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Aroma Active Volatiles in Four Southern Highbush Blueberry Cultivars Determined by Gas Chromatography−Olfactometry (GC-O) and Gas Chromatography−Mass Spectrometry (GC-MS)

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ABSTRACT: Aroma active volatiles in four southern highbush blueberry cultivars ('Prima Dona', 'Jewel', 'Snow Chaser', and 'Kestrel') were determined using solid phase microextraction (SPME) in combination with gas chromatography−olfactometry (GC-O) and identified via GC-PFPD and GC-MS using retention indices of reference compounds and mass spectral data. The aromas of total, unseparated SPME extracts evaluated using GC-O were rated 8.2−9.0/10 for the four cultivars in terms of similarity to the original blueberry homogenates. In terms of GC-O aroma similarity, those aroma active volatile groups characterized as green, fruity, and floral were most intense. Of the 43 volatiles found to have aroma activity, 38 were identified and 13 had not been previously reported in blueberries. Although linalool and (*E*)-2-hexenal were common major aroma impact volatiles, dominant aroma-active volatiles were different for each cultivar. Principal component analysis confirmed that each cultivar possessed a unique aroma active profile as each cultivar was clustered into a separate score plot quadrant.

KEYWORDS: *southern highbush blueberry, blueberry aroma, GC-O, SPME*

INTRODUCTION

Next to strawberries, blueberries are the second most important berry crop in the United States.[1] The cultivated blueberry market has more than tripled since the 1970s. It has increased at a rate of 10−20% annually since 2000. The U.S. 2012 crop was worth $781.8 million.[2] The rapid increase in blueberry consumption is mainly driven by its reported nutraceutical functions, health benefits, and unique flavor.[3]

Blueberry is a plant native to North America. It is in the genus *Vaccinium*, section Cyanococcus. In this section, there are three main commercialized blueberries: highbush (*Vaccinium corymbosum*), lowbush (*Vaccinium angustifolium*), and rabbiteye (*Vaccinium ashei*). Highbush blueberry is the major planted cultivar, which accounts for approximately 95% of the total commercial production in the United States. Highbush blueberry can be further divided into northern highbush and southern highbush blueberry. Northern highbush is a high-chill blueberry, which has been optimized for high-chill zones. High-chill zones are those climates that experience a minimum of 800 h below 7.2 °C. Southern highbush is a hybrid between the northern highbush and various low-chill Cyanococcus species.[4] The northern highbush blueberry is the predominant cultivar grown in the major blueberry production areas in the northern United States: Maryland, Michigan, New Jersey, North Carolina, and Washington. The southern highbush blueberry is primarily grown in California and Florida.

Florida's southern highbush blueberries ripen earlier than blueberries in other growing areas and are the major producers of U.S. blueberries from the middle of April to late May. The early-market fruit commands a high price, and this has led to the expansion of Florida's blueberry acreage.[5] To be competitive in the blueberry market the fruit should be early ripening and of good quality. Flavor and aroma are two of the most important fruit quality characteristics and ultimately determine consumer acceptability and purchase decision. However, to the best of our knowledge, there is no study that evaluates the aroma volatiles of Florida southern highbush blueberry.

The study of blueberry flavor has been quite limited. A few of the major volatiles have been identified in various blueberry types. In highbush blueberries, the major volatile compounds were reported as ethyl acetate, (*E*)-2-hexenal, (*E*)-2-hexenol, hexanal, (*Z*)-3-hexenol, linalool, and geraniol.[6−8] Other compounds such as citronellol, α-terpineol, 2-phenylethanol, and vanillin have also been suggested to contribute to the typical aroma of highbush blueberries.[9] Few studies have examined the aroma composition in lowbush blueberries. Acetaldehyde, methyl acetate, ethyl acetate, methyl 2-methylbutanoate, methyl 3-methylbutanoate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, methyl butanoate, and linalool were reported as the major aroma compounds.[10−12] A greater number of esters have been reported in lowbush compared to highbush blueberries. Studies on rabbiteye blueberry aroma are also very limited. The major volatiles identified in rabbiteye blueberry are ethyl acetate, *p*-cymene, hexanol, (*Z*)-2-hexenol, heptanol, cinerolone, β-ionone, terpene-4-ol, 2-undecanone, α-terpineol, carveol, nerol, and eugenol.[13−15] A combination of (*E*)-2-hexenal, (*E*)-2-hexenol, (*Z*)-3-hexenol, and linalool has been reported as being responsible for the characteristic blueberry flavor.[14]

Because the identification of blueberry aroma active volatiles is limited and conflicting, especially because there seem to be no studies on the aroma composition of southern highbush blueberry, the object of this study was to investigate the aroma active compounds in two common and two new Florida

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southern highbush blueberry cultivars using a combination of SPME, GC-O, and GC-MS. A secondary goal was to evaluate the aroma active volatiles of these cultivars using GC-O to determine how similar or different the aroma profiles of these four cultivars were.

