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Evaluation of Volatiles from Two Subtropical Strawberry Cultivars Using GC−Olfactometry, GC-MS Odor Activity Values, and Sensory Analysis

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ABSTRACT: Most strawberry flavor studies have examined berries grown in temperate regions with long summer days. Few studies have examined berries harvested in winter months from subtropical regions with shorter photoperiods. Fruits harvested in February and March from two strawberry cultivars, 'Strawberry Festival' and 'Florida Radiance', were examined. Thirty odor active compounds were detected using time-intensity GC-O. Twenty-nine were identified. The major odor active volatiles in both cultivars were 2,5-dimethyl-4-methoxy-3(2*H*)-furanone (DMMF), 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (DMHF), methyl butanoate, γ-decalactone, unknown (grassy, LRI 1362, wax), (*E*)-2-hexenal, linalool, (*E*,*Z*)-2,6-nonadienal, geraniol, butanoic acid, methyl 2-methylbutanoate, and ethyl hexanoate. Over 90 volatiles were identified and 54 quantified using GC-MS including 33 esters, 6 alcohols, 5 acids, 3 aldehydes, 3 lactones, 2 ketones, and 2 furanones. Odor activity values (OAVs) were determined for 46 volatiles, of which 22 had OAV > 1. The highest OAVs for 'Strawberry Festival' were ethyl butanoate (461), DMHF (424), methyl butanoate (358), and linalool (102). A 14-member trained panel evaluated quartered fruit using quantitative descriptive analysis to quantify seven sensory attributes. Sensory "strawberry flavor" scores were positively correlated with sensory "sweetness" ($R^2 = 0.83$) as well as GC-MS methyl 3-methylbutanoate ($R^2 = 0.90$) and ethyl butanoate ($R^2 = 0.96$). These cultivars lacked methyl anthranilate and possessed an aroma pattern different from summer-grown strawberries.

KEYWORDS: strawberry volatiles, GC-O, OAV, descriptive sensory analysis

INTRODUCTION

Strawberry (*Fragaria* × *ananassa* Duch. ex Rozier) is among the most widely consumed fruit because of its attractive color and flavor as well as its nutritional value. It is consumed, either fresh or frozen or in prepared foods such as preserves, juice, and pies. The United States is one of the larger strawberry producers in the world, and California alone accounts for 80% of the total U.S. production. However, the fresh strawberry season in California typically ends by the end of October, and Florida strawberry growers have selected cultivars that come into production during the winter months when there are few other domestic sources. Florida is the largest supplier of fresh strawberries to the eastern and midwestern United States during December, January, and February.[1]

Florida has developed specialized strawberry cultivars that have been adapted to the subtropical winter growing environment, shorter days, and unique soil types.[2] Various strawberry cultivars have been developed and released commercially from the University of Florida strawberry breeding program. Of these released cultivars, 'Strawberry Festival' is a major commercial strawberry cultivar in Florida. The fruits are firm and deep red and have excellent flavor. However, the production of 'Strawberry Festival' is low during the early season of November and December, and the size and sugar content tend to be less than optimum in the late season of March.[3] 'Florida Radiance' is a newly released strawberry cultivar, which appears to be a candidate cultivar to complement the production characteristics of 'Strawberry Festival'.[4]

Strawberry aroma is complex and chemically diverse. It is not surprising that approximately 1000 cultivars are preserved in germplasm collections worldwide.[5] Strawberry volatile composition has been extensively studied. Although a wide diversity of aroma patterns exists, strawberry aroma is generally a complex mixture of esters, furanones, and terpene alcohols with smaller amounts of lactones, aldehydes, alcohols, and sulfur compounds.[6−9]

Although strawberry aroma has been extensively studied in fruit grown in temperate climates throughout the world, little information could be found on aroma profiles for strawberries grown in subtropical regions. Florida cultivars have been bred for the southern climate, which may result in different aroma and flavor characteristics from their temperate counterparts. In addition, temperature and day length of the fruit grown November through March in Florida may affect flavor parameters as well. Limited volatile information was found for 'Strawberry Festival', the major cultivar grown in Florida.[10] No aroma information for 'Florida Radiance' was found. Therefore, the objective of this study was to characterize the aroma profiles of these two Florida strawberry cultivars using gas chromatography−olfactometry (GC-O), mass spectrometry (MS) determined odor activity values (OAVs), and human sensory evaluation.

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MATERIALS AND METHODS

Chemicals. Pure standards of ethyl acetate, methyl butanoate, butyl acetate, methyl 2-methylbutanoate, ethyl 2-methylbutanoate, ethyl butanoate, propyl butanoate, methyl hexanoate, ethyl hexanoate, (*E*)-2-hexenyl acetate, octyl butanoate, methyl isovalerate, ethyl isovalerate, methyl octanoate, isoamyl butanoate, methyl thioacetate, methyl thiobutanoate, 1-hexanal, (*E*)-2-hexenal, (*Z*)-3-hexenol, (*E*)-2-hexenol, 1-octen-3-ol, (*E*,*Z*)-2,6-nonadienal, linalool (3,7-dimethylocta-1,6-dien-3-ol), nerolidol (3,7,11-trimethyl-1,6,10-dodecatrien-3-ol), γ-decalactone, 2-methylbutanoic acid, 2,5-dimethyl-4-methoxy-3(2*H*)-furanone (DMMF), butanoic acid, hexanoic acid, 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (DMHF), and 3-heptanone (internal standard) were obtained from Aldrich Chemical Co., Inc. (Milwaukee, WI). Hexyl acetate was obtained from Fluka (Buchs, Switzerland). Geraniol (3,7-dimethylocta-2,6-dien-1-ol) was obtained from Acros Organics (Fair Lawn, NJ). Sodium fluoride (ACS graded) was also obtained from Acros Organics. Sodium chloride, anhydrous citric acid, sucrose, and methanol were purchased from Fisher Scientific (Fair Lawn, NJ).

