JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Comparison of Fast Gas Chomatography−Surface Acoustic Wave (FGC-SAW) Detection and GC-MS for Characterizing Blueberry Cultivars and Maturity

Xiaofen Du,[†] James Olmstead,[‡] and Russell Rouseff*[,†]

[†]Citrus Research and Education Center, University of Florida, 700 Experiment Station Road, Lake Alfred, Florida 33850, United States

[‡]Horticultural Sciences Department, University of Florida, Gainesville, Florida 32611, United States

ABSTRACT: A novel analytical method using fast gas chromatography−surface acoustic wave detection (FGC-SAW) was employed to rapidly characterize blueberry volatile profiles according to genotypes and fruit maturity. Fourteen FGC-SAW peaks were observed and 11 peaks were tentatively identified in the 15 s chromatogram. Peak identifications were confirmed by matching retention index values with similar values from GC-MS analyses of the same samples. Eighty peaks were observed in the 40 min GC-MS analysis of identical samples. Principal component analysis (PCA) score plots of FGC-SAW and GC-MS data both differentiated blueberries according to genotype, maturity stage, and harvest date even though FGC-SAW PCA's used far fewer peak area values. PCA plots clearly separated 'FL02-40', 'Snowchaser', 'Jewel', and 'Primadonna' blueberry cultivars into four quarters using two-dimensional PCA projections. FGC-SAW was also successful in differentiating three berry maturity stages in PCA score plots for both 'Jewel' and 'Primadonna' cultivars. FGC-SAW is an effective technique for rapid analysis of major blueberry volatiles, but could not determine many mid- and low-level volatiles as they were often coeluted with higher concentration volatiles.

KEYWORDS: *zNose, blueberry volatiles, blueberry cultivars, blueberry maturity*

INTRODUCTION

Blueberry is second only to strawberry in importance among berry fruits in America. Blueberries are grown to some extent in 39 states on nearly 30,000 ha, generating several hundred million dollars in sales every year.[1,2] Blueberries can be consumed fresh or processed in a variety of consumer goods such as jellies, jams, pies, muffins, snack foods, and cereals. Florida produces early-ripening blueberry fruit, which supplies northern U.S. markets when no other domestic blueberries are available.[3] In terms of fresh fruit quality, factors such as texture, taste (sugars and acids), and aroma (volatile profiles) are major attributes that influence consumer consumption and repurchase decisions.[4]

In practice, few quality analyses are performed for blueberry fruit from field to consumer. Aromatic flavor information is rarely, if ever, assessed. In packinghouses, blueberries are typically sorted, graded, and packed by visual assessment of color, size, uniformity, and surface defects. This is a subjective, labor-intensive process that can be standardized through the use of commercially available color and soft berry sorting equipment. Simple refractive index tests for sugars/dissolved solids are sometimes conducted for harvesting decisions and quality control. °Brix and titratable acidity generally correlate to sweet and sour taste, but they convey no direct information concerning fruit aroma quality. GC and GC-MS can be used to characterize and identify blueberry volatile components. However, GC-MS methods are time-consuming, costly, and limited to well-equipped laboratories. Therefore, there is a need for a rapid, simple, and relatively inexpensive instrumental technique to assess the aroma quality of blueberry fruit.

Solid state sensor array technology (e-nose) has been developed to analyze volatiles in an unseparated, relatively rapid, and simple way. E-nose technology has been explored intensively in recent decades, especially in the development of various sensor types and applications.[5,6] E-nose technology has been applied to a wide spectrum of raw product quality control issues such as the ripeness of apple,[7] peach,[8] and mango,[9] variety discrimination of apricot[10] and strawberry,[11] and shelf life of peach[8] and milk.[12] E-nose technology has also been investigated for quality sorting of blueberry.[13,14] However, e-nose has been associated with several problems such as sensor drift, sensor poisoning, and the influence of moisture.[6]

The fast gas chromatography−surface acoustic wave (FGC-SAW) instrument is not an electronic nose, even though it is sold commercially under the name zNose. An electronic nose samples the unconcentrated and unseparated volatiles using multiple sensors, which ideally are specific for certain volatiles. This is not how the FGC-SAW operates. FGC-SAW is a recent instrumental approach that traps and concentrates volatiles, before eluting them to be separated on a short chromatographic column and detecting them with a single quartz surface acoustic wave (SAW) detector. Generally, it can separate and analyze collected headspace volatiles within a few seconds. The dynamic headspace trapping and concentrating is simple and relatively brief (typically 5−20 s). FGC-SAW separation and

Received: February 16, 2012
Revised: April 26, 2012
Accepted: April 29, 2012
Published: April 29, 2012

detection is a robust technology well-suited for field work and has been verified by the U.S. Environment Protection Agency as being capable of providing useful data for environmental site characterization. This technology has also been used to detect and discriminate the aroma of lard and vegetable oil, to monitor storage stability, and to differentiate adulterations with different sources of oils.[15−19] The use of FGC-SAW to classify the flower source of honey on the basis of different volatile profiles, as well as the detection of sugar adulteration, has been reported.[20−22] FGC-SAW could also detect and discriminate lilac blossoms and *Thymus* cultivars, species, and geographical locations.[23,24] Aroma compounds in grapes and other plant volatiles have also been analyzed using FGC-SAW.[25,26] Collectively, these studies indicate that FGC-SAW has advantages of simplicity, speed of analysis, and lower costs in comparison to GC-MS. However, the application of FGC-SAW on fruit quality analysis is not fully exploited, and to our knowledge, FGC-SAW has never been employed for blueberry volatile analysis.

Over 100 volatiles have been reported in blueberries.[27] Overall, blueberry emits a low level of volatiles relative to other fruit, thus requiring a sensitive technique for detection and identification. Preliminary studies have shown that FGC-SAW could detect volatiles at microgram per liter (μg/L) levels and thus has potential use in screening and detecting blueberry volatiles. The objective of this study was to determine if FGC-SAW could differentiate blueberry fruit volatiles according to maturity stage and genotype.