MATERIALS AND METHODS

Chemicals. Pure standards of ethyl propionate, methyl 2-methylbutanoate, methyl 3-methylbutanoate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, (*Z*)-3-hexenyl acetate, (*E*)-2-hexenyl acetate, linalool, citronellol, nerol, geranylacetone, hexanal, (*Z*)-3-hexenal, (*E*)-2-hexenal, (*E*,*E*)-2,4-hexadienal, (*E*,*Z*)-2,6-nonedienal, (*E*,*E*)-2,4-nonedienal, (*Z*)-3-hexenol, 2-heptanol, 2-heptanone, 1-octen-3-one, 2-methylbutanoic acid, hexanoic acid, methional, Furaneol, *p*-cresol, and eugenol were purchased from Aldrich Chemical Co. Inc. (Milwaukee, WI, USA). Geranyl acetate, 1,8-cineole, α-terpineol, octanal, and decanal were supplied from Sunpure (Lakeland, FL, USA). Geraniol, pentanal, and dimethyl disulfide were purchased from Acros Organics (Fair Lawn, NJ, USA). 2-Nonanone was from Eastman Chemical Co. (Kingsport, TN, USA), whereas β-damascenone was from Givaudan Roure Corp. (Whippany, NJ, USA). Sodium fluoride (ACS grade) were obtained from Acros Organics. Sodium chloride and methanol were purchased from Fisher Scientific (Fair Lawn, NJ, USA).

Blueberry Samples. Highbush blueberry plants 'Jewel' and 'Prima Dona' were grown at a local blueberry farm in Haines City, FL, USA, whereas 'Snow Chaser' and 'Kestrel' were grown at a blueberry breeding center at the University of Florida, Gainesville, FL, USA. Blueberry samples were hand-harvested on April 27 (for 'Jewel' and 'Prima Dona') and May 10 (for 'Snow Chaser' and 'Kestrel') of 2011. All blueberries were grown in mounded pine bark and received similar fertilization. To obtain equal levels of fruit maturity, the berries were harvested in the more northern location (Gainesville) 2 weeks later than the berries grown in Haines City.

Blueberry samples were washed with distilled water and gently dried with tissue paper. Two hundred grams of distilled water and 200 g of berries were homogenized along with sodium chloride and sodium fluoride (final concentrations 20 and 1%, respectively) in a glass blender (Waring Products Div., Dynamics Corp. of America, New Hartford, CT, USA). Samples were blended in a high-speed pulse mode for 20 s. The puree was used for analysis.

SPME Blueberry Volatiles. SPME has been successfully coupled with GC-O to identify aroma active volatiles in foods for approximately 15 years now.[16] In this study 10 g of blueberry puree was added into a 40 mL vial and a 4 mm stir bar, which was flushed with nitrogen before sealing with a septum screw top. It was found that gentle stirring of the sample reduced equilibrium times and improved analytical precision. Samples were equilibrated at 40 °C in a water bath for 20 min. After equilibration, a 2 cm fiber coated with divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, Supelco, Bellefonte, PA, USA) was exposed to the headspace of the vial for 40 min at the same temperature. The three-phase fiber was chosen as it consistently produced the most representative collection of headspace volatiles compared to other fiber types. Forty minutes was chosen as an extraction time as it produced the largest amounts of volatiles and at the same time produced the most consistent results. The fiber was then introduced into the GC injection port for a 3 min desorption.

Direct GC-O. A 1 m deactivated silica column (0.32 mm i.d.) was installed on an Agilent 6890N gas chromatograph system (Palo Alto, CA, USA) directly from the injector to a high volume sniffing port (Datu, Geneva, NY, USA). The flow rate of the helium carrier gas was 20 mL/min. The injection port temperature was 220 °C, and the oven temperature was 150 °C (isothermal). The configuration allows for the evaluation of global odor of the extracts without chromatographic separation, and the entire analysis was completed in <30 s. Two trained panelists smelled the odor of the unseparated SPME extract at the GC sniffing port. Then, panelists opened a 40 mL vial to smell the original blended blueberry puree. Panelists rated the similarity between the unseparated SPME extract and the original puree odors using an 11-point scale ranging from 0 (far from the sample) to 10 (identical to the original sample). Each panelist had been screened from a larger pool of potential panelists using a set of 15 common fruit aroma active volatiles. Accepted panelists demonstrated that they could detect all separated volatiles in the training set and could reproduce the intensity of each volatile. Training time was approximately 3 weeks.

Time–Intensity GC-O Procedure. GC-O analysis was conducted using an Agilent 6890N gas chromatograph system equipped with a flame ionization detector (FID) with the previously described olfactometer. Samples were separated using a DB-Wax column (30 m × 0.32 mm i.d. cross-linked poly(ethylene glycol), 0.50 μm film thickness, J&W Scientific, Folsom, CA, USA). The column effluent was split 1:2 (by volume) into the FID and a heated sniffing port block, where the flow was mixed with humidified air. The column flow rate was 2.0 mL/min (helium) with injection in the splitless mode. Injector and detector temperatures were 220 and 250 °C. The initial oven temperature was 35 °C and held for 1 min before increasing to 190 °C at 4 °C/min and then to 240 °C at a rate of 8 °C/min, and holding at 240 °C for 5 min before cooling back to 35 °C.

Olfactometry analyses were performed by two trained panelists. Assessors rated aroma intensity continuously throughout the chromatographic run using a linear potentiometer from which output was recorded and quantified using Chromperfect software (Justice Innovations, Palo Alto, CA, USA). Retention times and verbal descriptors were recorded to permit aroma descriptors to be coupled with computerized aroma time–intensity plots. Triplicate analyses were performed for each sample by each panelist. Volatiles were not considered to be aroma active unless detected during at least half of the total sniffing trials. The intensity was the average from all panelists when an aroma was registered. Compound identification was based on matching retention times and aroma character with authentic pure standards, retention indices (RIs) that were determined using a series of standard linear alkanes C_5–C_{25}, GC-MS, or GC-PFPD (pulsed flame photometric detector).