Strawberry Samples. 'Strawberry Festival' and 'Florida Radiance' plants were grown at the University of Florida Gulf Coast Research and Education Center, Wimauma, FL. Strawberry fruits were hand-harvested at the fully mature stage (full-red) on February 9 and 25 and March 8 and 22 of 2010. After harvest, strawberries were stored at 5 °C overnight and then transferred to room temperature the next morning for processing.

For each cultivar/harvest, 2.7 kg of strawberries at the full-ripe stage were randomly harvested from approximately 500 plants. All of the berries in each sampling were washed and mixed. A final subset of 200 g of berries was randomly selected from the initial 2.7 kg harvest to make the final puree. Samples were pureed in a Waring blender (Waring Products Div., Dynamics Corp. of America, New Hartford, CT), with an equal weight of freshly distilled water, 20% (w/w) sodium chloride, and 1% (w/w) sodium fluoride. Sodium chloride was employed to inhibit enzyme activity, and sodium fluoride was used to inhibit microbial growth. Blending was performed in the high-speed pulse mode for 20 s, and the pureed fruit was immediately placed into individual sample vials and stored at −20 °C until analysis.

In addition, soluble solids content (SSC) and titratable acidity (TA, expressed as percent citric acid) were measured in duplicate on a subset of fruit (not salted) using a PAL-1 pocket refractometer (Atago USA, Inc., Bellevue, WA) for SSC and a Metrohm 808 Titrando (Metrohm USA, Westbury, NY) for TA.

Volatile Extraction with SPME. Ten grams of strawberry puree was added to a 40 mL glass vial, which was first flushed with nitrogen (ultrahigh-purity, 0.99999) and contained a 4 mm Teflon-coated stir bar. Fifty microliters of the internal standard (IS) solution (3-heptanone, 38.72 mg/kg in methanol solution) was added and mixed (IS was added to samples only for GC-MS quantification, not for GC-O). Each sample was equilibrated at 40 °C in a water bath for 20 min prior to analysis. After equilibration, a SPME fiber coated with divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, 2 cm, film thickness 50/30 μm, Supelco, Bellefonte, PA) was exposed in the vial headspace for 40 min at the same temperature (40 °C). The fiber was then introduced into a GC injection port at 220 °C for a 3 min desorption.

Multiple Standard Responses and OAVs. Calibration curves were constructed by adding known amounts of methyl butanoate, methyl 2-methylbutanoate, ethyl butanoate, hexanal, methyl hexanoate, (*E*)-2-hexenal, ethyl hexanoate, hexyl acetate, (*E*)-2-hexenyl acetate, (*Z*)-3-hexenol, (*E*)-2-hexenol, linalool, mesifurane, octyl butanoate, hexanoic acid, nerolidol, γ-decalactone, 2-methylbutanoic acid, and Furaneol to strawberry puree, the volatiles of which had been stripped using vacuum deaeration. Standards were added as mixtures so that final concentrations in the puree ranged from 10 μg/kg to 50 mg/kg. Fifty microliters of IS (3-heptanone) was also added to each sample. After equilibration, the added volatiles were extracted with SPME and analyzed with GC-MS as done for the samples. Calibration curves for each volatile were constructed using the peak area ratio of standards to internal standard and concentration ratio of standards to internal standard and were used to calculate the concentrations of volatiles in the samples. Samples of two cultivars from four harvest dates were determined using GC-MS. Duplicate analyses were performed for each sample and standard. OAVs were calculated by dividing the calculated concentrations with literature sensory thresholds in water.

Table 1. Reference Standards Used for Sensory Evaluation of Strawberry Samples

descriptor (intensity)	standard
sweet (2)	mixture of 0.025% citric acid and 1% sucrose
sweet (8)	mixture of 0.05% citric acid and 5% sucrose
sour (2)	mixture of 0.05% citric acid and 2% sucrose
sour (8)	mixture of 0.1% citric acid and 2% sucrose
strawberry flavor	strawberry purée made with a variety of ripe fruit, frozen and thawed before each panel; diluted 5% for the low level and neat for the high level; presented 7.5 mL in 32 mL soufflé cups
green flavor	(*Z*)-3-hexenal in water; 1 ppm for the low level and 3 ppm for the high level.
overripe/fermented	overripe fruit left on the bench overnight, cut in ~1 cm^3 pieces, and presented in 118 mL cups

GC-O. GC-O analysis was conducted using an Agilent 6890N gas chromatograph equipped with a flame ionization detector (FID) and a Datu olfactometer (Geneva, NY). 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) and a HP-5 column (30 m × 0.32 mm i.d. cross-linked phenyl-methyl polysiloxane × 0.25 μm film thickness, J&W Scientific). The column effluent was split 1:2 into a FID and a heated sniffing port. Column flow rate was 2.0 mL/min (helium), with injection in the splitless mode for 0.75 min. Injector and detector temperatures were 220 and 250 °C. The oven temperature was programmed at 35 °C for a 1 min hold and then to 190 °C at a rate of 4 °C/min and to 240 °C at a rate of 8 °C/min, with a 5 min hold at the final temperature.