MATERIALS AND METHODS

Chemicals. Methanol and sodium chloride were purchased from Fisher Scientific (Fair Lawn, NJ, USA), whereas sodium fluoride (ACS grade) was obtained from Acros Organics (Fair Lawn, NJ, USA). Pure standards of methyl 3-methylbutanoate, ethyl 2-methylbutanoate, (*Z*)-3-hexenyl acetate, (*E*)-2-hexenyl acetate, linalool (3,7-dimethylocta-1,6-dien-3-ol), hexanal, (*E*)-2-hexenal, 1-nonanal, (*E*,*Z*)-2,6-nonedienal, (*Z*)-3-hexenol, (*E*)-2-hexenol, 2-ethylhexanol, 1-octanol, 2-heptanone, 6-methyl-5-hepten-2-one, 2-undecanone, and hexanoic acid were from Aldrich Chemical Co. Inc. (Milwaukee, WI, USA). Limonene (1-methyl-4-(1-methylethenyl)cyclohexene) and hexyl acetate were supplied by Fluka Chemical (Milwaukee, WI, USA). 1,8-Cineole (1,3,3-trimethyl-2-oxabicyclo[2,2,2]octane) and α-terpineol (2-(4-methyl-1-cyclohex-3-enyl)propan- 2-ol) were supplied by Sunpure (Lakeland, FL, USA). Geraniol (3,7-dimethylocta-2,6-dien-1-ol), 1-pentanal, and 1-hexanol were purchased from Acros Organics. 2-Nonanone was from Eastman Chemical Co. (Kingsport, TN, USA).

Blueberry Samples. Four southern highbush blueberry cultivars (*Vaccinium corymbosum*), 'Snowchaser', 'Primadonna', 'Jewel', and 'FL02-40' (commercialized under the name 'Kestrel'), were investigated for the blueberry cultivar sorting assay. Fully mature blueberries (visually assessed by color) were hand-harvested from the University of Florida grower-cooperator farm near Gainesville, FL (29° 47′ 32″ N, 82° 07′ 22″ W), in the 2010 growing season. Harvest dates for 'Snowchaser' were May 10, 14, and 17. 'FL02-40' was harvested on May 10 and 14. 'Primadonna' and 'Jewel' were harvested on May 19 and 24.

'Primadonna' and 'Jewel' were two Haines City, FL, cultivars selected for the maturity study. The three fruit maturity/developmental stages were green-purple (fruit color was from green to purple), purple-blue, and blue-dark. Harvest dates for 'Primadonna' were May 5 and 11, whereas harvest dates for 'Jewel' were April 27 and May 5, 11, and 18, 2010.

Berries were randomly picked from the field, kept in plastic clamshells, and stored at 5 °C overnight for cleaning, sorting, and sample preparation the next morning. Berries were mixed and visually sorted into one of the three maturity stages with an emphasis on uniform coloration. Fruit with surface defects were discarded. Berries were then washed with distilled water and allowed to dry on paper towels. Two hundred grams of berries was blended in a glass Waring blender (Waring Products Div., Dynamics Corp. of America, New Hartford, CT), with equal weights of distilled water, 80 g of sodium chloride, and 4 g of sodium fluoride. Sodium chloride was employed to inhibit possible enzyme activity, and sodium fluoride was used to reduce microbial growth. The contents were blended in the high-speed pulse mode for 20 s, and the puree was stored under nitrogen in glass bottles at −20 °C until analysis (within 1 week).

Soluble solids content/°Brix (SSC/°Brix) and titratable acidity (TA) were measured on a subset of the same fruit (not salted) using a PAL-1 pocket refractometer (Atago USA, Inc., Bellevue, WA, USA) for SSC and a Metrohm 808 Titrando (Metrohm USA, Westbury, NY, USA) for TA. Titratable acidity was expressed as percent citric acid.

FGC-SAW Volatile Analysis. Duplicate 5 g samples of blueberry puree were weighed into 20 mL glass vials with silicone/PTFE septa (clean pack, Gerstel Inc., Linthicum, MD, USA) and sealed with aluminum crimp caps. Prior to headspace sampling, the samples were thermally equilibrated at 40 °C in a water bath for 20 min.

FGC-SAW (zNose 4500 ultrafast GC analyzer, Electronic Sensor Technology (EST), Newbury Park, CA, USA) consists of a GC sensor head, a support chassis, and a system controller (software). The sensor head is equipped with an inlet nozzle, six-port valve, Tenax trap, a short GC column and SAW detector. The support chassis contains a pump, a small helium gas container, and battery for electronic support. The chassis also has a built-in processor, which can be connected to a laptop via bluetooth. Analysis consists of two steps: headspace sampling and injection−separation. In sampling, a bent needle connected to the inlet nozzle (200 °C) was inserted through the septum of the sample vial, and headspace vapor was withdrawn at 30 mL/min. A hollow sparging needle was also separately inserted through the septum during the time of sampling, just above the bottom of the solution, to allow outside gases in, balancing the headspace gases pulled out by the pump. Sampling time was 20 s. After leaving the vial, headspace vapor passed through a heated valve (160 °C) and onto a Tenax trap (0.7 mm o.d. × 51 mm long containing approximate 2 mg of Tenax). The valve was then rotated to put the trap in line with the GC column to prepare for injection. The Tenax trap was quickly heated to 225 °C to release absorbed volatiles. The helium carrier gas transported the desorbed volatiles to a capillary column (DB5, 0.25 mm i.d. × 1 m length × 0.25 μm film thickness) at a rate of 3 mL/min. GC oven initial temperature was 40 °C and heated to 180 °C at a rate of 10 °C/s. A SAW crystal detected the volatiles exiting from the column. The crystal is mounted internally with a small thermoelectric cooler, which can provide cooling needed during vapor adsorption and heating needed to clean the crystal when required.

The complete analysis cycle was completed in approximately 60 s. This included headspace sampling (20 s), FGC-SAW analysis (26.3 s), and instrumental recycling (15 s). Between each sample measurement, at least one blank was run to ensure a clean system and eliminate sample carry-over. Duplicate analyses were performed for each sample. Because two samples were prepared and each sample was analyzed in duplicate, a total of four analyses were obtained for each fruit type.

Peak identifications were achieved by matching Kovats standardized retention index values (LRI, using standard *n*-alkanes of C_6−C_{22} in methanol, supplied by Electronic Sensor Technology) with those from GC-MS identification using the same type of column. Confirmation of peak identifications was achieved by matching LRI values with authentic standards in both systems. Chromatographic peak area values were used for data analysis.