GC-MS Identification Aroma Active Compounds. GC-MS analyses were performed using a PerkinElmer Clarus 500 gas chromatograph and a quadropole mass spectrometer (PerkinElmer, Waltham, MA, USA). Compound separation was achieved with a DB-WAX column (60 m × 0.25 mm i.d. cross-linked poly(ethylene glycol), 0.50 μm film thickness, J&W Scientific, Agilent Technique, Foster City, CA, USA). Column flow rate (helium) was 1.5 mL/min. Initial oven temperature was 35 °C. After holding for 1 min, the oven temperature was increased to 190 °C at 4 °C/min and then to 240 °C at 8 °C/min. The oven was maintained at 240 °C for 5 min before returning to 35 °C. Injection, MS transfer line, and ion source temperatures were 230, 240, and 180 °C, respectively. Fragmentation data from m/z 25 to 300 were collected in the scan mode. Compound identifications were made by comparing mass spectral data from the Wiley 275.L (G1035) database and confirmed by authentic pure standards and reported linear retention index (LRI; Kovats) values found in the literature.

GC-PFPD Identification of Sulfur Compounds. Sulfur volatile analyses were achieved using an Agilent 7890A gas chromatograph system equipped with a sulfur-specific detector: a PFPD (model 5380, OI Analytical Co., College Station, TX, USA). Volatiles were separated using a Stable-WAX column (30 m × 0.32 mm i.d. cross-linked poly(ethylene glycol), 0.50 μm film thickness, Restek, Bellefonte, PA, USA). Column flow was 1.5 mL/min (helium). Initial oven temperature was 35 °C. After holding for 1 min, the oven temperature was increased to 65 °C at a rate of 3 °C/min, then increased to 170 °C at a rate of 6 °C/min, and finally to 240 °C at a rate of 10 °C/min with a 5 min hold. The GC injection temperature was 200 °C, and the detector temperature was 250 °C. Sulfur gate time was 6–24.9 ms, and pulse frequency was approximately 3 pulses/s. Sulfur peaks from the PFPD detector were integrated using Chromperfect software version 5.0 (Justin Innovations, Inc., CA). Compound identification was based on matching LRI values from authentic sulfur standards and LRIs reported in the literature with those observed in the samples.

Multivariate Statistics. The aroma active volatile data set used average intensity ratings from the olfactory panel. Principal component

analysis (PCA) was carried out using Unscrambler version 10 from Camo (Woodbridge, NJ, USA). All 43 aroma active volatile values were used in the calculations. Graphical score and loading plots were superimposed into a single figure using the Unscrambler biplot graphing feature.

RESULTS AND DISCUSSION

Global Blueberry Aroma Using SPME. Although blueberry volatile composition has been studied by several groups, there is some disagreement, some of which can be attributed to volatile extraction and identification techniques employed. Traditional techniques such as distillation, liquid−liquid extraction, and headspace sampling are frequently used for volatile isolation in berry fruit.[17] However, most of these techniques are labor-intensive and can produce artifacts. All aroma extraction techniques produce extracts favoring some chemical classes and minimizing others or (in the case of solvent extraction) extract volatiles that have very little volatility at room temperatures but are 100% volatilized in the GC injector. Thus, GC-O results need to be interpreted with care. Static headspace solid phase microextraction (SPME) is an alternative volatile extraction technique that employs a fused silica fiber coated with an appropriate sorbent stationary phase which collects and concentrates only volatiles. The main advantages of SPME are speed, simplicity of operation, and lack of a solvent peak to obscure highly volatile components from the sample. SPME has been widely used to extract volatiles in a wide range of food samples.[18] However, not all SPME fibers produce the same results. The three-phase fiber was used in this study as we have found it to produce the most representative extracts.

When static headspace volatile collection is employed, berry samples can be present as whole fruit, sliced fruit, or blended puree. Blending is more commonly employed for berries because blending can increase volatile release from the berry matrix. However, the reaction of native enzyme activity can distort the original aroma by converting nonvolatiles into aroma active volatiles. In this study, 20% sodium chloride and 1% sodium fluoride (w/w) were added as part of the blending mixture to prevent or reduce this effect. Sodium chloride was used to inhibit lipoxygenase activity, whereas sodium fluoride was used to inhibit polyphenol oxidase activity and microbial growth during equilibrium and volatile extraction. Preliminary experiments indicated that when these salts were not employed, blueberry purees had more than 200 times the total "green volatiles" (hexanal, (*Z*)-2-hexenal, (*E*)-2-hexenal, hexanol, (*Z*)-3-hexenol, and (*E*)-2-hexenol). The rate of formation for each "green compound" appeared to be cultivar and time dependent. Informal sensory evaluations of these samples (blended with or without salts) indicated that berry purees with no added salt had much stronger "green" flavor than those with the added salt. Therefore, salts were added to the berries during blending to prevent the distortion of the native aroma profile.