Olfactometry analyses were conducted by two trained panelists on strawberry samples harvested on February 9, 2010. Each panelist evaluated the odors from individual 10 g aliquots from the same 200 g sample. The odor intensities were evaluated using a 15-point intensity scale, where 1 corresponded to slight aroma intensity, moderate intensity was 7, and 15 was for extreme intensity. 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 both panelists when an aroma was registered. Identifications were based on matching retention times and aroma character with authentic standards and retention indices (RIs) were determined using a series of standard linear alkanes C_5-C_{25}.

GC-MS Identification of Aroma Compounds. GC-MS analyses were performed using a PerkinElmer Clarus 500 GC-quadrupole MS (PerkinElmer, Waltham, MA). Separations were achieved using a DB-WAX column (60 m × 0.25 mm i.d. cross-linked polyethylene glycol, 0.50 μm film thickness, J&W Scientific, Agilent Technique, Foster City, CA). Column flow rate was 2.0 mL/min (helium). The oven temperature was programmed at 35 °C for a 1 min hold and then to 190 °C at a rate of 4 °C/min and to 240 °C at a rate of 8 °C/min, with a

Table 2. GC-O Identified Aroma Compounds in 'Strawberry Festival' and 'Florida Radiance' Strawberries with WAX and DB5 Columns

LRI				intensity[a] (WAX)		
WAX	DB-5	compound[a]	descriptors	Festival	Radiance	identification[b]
989	717	methyl butanoate	apple	9	8	RI, AD, MS, Std
1580	1069	DMMF (2,5-dimethyl-4-methoxy-3(2*H*)-furanone)	sweet, caramel	9	9	RI, AD, MS, Std
2040		DMHF (2,5-dimethyl-4-hydroxy-3(2*H*)-furanone)	sweet, caramel, candy	9	9	RI, AD, MS, Std
1006		methyl 2-methylbutanoate	green apple, fruity, sweet	8	6	RI, AD, MS, Std
1213	847	(*E*)-2-hexenal	grassy, pungent	8	8	RI, AD, MS, Std
1362	984	unknown	grassy	8	9	
1545	1098	linalool (3,7-dimethylocta-1,6-dien-3-ol)	citrus, fruity/floral	8	8	RI, AD, MS, Std
1572	1151	(*E*,*Z*)-2,6-nonadienal	green, fresh cucumber	8	8	RI, AD, MS, Std
1613		butanoic acid	sour, cheesy	8	7	RI, AD, MS, Std
1850	1258	geraniol (3,7-dimethylocta-2,6-dien-1-ol)	sweet, berry, floral	8	8	RI, AD, Std
2129	1486	γ-decalactone	peach, sweet	8	10	RI, AD, MS, Std
1033	798	ethyl butanoate	fruity, sweet, pineapple	6	8	RI, AD, MS, Std
1058	692	methyl thioacetate	sulfurous, cheesy	7	7	RI, AD, MS, Std
1060		ethyl 3-methylbutanoate	fruity, apple, pineapple	7	7	RI, AD, MS, Std
1070		hexanal	fresh, green	7	6	RI, AD, MS, Std
1191	966	methyl thiobutanoate	sulfury, cheesy, cabbage	7	7	RI, AD, MS, Std
1229	999	ethyl hexanoate	fruity, sweet, pineapple	7	8	RI, AD, MS, Std
1838		hexanoic acid	sweaty, cheesy	7	7	RI, AD, MS, Std
	594	ethyl acetate	sweet fruit, grape	7	7	RI, AD, MS, Std
1181	922	methyl hexanoate	fruity, pineapple	6	6	RI, AD, MS, Std
1382	1120	methyl octanoate	waxy, sweet, orange	6	6	RI, AD, MS, Std
1661	1036	2-methylbutanoic acid	sour, cheesy, sweaty	6	6	RI, AD, MS, Std
	1058	2-methylbutyl butanoate	fruity, apple, spicy	6	7	RI, AD, MS, Std
1064	811	butyl acetate	fruity, banana	5	5	RI, AD, MS, Std
1267	1013	hexyl acetate	fruity, green apple, banana	5	5	RI, AD, MS, Std
1441	980	1-octen-3-ol	mushroom	5	6	RI, AD, MS, Std
	460	acetaldehyde	green apple	5	5	RI, AD, MS
	887	propyl butanoate	fruity, sweet, pineapple	5	6	RI, AD, MS, Std
1014		methyl 3-methylbutanoate	fruity, apple, pineapple	4	4	RI, AD, MS, Std
1046		ethyl 2-methylbutanoate	green apple, fruity, sweet	4	5	RI, AD, MS, Std

[a] Underscored volatiles and intensities were determined solely from DB-5 data and were not detected using WAX columns. [b] RI, retention index; AD, aroma description; Std, pure chemical standard; MS, mass spectrum.

5 min hold at the final temperature. Injection, MS transfer line, and ion source temperatures were 220, 240, and 180 °C, respectively. Electron ionization mass spectrometric data from m/z 25 to 300 were collected in the scan mode, with an ionization voltage of 70 eV. Compound identifications were made by comparing mass spectral data from the Wiley 275.L (G1035) database and confirmed by authentic pure standards. RIs were determined and used to identify aroma compounds in GC-O study.

Sensory Analysis. Sensory analyses were conducted at the Citrus and Subtropical Products Research Laboratory, USDA-ARS, Winter Haven, FL, using 14 trained panelists. Panelist ages ranged from 25 to 65 years.

