Quantification of Selected Aroma-Active Compounds in Strawberries by Headspace Solid-Phase Microextraction Gas Chromatography and Correlation with Sensory Descriptive Analysis

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ABSTRACT: Selected aroma-active compounds in strawberries were quantified using headspace solid-phase microextraction and gas chromatography. Ten strawberry cultivars grown in California and Oregon were studied. The standard curves were built in a synthetic matrix and quantification was achieved using multiple internal standards. Odor activity values (OAVs) of the aroma compounds were calculated to understand their contribution to the overall aroma. Although the concentrations of the aroma compounds varied depending on the cultivars, in general, ethyl butanoate, mesifurane, ethyl hexanoate, ethyl 3-methylbutanoate, hexyl acetate, and γ-dodecalactone had the highest OAVs. Descriptive sensory analysis was performed by a trained panel of 10 members. A PCA plot was built to understand the aroma contribution of principal components. The chemical results were compared with sensory data. The OAV of esters correlated well with the floral, pineapple, and banana notes. The green notes did not correlate with the concentration or OAVs of aldehydes or C6 alcohols. It is assumed that the higher amounts of green, sulfur, musty, and waxy notes in some cultivars were due to the lack of fruity notes.

Keywords: odor activity value, quantification, SPME, strawberry

Introduction

Strawberries (*Fragaria* × *ananassa* Duch. ex Rozier) are highly valued for their delicious flavor and nutritional value. Aroma compounds in strawberry have been studied extensively, and it is generally considered that a complex mixture of furanone, esters, aldehydes, alcohols, and sulfur compounds is responsible for the aroma of strawberry (McFadden and others 1965; Pyysalo and others 1979; Dirinck and others 1981; Perez and others 1992).

2,5-Dimethyl-4-hydroxy-3(2H)-furanone (furaneol) and 2,5-dimethyl-4-methoxy-3(2H)-furanone (mesifurane) are considered to be the 2 most important furanones in strawberry (Pyysalo and others 1979; Honkanen and others 1980; Pickenhagen and others 1981; Douillard and Guichard 1989). Furaneol was identified for the 1st time as a natural aroma component in pineapples (Rodin and others 1965). It has since been detected in many fruits such as strawberry (Pyysalo and others 1979; Hirvi and Honkanen 1982; Douillard and Guichard 1989; Song and others 1998), raspberry (Honkanen and others 1980), mango (Pickenhagen and others 1981), tomato (Buttery and others 1995), and many other fruits. Furaneol is not stable and its degradation depends on pH and temperature (Hirvi and others 1980; Shu and others 1985). Because of its liability in aqueous solutions (Hodge and others 1963), recovery and detection of furaneol were affected by the conditions of isolation and detection technique (Williams and Mottram 1981). Mesifurane is more stable than furaneol (Hirvi and others 1980). Although it resembles the typical aroma of wild strawberry, much higher concentration of this compound has been found in some cultivated varieties (Pickenhagen and others 1981). Mesifurane and furaneol content increase with the ripening (Perez and others 1996) and give the characteristic caramel-like, sweet, floral, and fruity aroma (Tonsbeek and others 1968; Miller and others 1973).

Esters are another important class of aroma-active compounds in strawberry (Perez and others 1992). Esters encompass 25% to 90% of the total number of volatiles in ripe strawberry fruit (Forney and others 2000). Methyl butanoate, ethyl butanoate, butyl acetate, methyl hexanoate, and ethyl hexanoate have been reported to be the major esters. Butanoates and hexanoates account for 50% to 80% of total number volatiles in fresh strawberries (Forney and others 2000; Hakala Mari and others 2002). The ratio of ethyl and methyl esters depends on the genotype (Schreier 1980; Dirinck and others 1981; Perez and others 1992; Larsen and Poll 1995) and the growing conditions (Hakala and others 2002). Formation of esters has been reported to occur only in the mature fruit stage because the esterase activity is absent at immature stages (Yamashita and others 1977).