After blending, blueberry puree volatiles were extracted in the static headspace mode using a 2 cm, triphase SPME fiber (DVB/CAR/PDMS). This fiber was chosen because it had been found to extract the most representative strawberry volatiles.[19] To confirm the representativeness of the SPME extracts, the global aromas of the extracts were evaluated using the unseparated GC-O method and compared to the original blended samples. The global aroma of the extracts and aroma description of the original blended samples including 'Jewel', 'Prima Dona', 'Snow Chaser', and 'Kestrel' are presented in Table 1. Overall, the aroma of the homogenized blueberry samples could be described as "fresh green", "grassy", "fruity", "floral", "blueberry-like", and "tea-like". However, the top aroma impression for each blueberry cultivar was different. 'Jewel' gave the strongest aroma notes of "grassy" and "fresh green", whereas 'Snow Chaser' had the highest level of "floral" note. 'Prima Dona' had the top aroma notes of "fresh green" and "fruity". 'Kestrel' had a similar top aroma of "fresh green" and "fruity"; however, its intensity was much higher than that of 'Prima Dona'. Overall, the global aroma of SPME extracts was quite similar to that of the original blueberry homogenates (Table 1). Compared to the reference purees, the global aroma of SPME extracts had average rates of 8.5, 9.0, 8.2, and 8.9 for 'Jewel', 'Prima Dona', 'Snow Chaser', and 'Kestrel', respectively (Table 1). The results suggested that collected SPME volatiles generated an odor that was representative of the original sample.

Table 1. Global Aroma of SPME Headspace Extracts of Four Florida Southern Highbush Blueberries Scores Compared to Aroma of Original Blended Samples

cultivar	aroma of original blended sample	global aroma of SPME extract	score
Jewel	grassy, fresh green, fruity, blueberry-like	grassy, green, fruity, blueberry-like, red tea-like	8.5
Prima Dona	fresh green, fruity, blueberry-like, green tea-like	fresh green, blueberry-like, fruity, red tea-like	9.0
Snow Chaser	floral, fruity, fresh green, blueberry-like	rosy, fresh green, floral, fruity	8.2
Kestrel	fresh green, fruity, floral, green tea-like, blueberry-like	fresh green, fruity, blueberry-like, floral, red tea-like	8.9

GC-O Analysis. Shown in Figure 1 are the average aromagrams from 'Prima Dona' and 'Kestrel' southern highbush blueberry cultivars. The 'Kestrel' aromagram is inverted for comparison purposes. Both qualitative (missing aroma volatiles) and quantitative (aroma intensity) differences are apparent. One of the more obvious features is the high number

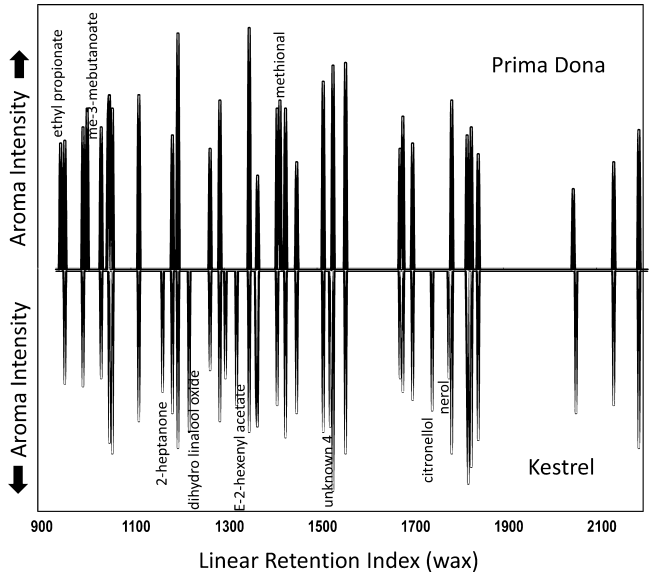

Figure 1. Aromagrams of average aroma intensities from 'Prima Dona' (upper) and 'Kestrel' (lower and inverted).

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Table 2. Identified GC-O Aroma-Active Compounds in 'Jewel', 'Prima Dona', 'Snow Chaser', and 'Kestrel' Blueberries