Fresh strawberries were harvested on February 25, March 8, and March 22, 2010. The next day they were rinsed with water and cut into fourths. Five pieces of fruit were placed in 118 mL (4 oz) plastic soufflé cups with lids (SOLO Cup Co., Urbana, IL) coded with three-digit numbers and served at room temperature. The order of presentation followed a "Williams" design (completely balanced pairwise), and Compusense 5 was used for data acquisition (Compusense Inc., Guelph, ON, Canada). Panelists were asked to rate samples for firmness, sweetness, sourness, astringency, strawberry flavor, green flavor, and fermented/overripe flavor using a 10-point intensity scale anchored with the words "low" (0−1), "medium" (5), and "high" (10), except for firmness, which was anchored with the words "soft" and "very firm" at each end. Reference standards (Table 1) were provided, and panelists were instructed to review standards before tasting samples and, as necessary, during the session. Sensory evaluation was conducted in individual booths under red lights to prevent color bias. Unsalted crackers and spring water were provided for cleansing the palate between samples.

Statistical Analysis. Correlation matrix analysis of correlating sensory data with chemical analysis was determined using Statistica version 9 software (StatSoft Inc., Tulsa, OK). Principal component analysis (PCA) of relative concentrations of the 12 key strawberry volatiles[11] was calculated using Unscrambler multivariate statistical software (version 10.1 CAMO, Woodbridge, NJ).

RESULTS AND DISCUSSION

Odor-Active Compounds Determined Using GC-O. Thirty volatiles were found to have aroma activity using both polar and nonpolar columns from the two strawberry cultivars examined in

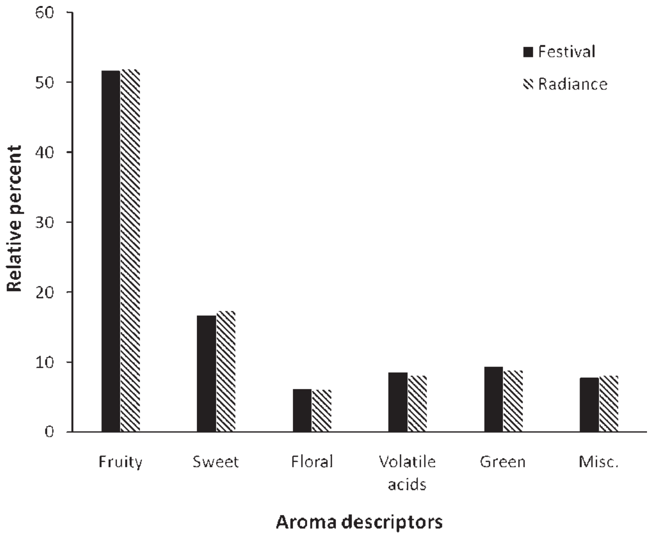

Figure 1. GC-O summation of the relative aroma contribution of individual aroma active volatiles grouped into six major groups in 'Florida Radiance' and 'Strawberry Festival' strawberry cultivars.

this study (Table 2). These aroma active substances included 16 esters, 4 aldehydes, 3 alcohols, 3 acids, 2 furanones, 1 lactone, and 1 unidentified compound. Early strawberry GC-O studies reported 22−26 aroma active volatiles.[11,12] More recent studies have reported as many as 30 among six different cultivars.[13] Many aroma active esters were reported, and furanones (DMHF and DMMF) were considered to be character impact volatiles.[11,12] Most of the strawberry aroma volatiles observed in this study have been previously reported. However, overall the aroma profiles from these winter-grown subtropical strawberries were quite different from those reported for summer-grown temperate region strawberries. Aroma active compounds observed in earlier studies such as 2,3-butanedione,[5] 2-pentanone,[11,13] β-ionone, and methyl anthranilate[11] were not observed in the Florida cultivars. On the other hand, three volatiles (geraniol, (*E*,*Z*)-2,6-nonadienal, and 1-octen-3-ol) were observed only in the Florida cultivars.

The volatiles in Table 2 are ranked in order of decreasing aroma intensity (T-I peak height) based first on 'Strawberry Festival' (as it is the most common cultivar) and then on 'Florida Radiance'. The 12 most potent odor active volatiles in 'Strawberry Festival' and 'Florida Radiance' in decreasing intensity were DMMF, DMHF, methyl butanoate, γ-decalactone, a grassy-smelling unknown (LRI 1362), (*E*)-2-hexenal, linalool, (*E*,*Z*)-2,6-nonadienal, geraniol, butanoic acid, methyl 2-methylbutanoate, and ethyl hexanoate. Five of these were also ranked as among the most intense strawberry volatiles in European cultivars.[14] However, most European cultivars have appreciable aroma activity due to dimethyl sulfide (garlic) and dimethyl trisulfide (cabbage),[13] which were not observed in the Florida cultivars. These two volatiles were observed as minor peaks in a recent sulfur-specific report of these cultivars.[9]

The aroma active volatiles from Table 2 with similar sensory descriptors were grouped into six general aroma categories on the basis of their primary aroma character: fruity, sweet, floral, volatile acids, green, and miscellaneous. The total aroma value for each category was the sum of the individual aroma values within each category. Results are displayed in Figure 1, where the contribution of each aroma category is shown as a percentage of the total aroma intensities of all aroma volatiles.

Fruity. Fruity is the major aroma character for both strawberry cultivars shown in Figure 1, comprising slightly more than 50% of the total aroma intensity. This group consists of 16 esters. Esters constitute one of the largest groups of volatile compounds in fruit. Strawberries are a particularly rich source of esters as approximately 130 different strawberry esters have been reported.[15] They constitute 25−90% of the total number of volatiles in ripe strawberry fruit.[7,16,17] In this study 33 esters were identified, of which 16 were aroma active. Even though they were the most numerous and as a group provided the predominant aroma character to strawberry, only methyl butanoate, methyl 2-methylbutanoate, and ethyl hexanoate were among the 12 most intense strawberry odorants.