Many other compounds are also identified to be important to strawberry aroma. Lactones, terpene alcohols can contribute to the pleasant coconut and citrus character in some cultivars. Volatile sulfur compounds, including hydrogen sulfide, methanethiol, dimethyl disulfide, methyl thioacetate, methyl thiobutyrate, dimethyl sulfide, and dimethyl trisulfide, have been

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identified in strawberry (Winter 1963; Dirinck and others 1981; Schulbach and others 2004) and some of these could be important to the aroma of some cultivars, even though they are present at very low concentrations. Hexanal, trans-2-hexenal, and cis-3-hexenol are important to strawberry aroma and contribute to the green, unripe note. Their concentrations depend on the cultivar as well as the degree of ripeness. Butanoic, 2-methylbutanoic, and hexanoic acids can also be important to the aroma of strawberries (Pyysalo and others 1979).

California is the leading state for strawberry production in the United States and is the leading production area in the world. The majority of the fruit grown in California is for the wholesale fresh market. Fruit for this market must be harvested, packed, shipped around the country, and still be appealing to consumers in distant marketplaces. Cultivars developed by public and private breeding programs in California for this market must be firm enough to handle picking and shipping, attractive for consumers, and have acceptable fruit quality for consumers. The most important strawberry cultivar in California in the early 2000s was "Camarosa" with some interest in the newer cultivars such as "Ventana." Some private companies such as Driscoll Strawberry Associates have their own cultivars such as "San Miguel" and "Venice" that are only available to their growers but whose fruits are sold very widely.

The Pacific Northwest is the 3rd most important production area in the United States after California and Florida. Historically the Pacific Northwest industry has produced fruit for the processing industry. Public breeding programs in the Pacific Northwest have developed cultivars specifically for this market. These cultivars have intense external and internal red color, high soluble solids, low drip loss, and intense flavor that make them ideal to be an ingredient in other products. In addition to being bred for processing, the environmental condition in the Northwest with warm, dry days with cool nights optimizes color and flavor development. "Totem" is the most widely grown cultivar in the Pacific Northwest and some other popular cultivars include "Hood," "Puget Reliance," "Puget Summer," and "Tillamook." Aroma profiles for these cultivars have not been developed and no previous studies had correlated chemical composition with sensory descriptive analysis. The objective of this work was to quantify the concentration of major esters and other selected aroma-active compounds in those cultivars using the solid-phase microextraction (SPME) technique, and correlate their odor activity values (OAVs) with sensory descriptive analysis. The results will be used to understand the flavor difference for strawberries targeted for fresh market versus for processing.

Materials and Methods

Strawberry fruit

Ventana, Camarosa, 13G97, San Miguel, and Venice strawberries were procured from Driscoll Strawberry Associates Inc. (Watsonville, Calif., U.S.A.). The fruits were harvested late, when fully colored for processing rather immature but firm for the fresh market. Totem, Hood, Puget Reliance, Puget Summer, and "Independence" were obtained from Norpac Foods (Stayton, Oreg., U.S.A.) and Townsend Farms (Fairview, Oreg., U.S.A.). All the berries were harvested fully colored and mature in summer 2004, individually quick frozen at −37 °C, and stored at −23 °C. Samples were analyzed within 9 mo.

