similar trends regarding linalool and (Z)-linalool oxide were found, and the whole OID class generally increased with increasing maturity (data not shown). OID compounds displayed the greatest significant F values in FR fruit (Table 4). According to weighted F values, (E)-2-hexenal, (E)-linalool oxide, and α-terpineol displayed a greater significance (magnitude) in FR and SR compared with MG and pink fruit (Table 6).

On a relative percentage basis (data not shown), EST (11 compounds) were exceedingly low at MG and pink stages and increased massively, similar to cantaloupe esters (Beaulieu, 2006), in all five cultivars with SR having the greatest integrated ion content, oftentimes 5- to 10-fold relative increases from FR to SR. However, on data transformation, the means only reflected significant increases in EST in FR and SR fruit in 'Powder Blue' and 'Premier'. OID (17 compounds) were generally lowest in MG fruit and increased steadily through the SR stage in all five cultivars (data not shown). FUR (one compound) generally declined from MG to pink and was not observed in FR and SR maturity stages, and ONE (four compounds) displayed no clear trends (data not shown).

Compound variation by maturity and cultivar. Based on consensus from only upright blueberry (NHB, RAB, and SHB) volatile literature, there are 33 compounds recovered in substantial levels or believed to have aroma impact (J.C. Beaulieu, unpublished data). These are: ethyl acetate, (E)-2-hexanal, (Z)-3-hexenal, hexanal, heptanol (E,Z)-2,6-nonadienal, (E)-2hexenol, (Z)-2-hexenol, (Z)-3-hexenol, hexanol, ethyl butanoate, methyl 2-methylbutanoate, methyl 3-methylbutanoate, ethyl 2methylbutanoate, ethyl 3-methylbutanoate, (Z)-3-hexenyl acetate, (E)-3-hexenyl acetate, hexyl acetate, linalool, p-cymene, cinerolone, β-ionone, 1,8-cineole, terpene-4-ol, limonene, α-terpineol, carveol, geraniol, eugenol, geranylacetone, 2undecanone, 2-butanone, and damascenone (Du et al., 2011; Hirvi and Honkanen, 1983a, 1983b; Horvat et al., 1983; Horvat and Senter, 1985; Parliment, 1977; Parliment and Kolor, 1975; Zhang et al., 2009). From that group of compounds, 17 were recovered here by SPME across the five cultivars at four maturities [ethyl acetate, (E)-2-hexanal, (Z)-3-hexenal, hexanal, (E)-2-hexenol, (Z)-3-hexenol, hexanol, methyl 3-methylbutanoate, ethyl 2-methylbutanoate, ethyl 3methylbutanoate, (Z)-3-hexenyl acetate, hexyl acetate, linalool, p-cymene, 1,8-cineole, limonene, and α-terpineol]. Presentation of individual compounds (Tables 5 and 6) will therefore be focused within this group that delivered significant overall and treatment combination effects in the ANOVA (Table 5). Effectively, this caused ethyl 3-methylbutyrate, ethyl 2methylbutyrate, and (Z)-3-hexenol to be removed from consideration. Once compound F values decreased and were predominantly

insignificant, compounds were not tabled. Because OID and LOO classes were demonstrated to be the most F significant compound classes, compounds within those classes with highly significant F values are also tabled.

Only one laboratory has reported volatiles in the hexaploid (2n = 6x = 72) RAB and they found the major volatiles were ethyl acetate, p-cymene, hexanol, (Z)-2-hexenol, heptanol, cinerolone, β -ionone, terpene-4-ol, 2-undecanone, α -terpineol, carveol, nerol, and eugenol (Horvat et al., 1983; Horvat and Senter, 1985). Although volatile constituents are different among different species of blueberry, (E)-2-hexenal (E)-2hexenol, (Z)-3-hexenol, linalool, and geraniol have been found in most types and are considered typical aroma compounds for blueberry (Du et al., 2011; Hirvi and Honkanen, 1983a, 1983b; Horvat et al., 1996; Horvat and Senter, 1985; Parliment and Kolor, 1975). Aside from geraniol (not recovered or resolved herein), the other four compounds were isolated but (Z)-3hexenol was not significant. Surprisingly, a patent claims that combinations of just four compounds [linalool, (E)-2-hexanal, (E)-2-hexenol and (Z)-3-hexenol mimic blueberry flavor (Parliment, 1977). However, this was based on knowledge obtained for NHB cultivars, which are tetraploids (2n = 4x =48). Furthermore, within the SHB types with a markedly diverse parental lineage (ploidy varies as a result of complicated interbreeding among diploids, tetraploids, and hexaploids), there were often marked volatile differences between cultivars (Du et al., 2011).