SPME-GC-MS Volatile Analysis. Ten grams of blueberry puree was added into a 40 mL vial with a 4 mm Teflon stir bar, flushed with nitrogen, and sealed with a screw-cap Teflon-coated septum. The sample was equilibrated at 40 °C in a water bath for 20 min. After equilibration, a fiber coated with divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, 2 cm, 50/30 μm film thickness, Supelco, Bellefonte, PA, USA) was exposed to the headspace of the vial for 40 min at 40 °C. The fiber was then introduced into a heated GC injection for a 3 min desorption.

GC-MS analyses were performed using a PerkinElmer Clarus 500 gas chromatograph and quadropole mass spectrometer (PerkinElmer, Waltham, MA, USA). Compound separation was achieved with an Rtx-5 column (60 m × 0.25 mm i.d., crossbond 5% diphenyl−95% dimethyl polysiloxane × 0.50 μm film thickness, Restek Corp., Bellefonte, PA, USA). The column flow rate was 2.0 mL/min. The oven temperature started at 40 °C and held for 1 min, then increased to 190 °C at a rate of 4 °C/min, and finally to 250 °C at a rate of 8 °C/min, with a 5 min hold at the final temperature. Injection, MS transfer line, and ion source temperatures were 230, 240, and 180 °C, respectively. Electron ionization mass spectrometric data from m/z 25 to 300 were collected using an ionization voltage of 70 eV. Compound identifications were made by comparing mass spectral patterns with those from the NIST or Wiley 275.L (G1035) databases and confirmed by comparisons with authentic standards. Linear retention index (LRI) values were calculated using standard n-alkanes of C_5−C_{25}.

Statistical Analysis. One-way analysis of variance (ANOVA) was performed for each compound within each treatment including the factors of cultivars and maturity stages. ANOVA values were calculated using Statistica software (version 9, StatSoft Inc., Tulsa, OK, USA). Mean volatile data were evaluated using Tukey's Honestly Significant Difference (HSD) test for multiple comparisons. Unscrambler version 10.0.1 software (CAMO ASA, Oslo, Norway) was used for principal component analysis (PCA) calculations.

RESULTS AND DISCUSSION

Identification of FGC-SAW Blueberry Volatile Peaks. Even though the FGC-SAW employs only a short 1 m column, additional separation is achieved from the thermal gradient used to desorb volatiles from the Tenax trap. The degree of separation obtained in a few seconds is quite acceptable for many applications. As the volatiles sequentially elute from the short column they are absorbed on the crystalline surface of a single SAW detector. The added mass on SAW detector reduces the oscillating frequency, which is in direct proportion to the mass of the volatile eluted. The frequency change is electronically measured, recorded, and converted to a chromatogram, which is similar to an inverted GC chromatogram. The area of each peak is correlated to its concentration, and detection limits as low as 200 μg/kg for hexanal in grapes have been reported.[25]

The SAW output for volatiles in a typical blueberry sample has been inverted in Figure 1 to make it appear more like a typical chromatogram. Because of limited resolution, FGC-SAW cannot separate all of the sample's volatiles into individual peaks, and many peaks will contain more than a single component. Calibration plots of a single volatile can be created to obtain a relationship between peak area and concentration. However, calculations based on these response factors should be interpreted with caution because many peaks will contain more than a single component. Alternatively, the information in the chromatogram can be considered as a unique set of retention time−peak area values, which can be used with pattern recognition programs to differentiate or discriminate between different samples.

Even with limited resolution, 14 peaks were observed in blueberry purees (Figure 1). The peak identifications were confirmed by matching retention times with authentic standards and comparing FGC-SAW standard retention (LRI) values obtained using GC-MS. A more complete chromatographic separation of blueberry volatiles is shown in the GC-MS chromatogram in Figure 2. Volatiles from the blueberry cultivars examined in this study using both analytical techniques are compared in Table 1. A single peak numbering system was employed to make comparisons between the total ion chromatogram (TIC) GC-MS chromatograms and the FGC-SAW chromatograms less confusing. The same peak number corresponds to the same volatile in both chromatograms shown in Figures 1 and 2. For example, (E)-2-hexenal, (E)-2-hexenol, and hexanol all eluted as a single large FGC-SAW peak in the chromatogram shown in Figure 1. All three volatiles produced identical retention times of 1.88 s and a corresponding LRI value of 856 (Table 1) when injected as separate standards.

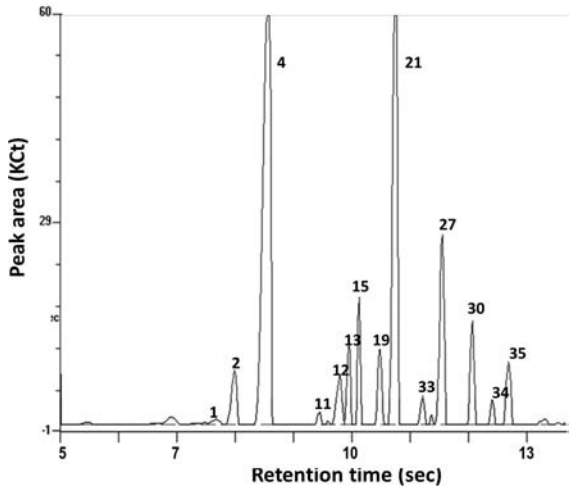

Figure 1. Typical FGC-SAW blueberry "chromatogram" (specifically, 'FL02-40' harvested on May 10, 2010). Peaks: 1 = methyl 3-methylbutanoate; 2 = 1-hexanal; 4 = (E)-2-hexenal; 5 = (E)-2-hexenol; 6 = hexanol; 11 = 6-methyl-5-hepten-2-one; 12 = (Z)-dehydrolinalool oxide; 13 = (E)-dehydrolinalool oxide; 15 = 2-ethylhexanol; 19 = pinol; 21 = linalool; 27 = α-terpineol; 30 = geraniol; 33 = unknown 1; 34 = unknown 2; 35 = unknown 3. See Table 1 for retention times of minor coeluted volatiles.