Lactones such as γ-decalactone are cyclic esters and also contribute to fruity aroma but for the purpose of discussion were considered as a separate group. As shown in Table 2, it is one of the most intense and important strawberry aroma volatiles. γ-Decalactone possesses a "peach", "strawberry-like" aroma and has been found in high concentrations in some strawberry cultivars. It is among the key strawberry flavor volatiles in many cultivars.[18]

Sweet. Sweetness is generally considered as a taste due to sugars rather than a volatile attribute and often associated with "fruity". However, some volatiles have a sweet component by association with sweet foods. As seen in Table 2, DMHF and DMMF were the two most intense odorants in strawberry and are generally described as smelling like "cotton candy" and "caramel", hence the "sweet" descriptor. They were major contributors to the sweet aromas shown in Figure 1. Sweetness was the second most intense aroma component in strawberry. Furanones such as DMMF and DMHF are found in a wide variety of fruits such as strawberry, blackberry, pineapple, raspberry, mango, and tomato.[19−25] They are considered to be strawberry character impact compounds.[7,18,20] Both DMMF and DMHF have very low organoleptic sensory thresholds, 16[7] and 10 μg/kg[26] in water, respectively.

Floral. This is the least intense strawberry aroma category shown in Figure 1. Terpene alcohols such as linalool and geraniol provided "fruity", "floral", and "berry-like″ aroma notes to strawberries and have been placed in the floral category. Linalool occurs naturally in various fruits, and it is present in large amounts in some strawberry cultivars.[7,27] However, few studies report geraniol as an important aroma compound in strawberries. This could be a unique feature of Florida cultivars or the subtropical climate. It is also worth noting that geraniol is an extremely potent volatile with a threshold of 10 μg/kg[28] and is present at trace levels. Thus, it can be perceived by GC-O even though it fails to produce a TIC or FID peak.

Volatile Acids. Various aromas are produced by volatile organic acids. As shown in Figure 1, they are minor components of strawberry aroma. Even though there are only three organic acids identified in Table 2, two of them, butanoic and hexanoic acids, have fairly strong aroma intensities.

Green. (*E*)-2-Hexenal is a very common aroma compound in fresh fruits, which can contribute a "green" and "fresh fruity" aroma. Hexanal, (*E*)-2-hexenal, and (*Z*)-3-hexenol are primarily responsible for the green/vegetative aroma component in strawberries.[7] (*E*)-2-Hexenal is thought to be responsible for the "fresh strawberry" flavor in 'Strawberry Festival' and 'Florida Radiance' strawberries.

Miscellaneous. Sulfur esters such as methyl thioacetate and methyl thiobutanoate have been recently identified in strawberries.[29] These sulfur esters generally do not possess fruity

Table 3. Volatiles Identified and Quantified Using GC-MS in 'Strawberry Festival' and 'Florida Radiance' Strawberries Listed in Decreasing Odor Strength (OAV)[a]

			Festival			Radiance	
LRI (WAX)	compound	threshold[b] (μg/kg)	mean	OAV	rank	mean	OAV
1046	ethyl butanoate	1	461	461	7	553	553
2057	DMHF (2,5-dimethyl-4-hydroxy-3(2*H*)-furanone)	10	4240	424	2	3590	359
1003	methyl butanoate	10	3580	358	3	2610	261
1554	linalool (3,7-dimethylocta-1,6-dien-3-ol)	1	102	102	13	162	162
1236	(*E*)-2-hexenal	82	5790	71	1	4400	54
1021	methyl 2-methylbutanoate	0.4	21	51	28	12	30
1615	DMMF (2,5-dimethyl-4-methoxy-3(2*H*)-furanone)	16	460	29	8	520	32
1680	2-methylbutanoic acid	100	2590	26	4	5260	53
1241	ethyl hexanoate	1	16	16	30	16	16
2184	γ-decalactone	11	134	12	11	683	62
1059	ethyl 2-methylbutanoate[c]	0.3	2.5	8.3	41	1.5	4.9
1090	hexanal	21	165	7.9	10	185	8.8
2416	γ-dodecalactone	7	50	7.2	16	71	10
1073	ethyl 3-methylbutanoate[c]	0.4	2.6	6.5	40	2.7	6.9
1640	butanoic acid[d]	100	639	6.4	6	426	4.3
1061	methyl thioacetate[c]	5	25	4.9	24	28	5.6
1194	methyl hexanoate	70	211	3.0	9	204	2.9
1854	hexanoic acid	1000	2130	2.1	5	2480	2.5
1029	methyl 3-methylbutanoate[e]	4.4	7.6	1.7	35	9.8	2.2
1280	hexyl acetate	10	15	1.5	31	19	1.9
1457	1-octen-3-ol[f]	10	12	1.2	32	8.7	0.9
1139	ethyl pentanoate[c]	1.5	1.7	1.1	45	3.3	2.2
1394	(*Z*)-3-hexenol	50	37	0.7	18	26	0.5
1079	butyl acetate[c]	43	25	0.6	22	39	0.9
2049	nerolidol (3,7,11-trimethyl-1,6,10-dodecatrien-3-ol)	120	70	0.6	14	145	1.2
1723	methyl hexanethioate[g]	0.3	0.1	0.5	51	0.1	0.4
1434	(*E*,*E*)-2,4-hexadienal[f]	60	22	0.4	25	14	0.2
1416	(*E*)-2-hexenol	100	31	0.3	20	38	0.4
1953	γ-octalactone[h]	14	3.9	0.3	38	5.8	0.4
1351	6-methyl-5-hepten-2-one[i]	160	40	0.2	17	41	0.3
1326	(*Z*)-3-hexenyl acetate[j]	16	3.7	0.2	39	5.5	0.3
1180	pentyl acetate[c]	43	9.2	0.2	34	11	0.2
1224	butyl butanoate[k]	110	21	0.2	27	27	0.2
998	2-heptanone[l]	140	22	0.2	26	7.5	0.1
2068	octanoic acid[m]	910	114	0.1	12	116	0.1
1342	(*E*)-2-hexenyl acetate	210	26	0.1	21	30	0.1
1899	benzyl alcohol[m]	620	55	0.1	15	100	0.2
1961	heptanoic acid[m]	640	25	0.0	23	25	0.0
911	1-methylethyl acetate[n]	1700	32	0.0	19	70	0.0
903	ethyl acetate[n]	1000	18	0.0	29	24	0.0
1810	methyl salicylate[g]	40	0.6	0.0	49	1.1	0.0
1625	octyl butanoate	250	2.2	0.0	43	1.3	0.0
1398	methyl octanoate[o]	200	1.2	0.0	47	2.1	0.0
1470	(*Z*)-3-hexenyl butanoate[o]	320	0.6	0.0	48	1.1	0.0
991	propyl acetate[n]	2000	0.0	0.0	53	0.0	0.0
1145	butyl propanoate[c]	200	0.0	0.0	54	0.0	0.0
1149	methyl 4-methylpentanoate[c]		12		33	18	
1209	methyl thiobutanoate[k]		6.4		36	5.5	
1132	1-methylpropyl butanoate[c]		5.4		37	8.6	
1484	(*E*)-2-hexenyl butanoate[o]		2.5		42	5.3	
1118	methyl (*E*)-2-butenoate[c]		1.9		44	7.2	