Chemicals

Methyl butanoate, methyl hexanoate, ethyl butanoate, ethyl hexanoate, ethyl 3-methylbutanoate (ethyl isovalerate), butyl acetate, 3-methylbutyl acetate (isoamyl acetate), octyl acetate, butyl butanoate, trans-2-hexenyl acetate, hexyl hexanoate, trans-2-hexenal, hexanal, nonanal, 4-methoxy-2,5-dimethyl-3(2H)-furanone (mesifurane) δ-decalactone, γ-dodecalactone, 3,7-dimethyl-1,6-octadien-3-ol (linalool), 2-decanol, 2-methylbutanoic acid, and 6-(E)-3,7,11-trimethyl-1,6,10-dodecatrien-3-ol (nerolidol) were obtained from Aldrich Chemical Co. Inc. (Milwaukee, Wis., U.S.A.). Hexyl acetate, 3-heptanone, and γ-undecalactone were purchased from K & K Laboratories (Jamaica, N.Y., U.S.A.). Calcium chloride and sodium chloride were obtained from Fisher Chemical Company (Fairlawn, N.J., U.S.A.). Glucose and methanol were obtained from EMD Chemicals (Gibbstown, N.J., U.S.A.), and fructose and sucrose were obtained from J.T. Baker (Phillipsburg, N.J., U.S.A.).

Sample preparation and SPME extraction

Strawberries were slightly thawed at room temperature for 90 min. Calcium chloride (1%) and distilled water (10%) were added, and the berries were blended to a fine puree. Ten grams of puree was placed in a 40-mL amber glass vial (I-Chem, New Castle, Del., U.S.A.) with a polytetrafluoroethylene needle-pierceable septum screw cap. Internal standards at 0.5 ppm concentration and 2 g sodium chloride were added. A stirring bar was placed in the vial and the sample was equilibrated for 15 min in a circulating water bath at 50 °C prior to extraction. A Stableflex 50/30 μm DVB-Carboxen-PDMS fiber (2 cm, Supelco, Bellefonte, Pa., U.S.A.) was used for aroma extraction. Prior to use, the fiber was conditioned at 270 °C for 4 h. After equilibration, the SPME fiber was exposed to the headspace for 1 h at the same agitation speed and temperature. The SPME fiber was then introduced in the injector of the gas chromatography (GC) for desorption at 250 °C for 3 min in the splitless mode.

Gas chromatography (GC-FID)

The volatiles were chromatographed with a Hewlett-Packard 5890 gas chromatograph equipped with a flame ionization detector (FID) and a HP-wax column (30 m × 0.25 mm ID × 0.50 μm film thickness). Injection port was maintained at 250 °C and the detector at 270 °C. Carrier gas (nitrogen) flow rate was 2 mL/min measured at 35 °C under constant pressure. The oven temperature was programmed to hold at 35 °C for 5 min and then increased to 230 °C at a rate of 2 °C/min and held at 230 °C for 5 min. Hydrocarbon standards (C_8 to C_{40}) were injected using the same temperature program to determine the retention indices of the individual compounds using modified Kovats method (Van den Dool and Kratz 1963).

Gas chromatography-mass spectrometry (GC-MS)

Volatile identification was performed using an Agilent 6890 gas chromatograph equipped with an Agilent 5973 mass selective detector. A ZB-wax column (30 m × 0.25 mm ID × 0.25 μm thick film, Phenomenex, Torrance, Calif., U.S.A.) was used for the separation of the volatiles with the same oven temperature program at constant flow (2 mL/min). Electron impact mass spectrometric data from m/z 35 to 300 were collected using a scan rate of 5.27/s, with an ionization voltage of 70 eV. The volatile compounds were identified by comparing the mass spectral data with the Wiley library and retention indices.

Quantification

Based on previous research (Scherz 1994; Schieberle and Hofmann 1997), a synthetic matrix was developed using 4 g pectin, 23 g glucose, 23 g fructose, 10 g sucrose, 7 g citric acid, and 1 g malic acid dissolved in 1 L of millipore water. An internal standard solution containing 50 ppm each of 3-heptanone, 2-decanol, and