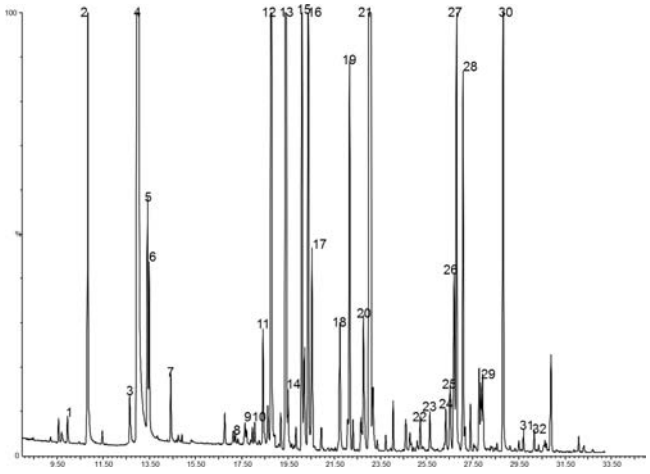

Figure 2. GC-MS chromatogram of the same blueberry sample as in Figure 1. Peaks: 1 = methyl 3-methylbutanoate; 2 = hexanal; 3 = (E)-2-hexenal; 4 = (E)-2-hexenal; 5 = (E)-2-hexenol; 6 = hexanol; 7 = 2-heptanone; 8 = (E)-2-heptenal; 9 = heptanol; 10 = 1-octen-3-ol; 11 = 6-methyl-5-hepten-2-one; 12 = (Z)-dehydrolinalool oxide; 13 = (E)-dehydrolinalool oxide; 14 = (E)-2-hexenyl acetate; 15 = 2-ethylhexanol; 16 = limonene; 17 = eucalyptol; 18 = octanol; 19 = pinol; 20 = α-terpinolene; 21 = linalool; 22 = perilla alcohol; 23 = nonanol; 24 = 4-terpineol; 25 = p-cymen-8-ol; 26 = limonene oxide; 27 = α-terpineol; 28 = dihydrocarvone; 29 = nerol; 30 = geraniol; 31 = γ-ionone; 32 = 2-undecanone.

dx.doi.org/10.1021/jf300697f | J. Agric. Food Chem. 2012, 60, 5099−5106

Table 1. Tentative Identification of Chromatographic Peaks from 'FL02-40' Blueberry Puree Comparing Data from both FGC-SAW and GC-MS

	retention time		retention index			
peak	SAW (s)	MS (min)	SAW	MS	MS area %	identification
1	1.28	9.95	781	778	0.16	methyl 3-methylbutanoate
2	1.46	10.83	800	802	3.64	hexanal
3		12.65		830	0.32	(*Z*)-3-hexenal
4	1.88	13.03	856	857	18.62	(*E*)-2-hexenal
5	1.88	13.42	856	859	1.23	(*E*)-2-hexenol
6	1.88	13.49	856	860	0.84	hexanol
7	2.06	14.43	891	896	0.42	2-heptanone
8		17.22		951	0.01	(*E*)-2-heptenal
9	2.64	17.65	970	962	0.17	heptanol
10	2.70	18.07	978	982	0.13	1-octen-3-ol
11	2.54	18.43	985	990	0.75	6-methyl-5-hepten-2-one
12	2.84	18.79	1003	998	7.59	(*Z*)-dehydrolinalool oxide
13	2.94	19.42	1014	1014	12.10	(*E*)-dehydrolinalool oxide
14	3.02	19.51	1020	1017	0.22	(*E*)-2-hexenyl acetate
15	3.10	20.11	1032	1031	3.58	2-ethylhexanol
16		20.38		1038	3.77	limonene
17	3.14	20.54	1035	1040	1.19	eucalyptol
18	3.41	21.75	1070	1072	0.91	octanol
19	3.42	22.17	1079	1082	2.53	pinol
20		22.78		1096	0.96	α-terpinolene
21	3.68	23.10	1102	1103	24.76	linalool
22		25.23		1138	0.22	perilla alcohol
23	4.28	25.65	1176	1155	0.23	nonanol
24	4.34	26.34	1183	1182	0.23	4-terpineol
25	4.40	26.52	1190	1189	0.45	*p*-cymen-8-ol
26		26.70		1194	1.17	limonene oxide
27	4.46	26.81	1190	1195	3.57	α-terpineol
28		27.09		1201	2.27	dihydro-carvone
29	4.76	27.95	1232	1250	0.47	nerol
30	4.98	28.82	1257	1259	3.32	geraniol
31		29.71		1279	0.13	γ-ionone
32	5.34	30.17	1293	1296	0.13	2-undecanone
33	4.12		1164			unknown 1
34	5.32		1312			unknown 2
35	5.62		1365			unknown 3

However, the volatile present in highest relative amount is (*E*)-2-hexenal (18.6 versus 1.2 and 0.8% according to MS data). Therefore, this peak is labeled "4" even though much smaller amounts of "5" and "6" are present. In a similar manner, the remaining FGC-SAW peaks shown in Figure 1 are labeled according to the volatile present in highest relative amount.

All FGC-SAW peak identifications were determined by matching retention times using standards identified from the GC-MS data. Twenty-seven FGC-SAW retention time values are listed in Table 1. Because LRI values were also determined using alkane standards, calculated FGC-SAW LRI values are also compared with those obtained from GC-MS for further identity confirmation. As shown in Table 1, the LRI values from the two systems are very similar (usually within ±5 LRI units).

Identified blueberry volatiles from both analytical systems are presented in Table 1. These volatiles were observed in all four cultivars examined in this study. Even though 14 peaks were observed in the FGC-SAW chromatograms, only 11 peaks could be identified with some assurance by matching retention time of standards as well as FGC-SAW retention index values with GC-MS retention index values of compounds identified from spectral libraries and standards (Figure 2). Thirty-two volatiles have been identified using MS in Table 1 and are labeled in Figure 2. Of the 14 FGC-SAW peaks shown in Figure 1, 11 could be associated with 27 volatiles, for which retention times are listed in Table 1. Three FGC-SAW peaks could not be matched with GC-MS identifications and LRI matches. They are labeled 33, 34, and 35. Because they elute late, they could be due to higher boiling (higher molecular weight) volatiles) that were trapped by the Tenax and not the SPME fiber. In addition, the types of headspace sampling were different as well. SPME is a static headspace technique, whereas a Tenax trap is employed in dynamic headspace sampling. Thus, it is possible that a slightly different set of volatiles was collected in the two techniques.