LRI	identity	aroma descriptors	Jewel	Prima Dona	Snow Chaser	Kestrel	identification basis[a]
949	ethyl propionate[b]	sweet, fruity, apple	5.5	4.7			RI, Std, AD
959	pentanal	malty, almond, sulfuric	5.6	4.8	4.6	4.2	RI, Std, AD, MS
997	methyl 2-methylbutanoate	green, fruity, pear		5.3	4	4.3	RI, Std, AD, MS
1005	methyl 3-methylbutanoate	fruity, apple, pineapple	6	6	5.3		RI, Std, AD, MS
1036	ethyl 2-methylbutanoate	fruity, apple	5.8	5.3		4	RI, Std, AD, MS
1050	ethyl 3-methylbutanoate	fruity, apple, sweet	6.5	5.5			RI, Std, AD, MS
1054	dimethyl disulfide	sulfuric, rotten egg	8.3	6.4	5.8	6.4	RI, Std, AD, PFPD
1061	hexanal	fresh, green, fruity	6.3	6	5.5	6.8	RI, Std, AD, MS
1117	(*Z*)-3-hexenal	green, leafy	7	6.5	5	5.6	RI, Std, AD, MS
1168	2-heptanone	fruity, spicy, cheesy	4			4.5	RI, Std, AD, MS
1188	1,8-cineole	minty, woody, herbaceous	3.7	5	6	5.3	RI, Std, AD, MS
1202	(*E*)-2-hexenal	grassy, pungent	8.3	8.8	8	6.6	RI, Std, AD, MS
1224	dihydrolinalool oxide	moldy, fatty, fruity	5.8		6.3	6	RI, MS
1270	octanal	citrus, fatty	4	4.5		3.7	RI, Std, AD, MS
1291	1-octen-3-one[a]	mushroom	6.3	6.3	5.2	5.6	RI, Std, AD
1302	(*Z*)-3-hexenyl acetate	green, fruity, woody	4.5		5	4	RI, Std, AD, MS
1326	(*E*)-2-hexenyl acetate	green, leafy, apple			4.7	5	RI, Std, AD, MS
1353	unknown 1	sulfurous, grassy	8.5	9	6.8	6	
1370	(*Z*)-3-hexenol	green, grassy				4.8	RI, Std, AD, MS
1373	2-nonanone	fruity, earthy, herb	4.3	3.5	4	5.7	RI, Std, AD, MS
1413	(*E*,*E*)-2,4-hexadienal	leaf, fatty, green	6	6	5	5	RI, Std, AD, MS
1419	unknown 2	floral, perfume	5.6	6.3			
1432	methional	cooked potato	6	6	5.8	6.2	RI, Std, AD, PFPD
1456	unknown 3	fresh, fruity, rosy	5.7	4	5.3	5.3	
1513	2-heptanol[b]	woody, musty, moldy	6.7	7	5.3	6	RI, Std, AD
1528	unknown 4	fresh green, fruity, fatty	4.8	-	4.5	5.8	
1534	linalool	green, rosy, floral	8	7.6	8	8.2	RI, Std, AD, MS
1561	(*E*,*Z*)-2,6-nonadienal	fresh, green, cucumber	6.8	7.7	6.3	6.8	RI, Std, AD, MS
1651	2-methylbutanoic acid[b]	cheesy, sour	5		3.5		RI, Std, AD
1677	α-terpineol	woody, piney, citrus	4	4.5	4	4	RI, Std, AD, MS
1684	(*E*,*E*)-2,4-nonedienal	fatty, green, cucumber	6.3	5.7	6	4.5	RI, Std, AD, MS
1706	geranyl acetate	woody, floral, rosy	5	4.7	5	4.8	RI, Std, AD, MS
1747	citronellol	floral, rosy, sweet				5.2	RI, Std, AD, MS
1783	nerol	floral, citrus, sweet				4	RI, Std, AD, MS
1790	β-damascenone[b]	floral, fruity, tea	7.2	6.3	5.8	6.8	RI, Std, AD
1824	hexanoic acid	cheesy, sour	4	5	6	4.5	RI, Std, AD, MS
1825	geraniol	sweet, fruity, berry			6	7.8	RI, Std, AD, MS
1832	geranylacetone	floral, rosy, sweet	5.9	5.3	6.2	7.3	RI, Std, AD, MS
1846	decanal	fruity, citrus, orange	5.6	4.3	4	6.3	RI, Std, AD, MS
2049	Furaneol[b]	sweet, candy, camarel	4.8	3			RI, Std, AD
2057	*p*-cresol[b]	fecal, sulfurous			3.5	5.3	RI, Std, AD
2138	eugenol	sweet, clove, tobacco	4	4	6.5	5	RI, Std, AD, MS
2191	unknown 5	smoky, earthy, moldy	6.7	5.2	6.6	6.6	

[a]RI, retention index; Std, standard; AD, aroma description. [b]Tentatively identified.

(28) of shared aroma active volatiles. It is also worth noting that the aroma strengths of these aroma active volatiles are quite different. For example, the most intense odorant in the 'Kestrel' cultivar was linalool, whereas the most intense odorant for 'Prima Dona' was an unknown described as having a sulfurous, grassy aroma and eluted at a wax LRI of 1353. There were, however, nine volatiles that were found in only one cultivar and not the other. The relatively new 'Kestrel' cultivar contained six aroma active volatiles that were not observed in the more established 'Prima Dona' cultivar. This is not to say that the six aroma active volatiles labeled in Figure 1 were not present in 'Prima Dona'; it means only if they were present, their concentrations were below their respective thresholds. Conversely, the 'Prima Dona' cultivar contained three aroma active volatiles not observed in the 'Kestrel' cultivar. These peaks are labeled as well.

Aroma Volatiles in Southern Highbush Blueberry. A summary of aroma active volatile from all four southern highbush blueberry cultivars is shown in Table 2. A total of 43 aroma active volatiles are observed in the GC-O experiments. Thirty-eight of these aroma active compounds were identified using the techniques listed in the last column in the table. Five aroma active volatiles remain unidentified/unknown. There were nine aldehydes, eight esters, seven terpenes, five ketones, two alcohols, two acids, two sulfurs, and three miscellaneous compounds.

Aldehydes are the most abundant chemical group with aroma activity found in southern highbush blueberry. Hexanal, (*Z*)-3-hexenal, (*E*)-2-hexenal, (*E*,*Z*)-2,6-nonadienal, and (*E*,*E*)-2,4-

nonedienal contributed "fresh green", "grassy", and "fruity" aroma notes, whereas pentanal, octanal, (*E*,*E*)-2,4-hexadienal, and decanal contributed "fatty" and "citrus" aroma notes (Table 2). Unsaturated aldehydes such as (*Z*)-3-hexenal, (*E*,*E*)-2,4-hexadienal, (*E*,*Z*)-2,6-nonadienal, and (*E*,*E*)-2,4-nonedienal have not been previously reported in blueberries. These unsaturated aldehydes are generally considered as the oxidized products of unsaturated fatty acids and should be considered as natural in vivo products of blueberry maturation as the added salt should have inhibited enzyme activity during and after sample blending. Aldehydes as a group make a major contribution to blueberry aroma, in agreement with previous results.[14]