Table 3. Continued

LRI (WAX)	compound	threshold[b] (μg/kg)	Festival mean	Festival OAV	rank	Radiance mean	Radiance OAV
1751	phenylmethyl acetate[g]		1.4		46	2.7	
1818	octyl hexanoate[g]		0.2		50	0.2	
1336	5-hexenyl acetate[j]		0.1		52	0.2	

[a] Volatiles are listed in decreasing calculated OAV value using literature thresholds. Volatiles present below threshold values are listed as zero. [b] Thresholds are referred to ref 30. [c] Concentration determined using the response factor of ethyl butanoate. [d] Determined using the response factor of 2-methylbutanoic acid. [e] Concentration determined using the response factor of methyl 2-methylbutanoate. [f] Determined using the response factor of (*E*)-2-hexenol. [g] Concentration determined using the response factor of octyl butanoate. [h] Determined using the response factor of γ-decalactone. [i] Determined using the response factor of (*Z*)-3-hexenol. [j] Concentration determined using the response factor of by hexyl acetate. [k] Concentration determined using the response factor of methyl hexanoate. [l] Concentration determined using the response factor of hexanal. [m] Determined using the response factor of hexanoic acid. [n] Concentration determined using methyl butanoate's response factor. [o] Concentration determined using the response factor of (*E*)-2-hexenyl acetate.

odors at the concentrations found in this study. Their odors are often described as "cabbage", "garlic", "onion", or "rotten egg-like". The role of sulfur esters in fresh strawberry aroma is unclear, and they were included in the miscellaneous category. However, because their concentrations increase during the thermal process of making strawberry jam,[29] they are thought to contribute to the cooked aroma observed in strawberry jams. The mushroom-smelling 1-octen-3-ol was also a member of this minor group.

Cultivar Comparison. As shown in Figure 1 and Table 2, the overall GC-O profiles of 'Strawberry Festival' and 'Florida Radiance' strawberries were similar. Preliminary sensory evaluation during variety selection also indicated that the flavor of 'Florida Radiance' was similar to that of 'Strawberry Festival'. Because all strawberry genotypes were bred from the cultivated species *F.* × *ananassa*, the volatile profiles would be expected to be similar. The °Brix (sugar content) and titratable acidity (represented as percentage of citric acid) for the two cultivars were also similar. The °Brix (average of four harvests) was 8.4 ± 1.3 for 'Strawberry Festival' and 7.3 ± 1.5 for 'Florida Radiance'. Titratable acidity (average of four harvests) of 'Strawberry Festival' was 0.89 ± 0.10 and that of 'Florida Radiance', 0.76 ± 0.08.

GC-MS Volatile Identification and Calculated Odor-Activity Values (OAVs). The OAVs, shown in Table 3, were calculated from the averages of analytical concentrations determined on four sampling dates and published odor thresholds in water. Typically, odor active compounds with high OAVs are more likely to be major contributors to strawberry aroma, although aroma synergy and suppression probably exist. The 54 volatiles in Table 3 are listed in terms of decreasing OAVs for 'Strawberry Festival'. Twenty-two compounds possessed OAVs >1, but eight volatiles had no OAVs as no thresholds were available.