A number of minor volatiles that coeluted with some of the major peaks are labeled in Figure 1. Because the number of observed FGC-SAW peaks was much less than that observed using GC-MS, extensive coelution was expected. This was confirmed by the tight clusters of FGC-SAW retention time values for blueberry volatile standards listed in Table 1. Therefore, FGC-SAW analysis is best suited for situations when only the major volatiles need to be determined in the shortest possible time.

FGC-SAW Differentiation of Blueberry Volatile Patterns. Percent soluble solids content (°Brix, SSC) and titratable acidity (TA) were measured for all samples. °Brix/acid (SSC/TA) ratios for 'Jewel' blueberries from Haines City on multiple harvest dates are listed in Table 2. On average, fully mature fruit 'Snowchaser', 'FL02-40', 'Primadonna', and 'Jewel' from Gainesville had SSC of 13.3, 13.0, 15.1, and 12.0% (°Brix) and TA of 0.56, 0.43, 0.13, and 0.34% (% of citric acid), respectively. In contrast, fully mature fruit 'Primadonna' and 'Jewel' from Haines City had SSC of 13.0 and 10.2 and TA of 0.63 and 0.92%, respectively. Generally, SSC increased and TA decreased with increasing fruit maturity. Any variation in SSC and TA was likely a result of the highly variable weather conditions at that time of year in Florida. For example, temperature fluctuations at the beginning of the growing season (February 2010) ranged from 0 to 22 °C to the end of the harvest season (May 2010), when temperatures ranged from lows of 18 °C and highs of 33 °C. Differences between night lows and daytime highs on the same day ranged from 2 to 22 °C during this period in 2010.

Some of the major volatiles, such as (*E*)-2-hexenal, and lesser volatiles, such as hexanal and (*Z*)-dehydrolinalool oxide, did not change over the harvest season. However, other major volatiles such as 2-ethylhexanol and linalool generally (but erratically) increased during the harvest season. The results are quite comparable with those obtained using GC-MS. The different relative peak areas of the various volatiles from fruit at the same visual maturity but different harvest dates shown in Table 2 suggest that plants were exposed to different environmental conditions as documented in the preceding paragraph, which will influence fruit volatile metabolism.

There were some differences between the volatile patterns of the four blueberry cultivars examined. All cultivars had (*E*)-2-

Table 2. Soluble Solids/Titratable Acidity Ratios and Average FGC-SAW Peak Areas (kCts) of the Major Volatiles in 'Jewel' Blueberries Harvested from Haines City, FL, on Multiple Harvest Dates[a]

compound	peak	April 27	May 5	May 11	May 18
°Brix/acid ratio		8.8	11.0	10.3	16.7
methyl 3-methylbutanoate	1	69 AB	27 A	56 A	74 AB
hexanal	2	147 A	113 A	119 A	207 A
(*E*)-2-hexenal	4	3603 A	2852 A	3649 A	2948 A
6-methyl-5-hepten-2-one	11	94 B	55 A	94 B	83 AB
(*Z*)-dehydrolinalool oxide	12	265 A	285 A	189 A	243 A
(*E*)-dehydrolinalool oxide	13	67 A		171 B	
2-ethylhexanol	15	816 A	1501 C	1163 B	1611 C
pinol	19	270 AC	186 AB	168 AB	349 C
linalool	21	830 A	1172 AB	1554 B	1232 AB
unknown 1	33	285 B	255 B	242 AB	186 A
α-terpineol	27	491 D	315 AB	262 A	575 D
geraniol	30	39 A	123 AB	129 AB	190 B
unknown 2	34	530 A	491 A	815 B	669 AB
unknown 3	35	969 B	1024 B	1067 B	567 A
total		8475	8399	9678	8934

[a]Numbers followed by the same letter within each compound and cultivars are not significantly different by Tukey's test Studentized range (HSD) test (α = 0.05).

hexenal and linalool as the dominant volatiles, in agreement with our previous work.[28] However, from a qualitative point of view, methyl 3-methylbutanoate was not detected in 'Snowchaser' and 'FL02-40', whereas (*E*)-dehydrolinalool oxide was below detection limits in 'Primadonna' and 'Jewel' (data not shown). In terms of quantity, 'Primadonna' and 'FL02-40' had the highest levels of total volatiles, closely followed by 'Snowchaser' and 'Jewel'. The dominant compounds (*E*)-2-hexenal in 'Primadonna' and linalool and (*E*)-2-hexenal in 'FL02-40' distinguished these two cultivars from all others. Volatiles from these four cultivars differ in terms of what volatiles are present as well as how much of each volatile is present.

FGC-SAW and GC-MS Cultivar Pattern Recognition Analyses. Pattern recognition is a statistical technique used for data processing to characterize complex composition of samples without the need to identify and quantify individual components.[6] PCA is a common pattern recognition technique used to determine structure or patterns of samples with multiple measurements. It does not require that individual samples be classified into a particular category, but rather analyzes the data in such a way that samples with similar values cluster together. This is most effective when single or small numbers of measurements in the sample set are not differentiating. Because no single peak in the FGC-SAW differentiated all cultivars, the values from the common 14 peak FGC-SAW chromatographic data set were analyzed using PCA to determine if the set of sample measurements could differentiate between cultivars. GC-MS data for the four cultivars were analyzed in a similar fashion.