Esters are the next most abundant group, including ethyl propanoate, methyl 2-methylbutanoate, methyl 3-methylbutanoate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, (*Z*)-3-hexyl acetate, (*E*)-2-hexyl acetate, and geranyl acetate contributing "green", "sweet", "fruity", "apple", "banana", "pear", and "floral" aroma notes (Table 2). Ethyl propanoate, methyl 2-methylbutanoate, (*E*)-2-hexyl acetate, and geranyl acetate have not been previously reported in blueberry. Ethyl 2-methylbutanoate has been identified in northern highbush and lowbush blueberry,[6,10] whereas methyl 3-methylbutanoate, ethyl 3-methylbutanoate, and (*Z*)-3-hexyl acetate have been reported in lowbush blueberries.[10] Generally, esters are the major aroma components of fruits such as strawberry,[20] apple, and banana.[21] However, few esters have been identified in highbush blueberry. Esters are not considered as important as aldehydes to the aroma in northern highbush blueberries. They have been identified as important volatiles in some lowbush blueberries.[10] In terms of ester composition, the southern highbush blueberry is more similar to lowbush blueberry than to the northern highbush blueberry.

Terpenes such as linalool, citronellol, nerol, and geraniol contributed "sweet", "floral", "fruity", "citrus", and "berry-like" aroma notes. 1,8-Cineole, dihydrolinalool oxide, and α-terpineol contributed "woody", "herbaceous", and "piney" aroma notes (Table 2). Except for dihydrolinalool oxide, all of the other six terpenes had been previously reported in various blueberry types.[6,7,10,14] Linalool was a major aroma active volatile in all four southern highbush blueberry cultivars examined. This is consistent with literature data.[14] Geraniol was a major aroma volatile only in 'Snow Chaser' and 'Kestrel'.

Only two alcohols, including (*Z*)-3-hexenol and 2-heptanol, were aroma active (Table 2). We confirmed earlier reports that 2-heptanol contributed a "musty", "moldy" aroma to southern highbush blueberries. (*Z*)-3-Hexenol had been reported as one of the major "green compounds" in blueberries. However, in this study, it was observed in only a single cultivar ('Kestrel)' and only at midlevel intensity.

Only three ketones were found to have aroma activity. They included 2-heptanone, 1-octen-3-one, and 2-nonanone. They contributed "fruity" "mushroom", "earthy", and "cheese-like" aroma. These carbonyl compounds have not been previously reported as contributing to blueberry aroma. Acids such as hexanoic acid and 2-methylbuanoic acid contributed "cheesy" and "sour" sensory attributes and have been previously reported in blueberry.[7]

Sulfur compounds were identified using a special sulfur-specific detector, a PFPD. Using this highly sensitive and selective detector, several sulfur volatiles were tentatively identified using retention index matching. Identified volatiles included hydrogen sulfide, methanethiol, dimethyl sulfide, dimethyl disulfide, and methional. However, only dimethyl disulfide and methional were found to possess aroma actively in parallel GC-O studies. They were described as possessing "sulfurous rotten egg" and "cooked potato" aromas, respectively (Table 2). Methional had not been previously reported in blueberries, but dimethyl sulfide had been reported in some wild blueberries.[22]

Furaneol was tentatively identified in two southern highbush blueberries ('Jewel' and 'Prima Dona', Table 2). Furaneol has "sweet", "candy", and "caramel" aroma notes, with a very low organoleptic threshold, 10 μg/kg in water.[23] Furaneol has been reported in fruits such as strawberry,[23] pineapple,[24] and blackberry.[25] However, it has not been previously identified in blueberry. Two phenolic compounds, including *p*-cresol and eugenol, contributed "fecal" and "clove" aroma attributes to southern highbush blueberries. Both of these two compounds have been previously reported in blueberries.[7]

Comparison of Chemical Groups and Total Intensities. Not surprisingly, the four southern highbush blueberry cultivars share many common aroma volatiles. Of the 43 aroma active volatiles, 33 were common to at least three of the four cultivars. There were a total of 37 aroma volatiles in 'Kestrel', 36 in 'Jewel', 33 in 'Snow Chaser', and 32 in 'Prima Dona'. Aldehydes (9) were the most abundant volatile group, closely followed by esters (8) and terpenes (7). There was more variation within the terpene group than in any other chemical group. Only three aroma active terpenes were observed in 'Prima Dona', but seven terpenes were observed in 'Kestrel'.

Not only did the number of aroma active compounds vary among the four cultivars, but the aroma intensities also varied. 'Jewel' and 'Kestrel' had the highest total aroma intensities from all aroma active volatiles (both identified and unknown) of 209 and 204, respectively. In contrast, the total aroma intensities due to 'Prima Dona' and 'Snow Chaser' were only 180 and 179, respectively. The major contributors to total aroma intensity were aldehydes, esters, and terpenes. The aldehyde and terpene intensity values observed in this study were in agreement with literature reports that aldehydes and terpenes are considered the major volatiles contributing to blueberry aroma.[14]

Aroma Profiles of 'Jewel', 'Prima Dona', 'Snow Chaser', and 'Kestrel'. No single aroma active volatile was that much more intense than all the others. Only linalool and (*E*)-2-hexenal had aroma intensity >7.5 in all four cultivars. It has been previously reported that an aroma recombination of (*E*)-2-hexenal, (*E*)-2-hexenol, (*Z*)-3-hexenol, and linalool conveys the characteristic blueberry flavor.[14] In the current study (*E*)-2-hexenol was not detected and (*Z*)-3-hexenol was observed only as a midlevel aroma active volatile in a single cultivar ('Kestrel').