γ-undecalactone was prepared in methanol. An aliquot (0.1 g) of the internal standard solution was then added to 10 g of the synthetic matrix to yield a final concentration of 0.5 ppm. Standards of methyl butanoate, methyl hexanoate, ethyl butanoate, ethyl hexanoate, ethyl 3-methylbutanoate, 3-methylbutyl acetate, octyl acetate, butyl butanoate, hexyl acetate, trans-2-hexenyl acetate, trans-2-hexenal, hexanal, nonanal, mesifurane, δ-decalactone, γ-dodecalactone, linalool, 2-methylbutanoic acid, and nerolidol were divided into 3 groups for easy sample preparation and analysis. Standard stock solutions of 1000 ppm of each compound were prepared in high performance liquid chromatography grade methanol. The stock solutions were further diluted with methanol to get a final concentration of 6.25, 12.5, 25, 50, 100, 200, and 400 ppm. Standard solution (0.1 g) and internal standard solution were added to 10 g of the synthetic matrix to yield final concentrations of 0.06, 0.12, 0.25, 0.5, 1, 2, and 4 ppm. Aroma compounds were extracted using the same SPME fiber under the same conditions. The GC running conditions were the same as those used for the strawberry samples. The calibration curves and the corresponding internal standards (Table 1) were used to calculate esters, lactones, aldehydes, and other important aroma compounds in strawberry. In addition, the calibration curve of δ-decalactone was used to estimate the concentration of γ-decalactone, and the calibration curve of γ-dodecalactone was used to estimate jasmolactone. Similarly, the calibration curves of ethyl butanoate, hexanal, linalool, and 2-methylbutanoic acid were used to estimate other esters, aldehydes, terpene alcohols, and acids to compare the cultivars.

Sensory evaluation

Ten experienced panelists, 5 men and 5 women between the ages of 21 and 43 y, were chosen for the sensory study. A total of six 1-h training sessions and six 1-h testing sessions were conducted. During the 1st training session, all the test samples were provided to develop the descriptive terminology. The flavor descriptors floral, caramel, pineapple, peach, banana, and green were selected from previous research (Stampanoni 1997) and musty, waxy, sulfur, and citrus terms were identified by the panelists among the samples under study. In the subsequent training sessions, the panelists were trained to rate the intensity on a 0 to 15 scale for each aroma attribute. Vegetable oil (Wesson Oil, Conagra Foods, Los Angeles, Calif., U.S.A.), Hi-C Orange Lavaburst juice (Minute Maid, Houston, Tex., U.S.A.), grape juice (Welch's, Concord, Mass., U.S.A.), and cinnamon gum (Trident, Cadbury Adams, Parsippany, N.J., U.S.A.) were used as aroma intensity standards for 3, 7, 11, and 15, respectively (Lederer 1991). The standards and the samples (30 mL puree) were provided in an 8-ounce wine glass and the glass was covered with a plastic (nonodorous) lid (Solo Cup Co., Urbana, Ill., U.S.A.). Testing took place in individual testing booths under red lighting to mask the color differences among the samples. A randomized complete block design was used where each panelist received each sample 3 times (3 replications). Samples were coded with 3-digit random numbers.

Table 1—Regression equations for major compounds identified in strawberry

Compound	Internal standard	Regression equation	R^2
Methyl butanoate		$Y = 0.32x - 0.01$	0.994
Ethyl butanoate		$Y = 0.49x + 0.20$	0.974
Ethyl isovalerate		$Y = 1.01x + 0.13$	0.998
Hexanal		$Y = 0.54x + 0.27$	0.983
Butyl acetate		$Y = 1.46x + 0.18$	0.992
Isoamyl acetate	3-Heptanone	$Y = 0.96x + 0.17$	0.998
Methyl hexanoate		$Y = 1.79x + 0.17$	0.998
Trans-2-hexenal		$Y = 0.76x + 0.03$	0.990
Ethyl hexanoate		$Y = 3.06x + 0.69$	0.996
Hexyl acetate		$Y = 1.67x + 0.89$	0.972
2-methylbutanoic acid		$Y = 0.373x + 0.27$	0.972
Octyl acetate		$Y = 0.84x + 1.31$	0.992
Trans-2-hexenyl acetate		$Y = 3.26x + 1.64$	0.979
Linalool	2-Decanol	$Y = 2.11x + 0.49$	0.976
Nonanal		$Y = 13.95x - 3.59$	0.986
Mesifurane		$Y = 1.04x + 0.82$	0.972
Nerolidol		$Y = 16.11x + 3.27$	0.972
Δ-decalactone	γ-Undecalactone	$Y = 1.07x$	0.994
γ-dodecalactone		$Y = 1.58x$	0.998