The PCA score–loading biplot analysis of FGC-SAW data from four blueberry cultivars harvested on multiple harvest dates is shown in Figure 3. The first two principal components (PC) accounted for 66% of total variance, with PC1 contributing 42% and PC2 contributing 24% of the total variance, respectively. The PCA plot clearly demonstrated that FGC-SAW can be used to differentiate between these four major cultivars as all four are well separated from each other. 'Jewel' and 'Primadonna' were the most distant from each other and the other two cultivars. 'Snowchaser' and 'FL02-40' were still in separate clusters, but the proximity to each other suggested that they had many similar traits. It is worth noting that the values from duplicate samples of the same cultivar collected on the same date were closely paired in the PCA plot,

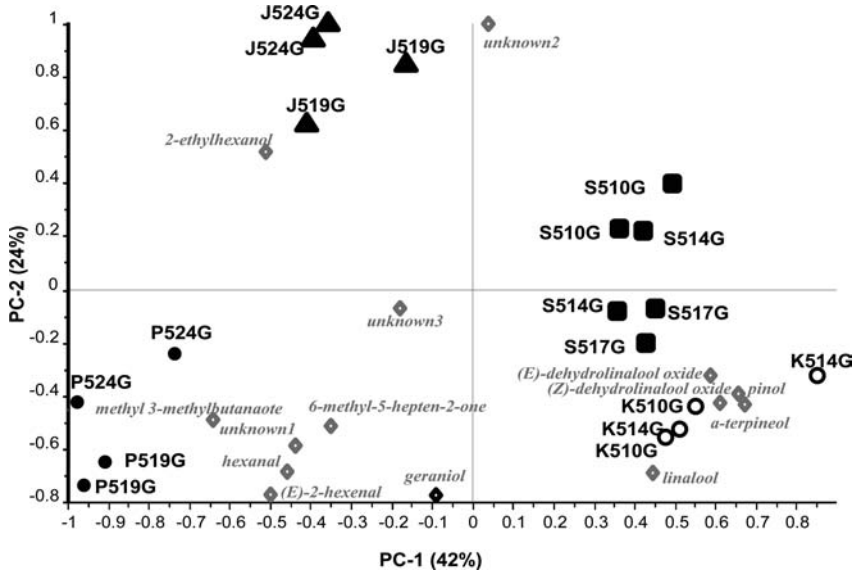

Figure 3. PCA score–load biplot of four blueberry cultivars, 'Snowchaser', 'FL02-40', 'Primadonna', and 'Jewel', harvested in Gainesville, FL, on multiple harvest dates using 14 FGC-SAW peak areas. Key: S = Snowchaser; K = FL02-40; P = Primadonna; J = Jewel; 510 = May 10; 514 = May 14; 517 = May 17; 519 = May 19; 524 = May 24; G = Gainesville, FL.

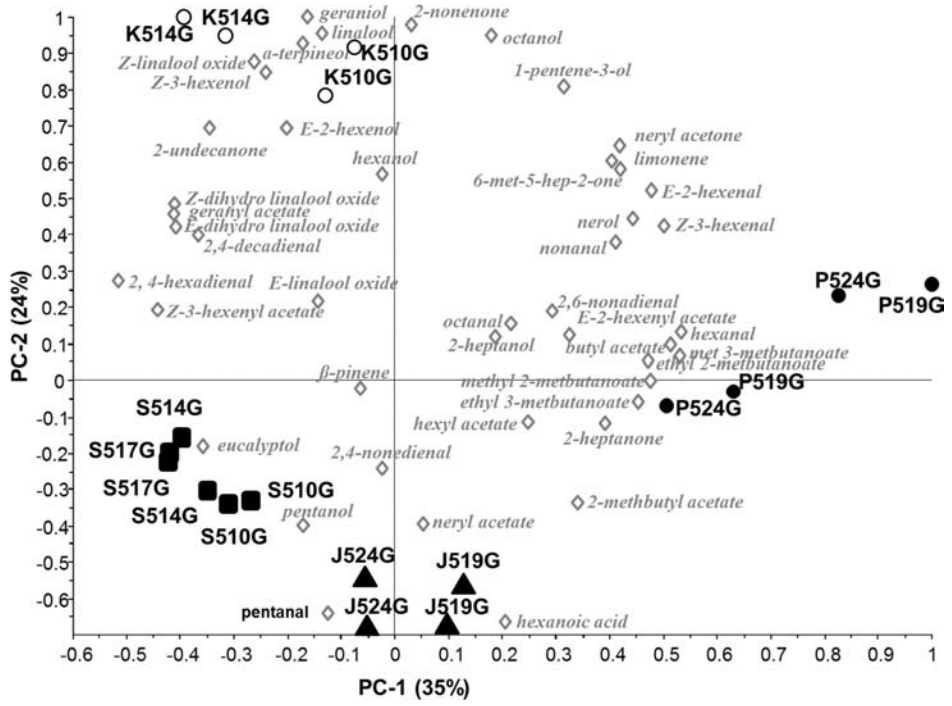

Figure 4. PCA score–load biplot of four blueberry cultivars, 'Snowchaser', 'FL02-40', 'Primadonna', and 'Jewel', harvested in Gainesville, FL, with multiple harvest dates differentiated by GC-MS. Key: S = Snowchaser; K = FL02-40; P = Primadonna; J = Jewel; 510 = May 10; 514 = May 14; 517 = May 17; 519 = May 19; 524 = May 24; G = Gainesville, FL.

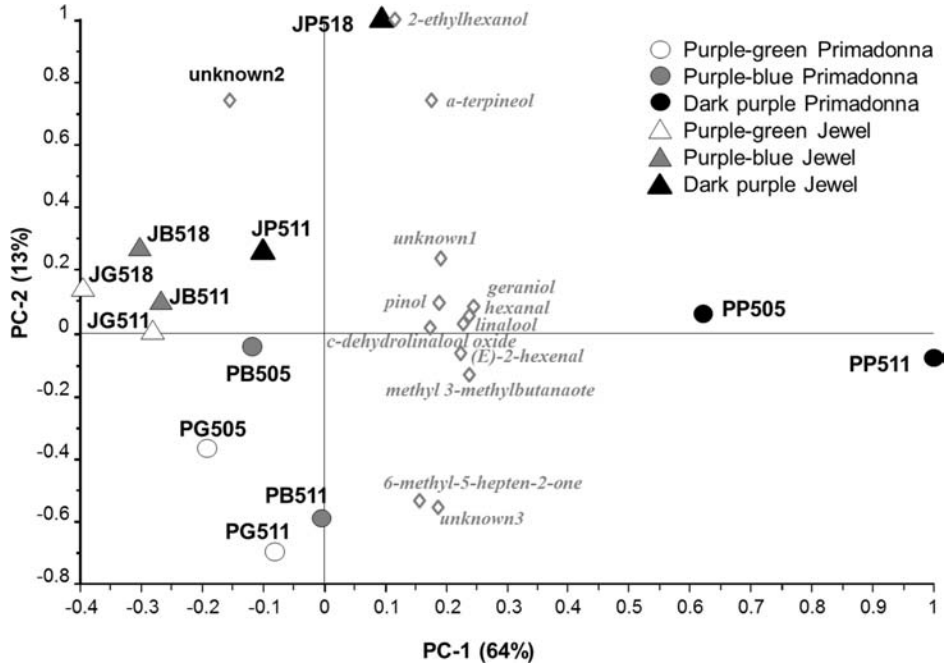

Figure 5. PCA score–load biplot of 'Primadonna' and 'Jewel' blueberries at three maturity stages on multiple harvest dates (Haines City, FL) differentiated by FGC-SAW. Key: PG = Primadonna green-purple; PB = Primadonna purple-blue; PP = Primadonna dark purple; JG = Jewel green-purple; JB = Jewel purple-blue; JP = Jewel dark purple; 505 = May 5; 511 = May 11; 518 = May 18.