Shown in Figure 2 is a summary of the total aroma intensities of all 43 aroma active volatiles grouped into eight groups based on aroma similarities. It can be seen that green, fruity, and floral aroma characters are the major contributors to blueberry aroma. Sulfury and moldy/musty are midlevel aroma attributes with the remaining three aroma categories contributing to background blueberry aroma. Therefore, blueberry aroma is more complex than a single aldehyde and a few 6- and 10-carbon alcohols.

The relative aroma strengths of these eight aroma categories varied somewhat between the four cultivars. 'Jewel' and 'Kestrel' possessed similar aroma strengths in the five strongest aroma categories. However, 'Jewel' contained relatively greater intensities in the low-level aroma categories of sweet and

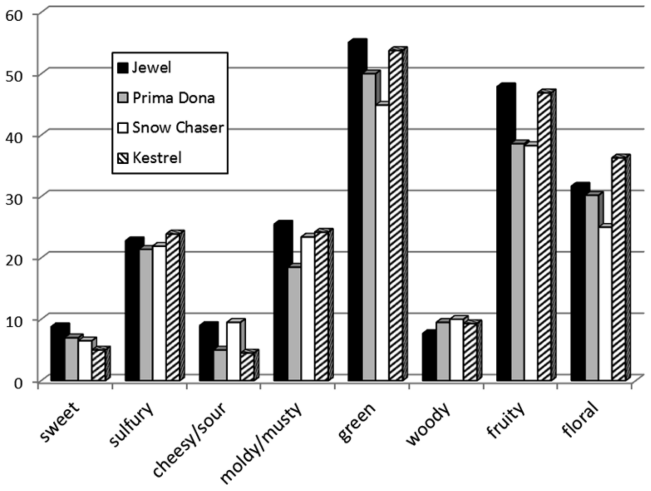

Figure 2. Comparison of GC-O aroma group profiles of four southern highbush blueberry cultivars grown in Florida.

cheese/sour than 'Kestral'. In a similar fashion, the aroma profile of 'Prima Dona' was similar to that of 'Snow Chaser", but 'Prima Dona' exhibited higher total levels in the major green and floral aroma categories.

Principal Component Analysis. To determine if there were appreciable differences in the aroma profiles of the four cultivars used in this study, the aroma volatile data were analyzed using PCA. Shown in Figure 3 are the score and

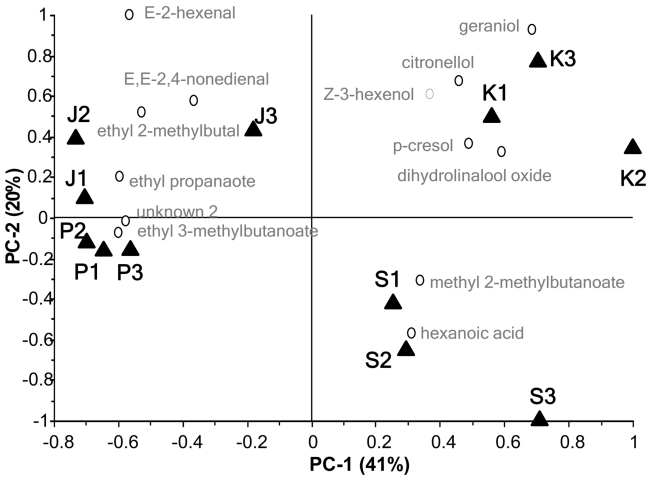

Figure 3. PCA biplot of eigenvalue score values (open triangles) and major loading values (solid circles). P, 'Prima Dona'; J, 'Jewel'; K, 'Kestrel'; S, 'Snow Chaser'.

loading plots of triplicate results for the four blueberry cultivars examined in this study. The first two principal components described 61% of the total variance. Furthermore, each of the four cultivars was clustered in the score plot with no overlap. This indicated that each of the four cultivars possessed a unique aroma profile. PC-1 cleanly separated 'Kestrel' and 'Snow Chaser' on the right-hand (+) side from the more commercially common 'Jewel' and 'Prima Dona' cultivars. The PC-1 axis was defined primarily by geraniol and dihydrolinalool oxide load values on the positive side and ethyl-3-methylbutanoate and unknown 2 loading values on the negative side. PC-2 completely separated 'Kestrel' from 'Snow Chaser' and 'Jewel' from 'Prima Dona'. However, as shown in Figure 3, the degree of separation in the latter case was not as profound. PC-2 was defined primarily but not exclusively by the loading values of citronellol, (*Z*)-3-hexenol, and *p*-cresol on the positive side and by hexanoic acid and methyl-2-methylbutanoate on the negative side. It should be kept in mind that the eigenvector values shown in the score plots were the results of many other variables with smaller loading values. Nevertheless, the complete separation of all four cultivars indicated that each possessed a unique aroma profile and that the close proximity (but not overlapping) of the 'Jewel' and 'Prima Dona' clusters indicated that their overall aroma profiles were similar but still distinct.

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REFERENCES

(1) Krewer, G.; NeSmith, D. S. The Georgia blueberry industry: its history, present state, and potential for development in the next decade. *Acta Hortic.* **2002**, *574*, 101−106.

(2) USDA National Agricultural Statistics Service, Blueberry statistics. March 2013, 395; http://www.nass.usda.gov/Statistics_by_Subject.