Individual compounds in Tables 5 and 6 have been ranked by their overall F values. In the top 16 highest ranked compounds, 10 of the compounds (bold font) are considered aroma-important or highly abundant in blueberry (Table 5). Hence, the following previously reported compounds were determined to be significant based on their F values: linalool, methyl 3-methylbutyrate, 1,8-cineole, (E)-2-hexenal, (Z)-3hexenal, (Z)-3-hexenyl acetate, limonene, hexyl acetate, hexanal, and α-terpineol. Compounds recovered that displayed significance that may contribute to aroma in RAB, which have not previously been delineated as such in the literature, were: methyl butyrate, 2-methyl-4-pentenal, 2-heptanone, (E,E)-2,4hexadienal, and (Z)-linalool oxide. Linalool and geraniol were considered to be major aroma active terpenoids in RAB (Horvat and Senter, 1985) and have also been reported to contribute to floral flavor (Rychlik et al., 1998). Aldehydes [e.g., hexanal and (E)-2-hexenal] have been commonly reported in several NHB blueberry (di Cesare et al., 1999; Hirvi and Honkanen, 1983a; Parliment and Kolor, 1975) and denoted in a patented blueberry (NHB) flavor (Parliment, 1977). Hexanal and (E)-2-hexenal were the major "green compounds," and (Z)-3-hexenol and (E)-2-hexenol were believed to be contributors to aroma as well (Horvat and Senter, 1985).

(E)-2-hexenal was a predominant compound reported in several SHB cultivars (Du et al., 2011) and had very high F values in 'Brightwell', 'Climax', and 'Powder Blue' (Table 6). However, the other generally dominant ALD, (Z)-3-hexenal, was only prevalent in 'Brightwell', 'Climax', and especially so in 'Tifblue'. Six carbon compounds such as hexanal, (Z)-3-hexenal, and (E)-2-hexenal have characteristic green/grassy odors, octanal and nonanal have citrus-like odor, (E,Z)-2,6-nonadienal has a characteristic cucumber odor, and (E,E)-2,4-nonadienal and (E,E)-2,4-decadienal have fatty/waxy odors (Rychlik et al., 1998; Schieberle et al., 1990). In RAB, (E)-2-hexenal, (E)-linalool oxide, α -terpineol, and β -caryophyllene

decreased with increasing maturity, whereas linalool and (*Z*)-linalool oxide increased with maturity (Horvat et al., 1996; Horvat and Senter, 1985). With the exception of geraniol (not recovered) and (*Z*)-3-hexenol, the remaining compounds yielded significant F values regarding overall magnitude in their F tests and both maturity and cultivar interaction effects (Tables 5 and 6).

The weight of compounds significance can also be viewed based on the df. The higher the df (e.g., 19), the more confidence in the delivered F value and significant contribution of a compound regarding a maturity or cultivar effect. This is largely the result of inference being based on more information because more treatments are being represented. However, this does not negate the fact that an individual compound may be highly significant even when not recovered in certain cultivars or maturities. For example, β-myrcene displayed the greatest overall significance in addition to maturity and cultivar interaction effects (Table 5). However, it was only recovered in half the treatment combinations (Tables 2 and 6). β-Myrcene was not recovered in MG or pink fruit in any cultivar, yet this illustrates how one compound with pleasant or desirable aroma attributes (e.g., grape, fruity, peach, must, rosy, citrus, mango) may indeed have important significance, especially because it only appeared in FR and SR fruit, similar in nature to nonoxygenated terpenes recovered in FR and SR fresh-cut mangos [Mangifera indica (Beaulieu and Lea, 2003)]. The same exact relationship occurred with methyl butanoate, which has been ascribed positively as well; e.g., as fruity, ethereal, apple, sweet (Beaulieu, 2005).