suggesting that the analytical precision and sampling techniques were satisfactory. The loading values (shown in gray) indicated that PC1 was based primarily on methyl 3-methylbutanoate and a combination of pinol and (*Z*)-dehydrolinalool oxide. This axis did not separate 'FL02-40' from 'Snowchaser' but did differentiate that pair from 'Primadonna' and 'Jewel'. On the other hand, PC2 was weighted most heavily with unknown 2 on one end and a combination of (*E*)-2-hexenol and geraniol on the other. This axis successfully separated 'Snowchaser' from 'FL02-40' and further separated 'Primadonna' from 'Jewel'.

The next objective was to determine if the greater chromatographic resolution from GC-MS would provide superior differentiation of the blueberry cultivars being examined. The score–loading PCA plot using the 32 peak

dx.doi.org/10.1021/jf300697f | J. Agric. Food Chem. 2012, 60, 5099–5106

areas from the GC-MS data common among the cultivars is shown in Figure 4. It is apparent that the four cultivars also cluster into separate groups using the GC-MS data. 'FL02-40' and 'Primadonna' are well separated from each other and the remaining two cultivars. 'Jewel' and 'Snowchaser' are also clustered separately but in close proximity to each other, suggesting that their GC-MS volatile patterns are somewhat similar. PC1 explains 35% of the variance and is most heavily influenced (according to loading values) by (*E*,*E*)-2,4-hexadienal and (*Z*)-3-hexenyl acetate in terms of negative values and hexanal with methyl 3-methylbutanoate among others for positive values. PC2 explains 24% of the total variance and is most heavily influenced by pentanal and hexanoic acid values of the negative side and geraniol with 2-nonenone for positive values.

It is useful to compare the resulting PCA plots in Figures 3 and 4 to determine if the increased resolution and longer analysis time with GC-MS has provided better information in terms of separating the four cultivars than FGC-SAW. Even though the four cultivar groups are in different relative locations, all four cultivars appear to be well separated using both data sets. In both cases, two of the cultivars are well separated from the other two. In both cases 'Primadonna' was well separated from the remaining three cultivars. However, in the FGC-SAW PCA 'Snowchaser' and 'FL02−40' were both on the same side and in proximity to each other, whereas in the GC-MS PCA, 'Snowchaser' and 'Jewel' were in proximity to each other. 'Snowchaser's being well separated from the other two cultivars in both data sets suggests that the volatiles used to calculate the score (eigenvalues) for 'Jewel' and 'FL02-40' were different. However, in the final analysis, it is apparent that both data sets provided equivalent information and that the extra analysis time and chromatographic resolution of GC-MS did not provide superior differentiation between the four cultivars.

FGC-SAW Volatile Differences due to Maturity. Because no single value was able to clearly differentiate between blueberries at three maturity stages, PCA was again employed to determine if the entire peak patterns could be used to differentiate blueberries at different maturity stages. Shown in Figure 5 is a PCA biplot of the FGC-SAW data for 'Primadonna' and 'Jewel' blueberries grown in Haines City, FL, and harvested at three maturity stages on multiple harvest dates. The first two PCs accounted for 77% of total variance; PC1 contributed 64%, and PC2 contributed 13%. PC1 is most heavily influenced by unknown 2 for negative values and a mixture of hexanal and methyl 3-methylbutanoate, among others, for positive values. PC2 was most heavily influenced by 2-ethylhexanol in terms of positive values and 6-methyl-5-hepten-2-one with unknown 3 for negative values. In this manner berries were separated by increasing maturity from left to right and from low to high on the PCA plot for both cultivars. PC1 differentiated the fully mature 'Primadonna' blueberry fruit (PP, dark purple) from green-purple (PG) and purple-blue (PB) berry fruit, with very limited separation between these two early stages. In contrast, 'Jewel' blueberries at the three maturity levels were also separated but not to the same degree as 'Primadonna'. The loading values indicated that the increased levels of hexanal, methyl 3-methylbutanoate, (*E*)-2-hexenal, (*Z*)-dehydrolinalool oxide, linalool, and geraniol were responsible for differentiating the fully mature (dark purple) stages in both cultivars from the two less mature stages. The greater degree of separation for the fully mature (dark purple) 'Primadonna' versus that in the 'Jewel' cultivar suggests there were greater increases in the levels of the secondary metabolites in 'Primadonna' than for 'Jewel'.

AUTHOR INFORMATION

Corresponding Author

*Phone: +1 (863) 956-8860. E-mail: rrouseff@ufl.edu.

Funding

This project was funded in part by a USDA Specialty Crops Research Initiative, "Increasing Consumption of Fresh Fruits & Vegetables by Enhancing Their Quality and Safety", as a joint research project with the University of California, Davis, and the University of Florida. The loan of the FGC-SAW instrument from the University of California, Davis, is gratefully acknowledged.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

Special thanks are given Kendra Blaker (Department of Horticulture Science, University of Florida) for harvesting blueberry samples in Gainesville, FL, and Mei Song for technical support.

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) U.-N.A.S. Service. *Noncitrus Fruits and Nuts 2010 Summary*; 2011.

(3) Williamson, J. G.; Lyrene, P. M. *Blueberry Varieties for Florida*; University of Florida IFAS Extension HS967; 2004; pp 1−9.

(4) Saftner, R.; Polashock, J.; Ehlenfeldt, M.; Vinyard, B. Instrumental and sensory quality characteristics of blueberry fruit from twelve cultivars. *Postharvest Biol. Technol.* **2008**, *49* (1), 19−26.