Statistical analysis

Analysis of variance (ANOVA) and principal component analyses (PCAs) among the cultivars for sensory analyses were done using SPSS statistical package (SPSS, Chicago, Ill., U.S.A.).

Results and Discussion

SPME extraction parameters

The sensitivity and accuracy of volatile analysis by SPME depend on the optimum extraction conditions. Extraction efficiency was evaluated for varying periods of extraction time (30, 45, 60, and 90 min) at different equilibration temperatures (30, 40, and 50 °C). The sensitivity of the volatiles was highest with 60 min of extraction with no major difference between 60 and 90 min (data not shown). Equilibration at 50 °C yielded higher amounts of higher boiling volatile compounds when compared with extraction at 30 and 40 °C. Various sample sizes (5, 10, 15, and 20 g) were evaluated and the maximum yield of volatile compounds was obtained at 10 g (data not shown). Some of the cultivar samples were very viscous and distilled water was added in varying amounts (10%, 20%, 30%, 40%, and 50%) to facilitate the action of the stir bar and subsequent release of volatiles. Water added at 10% gave the highest response (data not shown). Based on these results, all samples were mixed with 10% water, equilibrated at 50 °C for 15 min, and then extracted at the sample temperature for 60 min.

Aroma analysis

Many commonly used extraction methods have been used for strawberry aroma isolation. However, none of these methods can provide a complete aroma profile. The SPME technique has been used in the identification of key aroma compounds in strawberry (Song and others 1998; De Boishebert and others 2004), but it is difficult to quantify all the aroma compounds using SPME. Although the SPME fiber based divinylbenzene-polydimethylsiloxane-Carboxen (DVB-PDMS-Carboxen) has good sensitivity for a wide range of aroma compounds, it is not sensitive for short-chained acids and alcohols. Many short-chain alcohols and acids have been found in strawberries (Mussinan and Walradt 1975; De Boishebert and others 2004); however, since most of short chain alcohols and acids have very high sensory thresholds, they contribute very little to the overall aroma of strawberry and thus their concentrations were not quantified. Furaneol is one of the most important aroma compounds of strawberry; unfortunately, the DVB-Carboxen-PDMS fiber has a poor recovery of this compound, and the quantification was not reliable. The method was able to quantify all other important aroma-active compounds reported in literature for strawberry, including esters, aldehydes, C6 alcohols, lactones, terpenoids, as well as mesifurane.

Due to the wide range of aroma-active compounds in strawberry, 3 internal standards (1 ketone, 1 alcohol, and 1 lactone) were used to construct the standard curves for the aroma compounds. Good

Table 2—The concentration (mg/kg) of selected aroma-active compounds in 10 strawberry cultivars from Oregon and California