Compounds displaying the highest overall significance with all or almost all treatment combinations were generally in the OID and ALD classes (Tables 2 and 5). Again, linalool was the most significant OID and it conferred high significance across maturity within cultivar (Table 5) and displayed significant differences in 'Climax', 'Powder Blue', and 'Premier' (Table 6). Linalool also displayed the most significant differences in cultivar by maturity in FR and SR, similar to several other OID and ALD compounds denoted in Table 6. In general, compounds generally had greater significance for FR and SR maturity, pooled across cultivar, than MG or pink. Aside from two of the top three compounds ranked by overall F value (β-myrcene and methyl butyrate), the relative ranking (weight) of maturity effects per cultivar was significantly greater than cultivar differences pooled across maturity (Table 6). As noted earlier, this is somewhat expected because inbreeding depression has occurred in RAB as a result of a limited breeding background used during cultivar development (Aruna et al., 1995; Lyrene, 1981, 1983; Rowland et al., 2010; Stringer et al., 2009). Most current RAB cultivars originated from a narrow genetic base involving only four natively collected cultivars (i.e., Ethyl, Myers, Clara, and Black Giant) and their continued parental use resulted in inbreeding depression (Lyrene, 1983). For example, one of the standard cultivars assessed, Tifblue ('Ethel' × 'Clara') has common parental crosses also resulting in 'Brightwell' ('Tifblue' × 'Menditoo'), 'Powder Blue' ('Tifblue' × 'Menditoo'), and 'Premier' ('Homebell' × 'Tifblue') (Aruna et al., 1993; Eck, 1988; Okie, 2004). Nonetheless, minor volatile differences and profile dissimilarity resulting from growing location, year, harvest dates, and cultivar are common to several fruits, including SHB and RAB (Du et al., 2011; Horvat and Senter, 1985).

Across the five cultivars, based on relative percentage recovery (data not shown), the relative compound abundance in FR fruit was ALD (75.2%) > OLS (11.2%) > EST (6.9%) > OID (4.3%) > LOO(2.7%). On the other hand, in SR fruit, order of compound abundance was generally ALD (52.1%) > EST (32.3%) > OLS (8.2%) > OID (5.7%) > LOO (3.7%). Nonetheless, in general, there was a very similar pattern of dominant volatiles recovered in all five cultivars with 11 of the top 15 compounds (based on relative percentage) being similar: (E)-2-hexenal, ethyl acetate, hexanal, acetaldehyde, hexanol, (E,E)-2,4-hexadienal, 1-penten-3-ol, linalool, (Z)-3-hexenal, (E)-2-hexenol, and methyl 3-methylbutyrate (data not shown). The five most abundant compounds in most SR and FR cultivars were ethyl acetate, (E)-2-hexenal, acetaldehyde, hexanal, and (E,E)-2,4-hexadienal. The top 15 compounds in FR fruit comprised 97.5%, 97.1%, 96.6%, 96.5%, and 96.1% of the relative volatile contribution in cultivars Brightwell, Premier, Tifblue, Climax, and Powder Blue, respectively.

Herein, ANOVA provided F values measuring treatment differences for several compounds and compound groups in selected cultivars of RAB. Because those volatiles displaying no response were omitted rather than treated as zeros, for some compounds or compound groups, this resulted in entire treatments being eliminated. Given a factorial treatment structure of five cultivars × four maturity groups, a missing treatment combination resulted in a missing cell. Subsequently, ANOVA using the typical two-way with interactions sources of variance was not appropriate for all compounds. As a result of volatiles occasionally not being recovered, it was not wise to pool data and attempt to deliver horticultural-like quantitative or statistical appraisals. Fortunately, this was not the intended purpose for our analysis.

Industrially, many juices are made from concentrates and those fruit are often culls, residuals, and off-grades that are collected, refrigerated, and eventually bulk-transported to a limited number of concentrating plants nationwide. Subsequently, it is not uncommon to expect a vast mixture of cultivars, quality, and volatile attributes emanating from immature, ripe, overripe, and possibly fermenting product, which gets concentrated. Subsequently, because aldehydes are commonly reported as being important volatiles in blueberry, the method herein possibly deriving excess aldehydes is not considered undesirable. Indeed, ALD compounds were significant across cultivar and maturity. However, the data here indicate more importantly, similar to (Du et al., 2011), that OID and LOO compounds, along with ALD, likely confer the aroma importance based on recovery significance, trends, and previous literature. A sensory lexicon was recently published that will be used in further analysis to help gauge the relative importance of these compounds in blueberries and processed products (Bett-Garber and Lea, 2013). The qualitative SPME volatile data method reflecting a diverse array of mainly authenticated compounds recovered in RAB will be a useful database geared toward viewing changes in blueberry types, processed juice, byproducts, and value-added blueberry products.

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