(3) Diegidio, V.; Sinelli, N.; Casiraghi, E.; Spinardi, A.; Mignani, I. Evaluation of quality and nutraceutical content in blueberries (*Vaccinium corymbosum*) by near and mid infrared spectroscopy. *Postharvest Biol. Technol.* **2008**, *50*, 31−36.

(4) Lyrene, P. M. Breeding southern highbush and rabbiteye blueberries. *Acta Hortic.* **2006**, *715*, 29−37.

(5) Williamson, J. G.; Lyrene, P. M. Blueberry varieties for Florida. *Univ. Fla. IFAS Extension* **2004**, *No. HS967*, 1−9.

(6) Parliment, T. H.; Kolor, M. G. Identification of the major volatile components of blueberry. *J. Food Sci.* **1975**, *40*, 762−763.

(7) Hirvi, T.; Honkanen, E. The aroma of blueberries. *J. Sci. Food Agric.* **1983**, *34*, 992−996.

(8) Hirvi, T.; Honkanen, E. The aroma of some hybrids between high-bush blueberry (*Vaccinium corymbosum*, L.) and bog blueberry (*Vaccinium uliginosum*, L.). *Z. Lebensm. Unters. Forsch.* **1983**, *176*, 346−349.

(9) Hanoglu, A.; Pucarelli, F. Determination of key aroma-active compounds by GCO-aroma extract dilution analysis (AEDA) in cranberry and blueberry fruits. *Chem. Flavors* **2007**, 269−275.

(10) von Sydow, E.; Anjou, K. The aroma of bilberries (*Vaccinium myrtillus* L.). I. Identification of volatile compounds. *Food Sci. Technol. (London)* **1969**, *2*, 78−81.

(11) Von Sydow, E.; Andersson, J.; Anjou, K.; Karlsson, G. The aroma of bilberries (*Vaccinium myrtillus* L.). II. Evaluation of the press juice by sensory methods and by gas chromatography and mass spectrometry. *Food Sci. Technol. (London)* **1970**, *3*, 11−17.

(12) Hall, I. V.; Forsyth, F. R.; Lightfoot, H. J. Volatiles from developing fruit of *Vaccinium angustifolium*. *Can. Inst. Food Sci. Technol. J.* **1970**, *3*, 1−3.
(13) Horvat, R. J.; Senter, S. D.; Dekazos, E. D. GLC-MS analysis of volatile constituents in rabbiteye blueberries. *J. Food Sci.* **1983**, *48*, 278−279.
(14) Horvat, R. J.; Senter, S. D. Comparison of the volatile constituents from rabbiteye blueberries (*Vaccinium ashei*) during ripening. *J. Food Sci.* **1985**, *50*, 429−431.
(15) Horvat, R. J.; Schlotzhauer, W. S.; Chortyk, O. T.; Nottingham, S. F.; Payne, J. A. Comparison of volatile compounds from rabbiteye blueberry (*Vaccinium ashei*) and deerberry (*V. stamineum*) during maturation. *J. Essent. Oil Res.* **1996**, *8*, 645−648.
(16) Bazemore, R.; Goodner, K.; Rouseff, R. Volatiles from unpasteurized and excessively heated orange juice analyzed with solid phase microextraction and GC-olfactometry. *J. Food Sci.* **1999**, *64*, 800−803.
(17) Du, X. F.; Qian, M. Flavor chemistry of small fruits: blackberry, raspberry, and blueberry. In *Flavor and Health Benefits of Small Fruits*; ACS Symposium Series 1035; Qian, M. C., Rimando, A. M., Eds.; American Chemical Society: Washington, DC, USA, 2010; pp 27−43.
(18) Ferreira, I. M. P. L. V. O.; Pinho, O. Headspace solid-phase microextraction (HE-SPME) in combination with GC/MS or GC/FID for quantification of volatile compounds in food matrices. *Trends Chromatogr.* **2006**, *2*, 31−42.
(19) Jouquand, C.; Chandler, C.; Goodner, K.; Plotto, A. Optimization of strawberry volatile sampling by direct gas chromatography olfactometry. *Proc. Fla. State Hortic. Soc.* **2008**, *121*, 260−264.
(20) Latrasse, A. Fruits III. In *Volatile Composition in Foods and Beverages*; Maarse, H., Ed.; Dekker: New York, 1991; pp 329−387.
(21) Berger, R. G. Fruits I. In *Volatile Compounds in Foods and Beverages*; Maarse, H., Ed.; Dekker: New York, 1991; pp 283−304.
(22) Baloga, D. W.; Vorsa, N.; Lawter, L. Dynamic headspace gas chromatography-mass spectrometry analysis of volatile flavor compounds from wild diploid blueberry species. In *Fruit Flavors*; ACS Symposium Series 596; Rouseff, R. L., Leahy, M. M., Eds.; American Chemical Society: Washington, DC, USA, 1995; pp 235−247.
(23) Larsen, M.; Poll, L. Odor thresholds of some important aroma compounds in strawberries. *Z. Lebensm. Unters. Forsch.* **1992**, *195*, 120−1123.
(24) Pickenhagen, W.; Velluz, A.; Passerat, J. P.; Ohloff, G. Estimation of 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (Furaneol) in cultivated and wild strawberries, pineapples, and mangoes. *J. Sci. Food Agric.* **1981**, *32*, 1132−1134.
(25) Du, X. F.; Qian, M. Quantification of 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone using solid-phase extraction and direct microvial insert thermal desorption gas chromatography-mass spectrometry. *J. Chromatogr., A* **2008**, *1208*, 197−201.