		Concentration (mg/kg)									
Compound	Retention Index	Totem	Puget Reliance	Puget Summer	Hood	Independence	Ventana	Camarosa	San Miguel	Venice	13G97
Esters											
Methyl acetate	851	nd	0.04^{b}	0.02^{b}	nd	0.05 ± 0.02	0.02^{b}	0.02^{b}	0.10^{b}	0.39^{b}	0.02^{b}
Ethyl acetate	903	0.44 ± 0.02	0.60 ± 0.03	0.06^{b}	nd	0.02^{b}	0.05 ± 0.01	0.03^{b}	0.12^{b}	10.28 ± 0.06	0.03^{b}
Methyl butanoate	1001	0.97 ± 0.07	0.95 ± 0.02	0.82 ± 0.07	0.17 ± 0.04	0.56 ± 0.14	0.21 ± 0.02	0.36 ± 0.02	0.50^{b}	1.84^{b}	0.39 ± 0.05
Ethyl butanoate	1055	2.61 ± 0.01	3.33 ± 0.06	1.95^{b}	0.32 ± 0.02	0.02^{a}	0.27 ± 0.02	0.01^{a}	0.28^{b}	0.55 ± 0.01	0.07± 0.01
Ethyl isovalerate	1085	0.11 ± 0.01	0.12^{b}	0.01^{b}	nd	0.03 ± 0.01	0.01^{b}	0.03^{b}	0.01^{a}	0.05^{b}	0.02 ± 0.01
Butyl acetate	1117	0.15 ± 0.01	0.14^{b}	0.06^{b}	nd	Nd	< 0.01	nd	nd	0.10^{b}	0.01^{b}
Isoamyl acetate	1133	0.10 ± 0.01	0.01^{a}	0.02^{b}	0.01^{a}	0.03 ± 0.01	< 0.01	0.01^{b}	< 0.01	0.08^{b}	0.01^{b}
Ethyl pentanoate	1156	nd	nd	nd	nd	Nd	nd	nd	nd	1.76 ± 0.58	0.02^{b}
Methyl hexanoate	1197	0.06^{b}	0.07^{b}	0.01 ± 0.00	0.20 ± 0.10	0.32 ± 0.01	0.02b	0.02^{b}	0.03^{a}	0.11^{b}	0.08 ± 0.04
Butyl butanoate	1229	0.04^{b}	nd	nd	nd	Nd	nd	nd	nd	nd	nd
Ethyl hexanoate	1245	0.40 ± 0.02	0.68 ± 0.05	0.06^{b}	0.07 ± 0.05	0.07 ± 0.04	0.08 ± 0.01	0.06^{b}	0.05^{a}	2.08 ± 0.23	0.25 ± 0.20
Hexyl acetate	1284	0.06 ± 0.01	0.06 ± 0.01	0.05^{b}	0.08 ± 0.01	0.06 ± 0.01	< 0.01	0.02^{b}	<0.01	0.05 ± 0.01	0.04 ± 0.03
Cis-3-hexen-1-ol acetate	1314	nd	0.04 ± 0.01	0.02^{b}	nd	nd	0.01^{b}	nd	0.01^{a}	nd	nd
Trans-2-hexenyl acetate	1347	0.01^{a}	0.01^{a}	0.06^{b}	0.21 ± 0.04	0.02^{b}	< 0.01	0.01^{b}	<0.01	0.01^{b}	< 0.01
Hexyl butanoate	1426	0.13 ± 0.02	0.15 ± 0.02	0.14 ± 0.1	0.04 ± 0.01	0.01^{a}	0.02^{b}	0.22 ± 0.01	0.01^{a}	nd	0.02^{b}
Heptyl acetate	1590	nd	nd	nd	nd	0.02^{b}	nd	nd	nd	nd	nd
Octyl acetate	1487	nd	< 0.01	< 0.01	0.13 ± 0.01	0.03 ± 0.01	< 0.01	< 0.0	< 0.01	0.42 ± 0.01	0.03 ± 0.02
Hexyl hexanoate	1629	nd	0.03^{b}	0.03^{b}	1.55 ± 0.13	0.08^{b}	0.03^{b}	0.02^{b}	0.03^{a}	nd	1.55 ± 0.63