(5) Röck, F.; Barsan, N.; Weimar, U. Electronic nose: current status and future trends. *Chem. Rev.* **2008**, *108*, 705−725.

(6) Peris, M.; Escuder-Gilabert, L. A 21st century technique for food control: electronic noses. *Anal. Chim. Acta* **2009**, *638*, 1−15.

(7) Saevels, S.; Lammertyn, J.; Berna, A. Z.; Veraverbeke, E. A.; Natale, C. D.; Nicolaï, B. M. Electronic nose as a non-destructive tool to evaluate the optimal harvest date of apples. *Postharvest Biol. Technol.* **2003**, *30* (1), 3−14.

(8) Benedetti, S.; Buratti, S.; Spinardi, A.; Mannino, S.; Mignani, I. Electronic nose as a non-destructive tool to characterise peach cultivars and to monitor their ripening stage during shelf-life. *Postharvest Biol. Technol.* **2008**, *47* (2), 181−188.

(9) Lebrun, M.; Plotto, A.; Goodner, K.; Ducamp, M.-N.; Baldwin, E. Discrimination of mango fruit maturity by volatiles using the electronic nose and gas chromatography. *Postharvest Biol. Technol.* **2008**, *48* (1), 122−131.

(10) Solis-Solisa, H. M.; Calderon-Santoyoa, M.; Gutierrez-Martineza, P.; Schorr-Galindob, S.; Ragazzo-Sancheza, J. A. Discrimination of eight varieties of apricot (*Prunus armeniaca*) by electronic nose, LLE and SPME using GC−MS and multivariate analysis. *Sens. Actuators B: Chem.* **2007**, *125* (2), 415−421.

(11) Hirschfelder, M.; Hanrieder, D.; Ulrich, D. Discrimination of strawberry varieties by a gas sensory array in correlation with human sensory evaluation. *Semin. Food Anal.* **1998**, *3*, 27−36.

(12) Labreche, S.; Bazzo, S.; Cade, S.; Chanie, E. Shelf life determination by electronic nose: application to milk. *Sens. Actuators B: Chem.* **2005**, *106* (1), 199−206.

(13) Simon, J. E.; Hetzroni, A.; Bordelon, B.; Miles, G. E.; Charles, D. J. Electronic sensing of aromatic volatiles for quality sorting of blueberries. *J. Food Sci.* **1996**, *61* (5), 967−970.

(14) Li, C.; Krewer, G. W.; Ji, P.; Scherm, H.; Kays, S. J. Gas sensor array for blueberry fruit disease detection and classification. *Postharvest Biol. Technol.* **2010**, *55* (3), 144−149.

(15) Nurjuliana, M.; Che Man, Y. B.; Mat Hashim, D. Analysis of lard's aroma by an electronic nose for rapid *Halal* authentication. *J. Am. Oil Chem. Soc.* **2010**, *88* (1), 75−82.
(16) Che Man, Y. B.; Gan, H. L.; NorAini, I.; Nazimah, S. A. H.; Tan, C. P. Detection of lard adulteration in RBD palm olein using an electronic nose. *Food Chem.* **2005**, *90* (4), 829−835.
(17) Gan, H. L.; Che Man, Y. B.; Tan, C. P.; NorAini, I.; Nazimah, S. A. H. Characterisation of vegetable oils by surface acoustic wave sensing electronic nose. *Food Chem.* **2005**, *89* (4), 507−518.
(18) Gan, H. L.; Tan, C. P.; Che Man, Y. B.; NorAini, I.; Nazimah, S. A. H. Monitoring the storage stability of RBD palm olein using the electronic nose. *Food Chem.* **2005**, *89* (4), 271−282.
(19) Biswas, S.; Heindselmen, K.; Wohltjen, H.; Staff, C. Differentiation of vegetable oils and determination of sunflower oil oxidation using a surface acoustic wave sensing device. *Food Control* **2004**, *15* (1), 19−26.
(20) Tewari, J. C.; Irudayaraj, J. M. K. Floral classification of honey using mid-Infrared spectroscopy and surface acoustic wave based z-nose sensor. *J. Agric. Food Chem.* **2005**, *53* (18), 6955−6966.
(21) Veraverbeke, E. A.; Irudayaraj, J.; Lammertyn, J. Fast aroma profiling to detect invert sugar adulteration with zNose™. *J. Sci. Food Agric.* **2005**, *85* (2), 243−250.
(22) Lammertyn, J.; Veraverbeke, E. A.; Irudayaraj, J. zNose™ technology for the classification of honey based on rapid aroma profiling. *Sens. Actuators B: Chem.* **2004**, *98*, 54−62.
(23) Oh, S. Y.; Ko, J. W.; Jeong, S.-Y.; Hong, J. Application and exploration of fast gas chromatography-surface acoustic wave sensor to the analysis of *thymus* species. *J. Chromatogr., A* **2008**, *1205* (1−2), 117−127.
(24) Oh, S. Y.; Shin, H. D.; Kim, S. J.; Hong, J. Rapid determination of floral aroma compounds of lilac blossom by fast gas chromatography combined with surface acoustic wave sensor. *J. Chromatogr., A* **2008**, *1183* (1−2), 170−178.
(25) Watkins, P.; Wijesundera, C. Application of zNose™ for the analysis of selected grape aroma compounds. *Talanta* **2006**, *70*, 595−601.
(26) Kunert, M.; Biedermann, A.; Koch, T.; Boland, W. Ultrafast sampling and analysis of plant volatiles by a hand-held miniaturised GC with pre-concentration unit: kinetic and quantitative aspects of plant volatile production. *J. Sep. Sci.* **2002**, *25* (10−11), 677−684.
(27) Du, X.; Qian, M. Flavor chemistry of small fruits: blackberry, raspberry, and blueberry. In *Flavor and Health Benefits of Small Fruits*; Qian, M. C., Rimando, A. M., Eds.; ACS Symposium Series 1035; American Chemical Society: Washington, DC, 2010; pp 27−43.
(28) Du, X.; Plotto, A.; Song, M.; Olmstead, J.; Rouseff, R. Volatile composition of four Southern highbush blueberry cultivars and effect of growing location and harvest date. *J. Agric. Food Chem.* **2011**, *59* (15), 8347−8357.