It should be noted that there is good agreement between the olfactometer results and calculated OAVs in terms of the most intense odorants. Nine of the volatiles with the 10 highest OAV values were also observed with high GC-O intensities (score ≥ 7). This is shown in Tables 2 (GC-O) and 3 (MS-OAVs). The major exception was 2-methylbutanoic acid (ranked 8, OAV = 26), but was observed with only moderate GC-O intensity and ranked 22. Even though both approaches identified similar volatiles, the intensity rankings were not the same. Ethyl butanoate, DMHF, methyl butanoate, and linalool had the highest OAVs (all >100) in Table 3. In comparison, olfactometer results (Table 2) indicated that the most intense odorants were DMMF, DMHF, methyl butanoate, and γ-decalactone.

There were also some major differences in the results obtained from the two approaches. For example, ethyl butanoate had the highest OAV for both strawberry cultivars, suggesting that it was the most intense odorant. However, the olfactometer data indicated that ethyl butanoate possessed only a midlevel intensity. Some differences in the two approaches should be expected because the OAV method does not account for the psychophysical response (which is an exponential response to concentration), being solely based on a linear relationship based on ratios of concentration/thresholds. OAV values implicitly assume that the dose−response behavior of all volatiles is the same and do not account for the different relative responses for various volatiles.

The major advantage in calculating OAVs is that it utilizes the better reproducibility of instrumental versus human responses to determine aroma activity. However, the results are only as accurate as the odor thresholds used to divide the analytical concentrations. These thresholds are usually determined in water, and the specific matrix used in each study can profoundly modify these thresholds.

The results of this study demonstrate some of the limitations of the use of OAVs in determining food aroma profiles. The OAV approach requires that all aroma active volatiles are known and can be measured. It cannot assess new, unknown aroma components observed in GC-O studies (Table 2). For example, the strong grassy-smelling GC-O unknown with WAX and DB-5 retention index values of 1362 and 984 could not be assessed using OAVs as its analytical concentration and odor threshold are also unknown. The other major limitation of the OAV approach involves potent aroma volatiles present at trace levels. Trace level volatiles are often difficult to identify and are often not quantified. Examples in this study include geraniol and (*E*,*Z*)-2,6-nonadienal listed in Table 2. Both volatiles were strong odorants present at low levels and could not be easily quantified because of chromatographic coelution of interfering substances and low signal-to-noise ratios.

Volatile Patterns of Subtropical and Temperate Strawberry Cultivars. The results in Table 3 are profoundly different from recently published results of strawberry odorants OAVs.[13] Of the 22 volatiles in the literature study with OAVs >1, only 8 were also found in the Florida strawberries. Their relative rankings and OAVs also were profoundly different. For example, linalool had OAVs of 162 and 101 in Florida strawberries, whereas the European cultivars (*Fragaria vesca* L. Mieze Schindler, Polka, Elsanta, and Senga Gourmella) had OAVS ranging from 1.9 to 0.27. This suggests that the aroma volatiles of Florida strawberries may be different from those of strawberries grown in Europe.

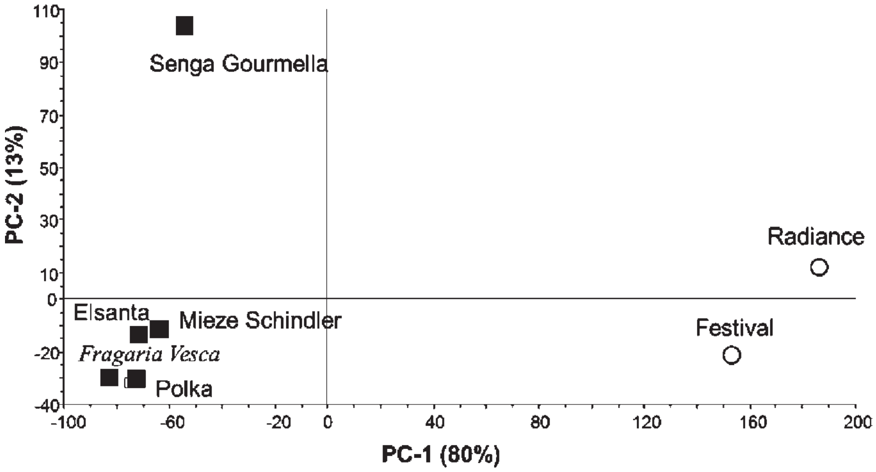

Figure 2. PCA score plot of five summer-grown European strawberry cultivars (■) reported by Ulrich et al.[11] compared with two winter-grown Florida cultivars (○).

To compare the relative aroma volatile concentrations of the winter-harvested subtropical cultivars from Florida with those from summer-harvested temperate European cultivars, the relative concentrations of 12 key strawberry volatiles were used for PCA calculations. The relative values for the 12 key volatiles reported by Ulrich et al.[11] from five European strawberries were used as examples of summer-harvested strawberries. Unfortunately, the specific concentrations of the 12 aroma inactive volatiles being compared from Ulrich et al.[11] were not given in terms of mass per unit volume but were given in terms of relative intensities based on a 100-point scale. Hexanoic acid was the volatile reported in highest relative concentration in all 12 volatiles. It had the highest relative value of the five European cultivars (Senga Gourmella specifically) with a high value of 94.3. The only way to compare the data in this study with those from the literature was to give the highest hexanoic acid value in this study a value of 94.3 as well and then all other volatiles in this study were given a proportional value normalized to the highest hexanoic acid value. The relative concentration values reported by Ulrich et al.[11] along with the normalized concentration values from the two Florida cultivars were then used to calculate PCA eigenvector values for all seven cultivars. The resulting score plot is shown in Figure 2. It is readily apparent that the volatile patterns of the subtropical cultivars are profoundly different from the temperate cultivars as all of the temperate cultivars are clustered on the left-hand side of the plot corresponding to negative PC1 values. The two Florida subtropical cultivars are grouped on the right-hand side of the plot corresponding to positive PC1 values. The PC1 axis explains 80% of the variance alone. The loading values of the 12 key strawberry volatiles used to calculate the score values for the seven cultivars are shown in Figure 3. PC1 can be thought of as a 2-methylbutanoic acid—methyl anthranilate axis. The Florida cultivars were characterized by having high 2-methylbutanoic acid values and zero (nondetectible) methyl anthranilate values. In contrast, the summer-harvested European cultivars were characterized as having low 2-methylbutanoic acid values, and at least two of them had detectable levels of methyl anthranilate. The Senga Gourmella cultivar was alone in a quadrant due to its having high γ-decalactone relative concentrations, and the high loading values are shown in Figure 3 for this volatile. The γ-decalactone value for this cultivar was 10 times greater than that of any of the other four cultivars.[11]

Although different sampling techniques may account for some differences in volatile profiles, it is highly unlikely that the qualitative and quantitative differences in the aroma profiles of subtropical strawberries compared to that from strawberries from temperate regions are due to different sampling techniques alone because actual concentrations were determined using internal standards. Therefore, genetic and environmental differences must also be considered major factors. Some slight differences between volatiles from different harvests might also be expected due to fluctuations in sunlight and temperature, even though the harvest period was only 6 weeks and the fruits at each harvest were picked at the same stage of maturity.

Sensory Analysis of 'Strawberry Festival' and 'Florida Radiance'. Sensory analysis differs from GC-O and OAV approaches in that the volatiles are examined in the food matrix (intact strawberries) and there is no separation prior to analysis. In sensory analysis both volatile and nonvolatile components are assessed simultaneously once the strawberries are placed in the mouth and chewed, whereas only volatiles are assessed in headspace techniques. In this study, seven attributes including "sweetness", "sourness", and "strawberry flavor" were used to describe 'Strawberry Festival' and 'Florida Radiance' using a trained panel. Because strawberry volatile composition varies among harvest dates, berries from three harvest dates were used for sensory analysis. A slight harvest date variation was observed. A spider plot of the average of each attribute from three harvests is presented in Figure 4. The highest sensory scores were for firmness, sourness, and strawberry flavor. Lowest scores were for fermented flavor. It was difficult to compare GC-O and OAV results with sensory profile results because they include only volatile components, whereas sensory attributes involved both volatiles and nonvolatiles as well as a physical measurement (firmness). "Strawberry flavor" is a complex mixture of various aroma compounds and was one of the highest sensory scores; it could be roughly compared to the results in Figure 1 demonstrating that fruity was by far the strongest aroma category according to GC-O. If the sweetness scores from DMHF and DMMF are added to the fruity values, the sum would

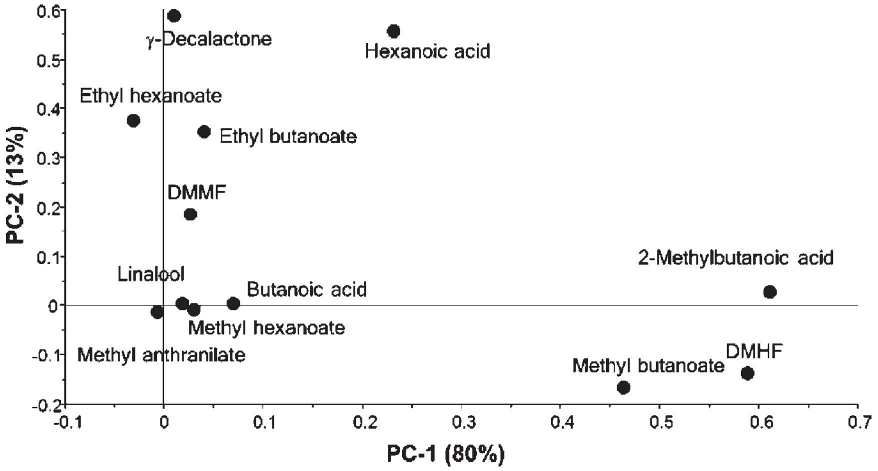

Figure 3. PCA loading values of the 12 key strawberry volatiles from the five European and two Florida cultivars.

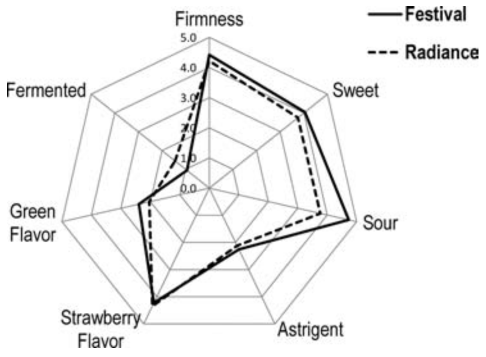

Figure 4. Spider plot of sensory scores (average of three harvest dates) for 'Strawberry Festival' and 'Florida Radiance' strawberries.

approximate sensory "strawberry flavor". Sensory "strawberry flavor" was also highly and significantly correlated to GC-MS methyl 3-methylbutanoate (R^2 = 0.90), ethyl butanoate (R^2 = 0.96), and methyl thioacetate (R^2 = 0.92) concentrations.

As shown in Figure 4, there was no difference in "strawberry flavor" between 'Strawberry Festival' and 'Florida Radiance'. With the exception of sourness, there was no appreciable difference in the sensory profiles from 'Strawberry Festival' and 'Florida Radiance' strawberries.

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