Octyl butanoate	1629	1.34 ± 0.52	nd	nd	nd	nd	nd	nd	nd	0.12^{b}	0.45 ± 0.13
Octyl 2-methylbutanoate	1651	0.65 ± 0.12	0.58 ± 0.11	0.16 ± 0.02	nd	0.03^{b}	0.11 ± 0.04	0.56 ± 0.07	0.18^{b}	0.24^{b}	0.16 ± 0.03
T-2-hexenyl hexanoate	1685	0.17 ± 0.01	nd	nd	nd	0.88 ± 0.07	nd	0.52 ± 0.06	< 0.01	< 0.01	0.13 ± 0.34
Decyl acetate	1701	nd	0.02^{b}	0.01^{a}	0.19 ± 0.05	nd	nd	0.02^{b}	nd	0.17^{b}	nd
Benzyl acetate	1743	1.34 ± 0.10	1.76 ± 0.32	0.53 ± 0.04	0.06 ± 0.01	0.68 ± 0.07	0.23 ± 0.06	0.11 ± 0.03	0.05^{b}	0.71 ± 0.02	0.41 ± 0.14
Pentyl hexanoate	1810	nd	0.09 ± 0.02	0.32 ± 0.05	0.15 ± 0.03	nd	0.21 ± 0.04	0.10 ± 0.02	0.07^{b}	0.14^{b}	nd
Decyl 3-methylbutanoate	1822	nd	0.32 ± 0.11	0.24 ± 0.01	0.66 ± 0.12	0.12 ± 0.01	0.12 ± 0.02	0.91 ± 0.05	0.06^{b}	0.23^{b}	0.07 ± 0.02
Ethyl dodecanoate	1827	0.24 ± 0.02	nd	nd	nd	nd	nd	nd	nd	nd	nd
Ethyl cinnamate	2160	0.33 ± 0.02	0.41 ± 0.02	0.07^{b}	nd	0.12 ± 0.03	0.55 ± 0.01	0.61 ± 0.15	0.45 ± 0.03	2.73 ± 0.05	0.36 ± 0.08
Cinnamyl acetate	2192	nd	0.24 ± 0.02	0.13 ± 0.02	nd	0.12 ± 0.02	0.19 ± 0.01	0.20 ± 0.01	0.13 ± 0.01	0.19^{b}	0.19 ± 0.02
Aldehydes											
Hexanal	1093	nd	0.05^{b}	0.06^{b}	0.09 ± 0.01	0.09^{b}	0.03^{b}	0.03^{b}	0.52^{b}	nd	0.05 ± 0.01
Trans-2-hexenal	1228	nd	0.16 ± 0.03	0.15 ± 0.01	0.11 ± 0.03	0.28 ± 0.05	0.01^{b}	0.26^{b}	0.02^{a}	0.06^{b}	0.09 ± 0.03
Octanal	1298	nd	0.01^{a}	nd	0.03^{b}	nd	nd	< 0.01	< 0.01	nd	nd
Nonanal	1374	nd	nd	nd	0.01^{a}	0.01^{a}	nd	< 0.01	nd	nd	0.01^{b}
Trans-2-octenal	1442	nd	0.43 ± 0.01	0.05 ± 0.01	0.02^{b}	0.01^{a}	0.01^{b}	0.01^{b}	< 0.01	nd	0.01^{b}
Benzaldehyde	1533	0.11 ± 0.02	0.16 ± 0.02	0.07 ± 0.01	0.03 ± 0.01	nd	0.11^{b}	0.06^{b}	0.06^{a}	0.18^{b}	0.08 ± 0.04
Alcohols											
3-Heptanol	1314	nd	nd	nd	nd	0.01^{a}	nd	< 0.01	nd	0.05^{b}	nd
1-Hexanol	1374	nd	0.01^{a}	< 0.01	nd	nd	< 0.01	nd	< 0.01	0.01^{b}	nd
Cis-3-hexen-1-ol	1401	nd	0.01^{a}	< 0.01	nd	0.01^{a}	< 0.01	< 0.01	< 0.01	nd	nd
1-Octanol	1578	0.02 ± 0.00	0.01^{a}	0.01^{a}	0.21 ± 0.01	nd	0.01^{b}	0.04^{a}	0.02^{b}	0.07 ± 0.01	0.07 ± 0.04
Benzene methanol	1892	nd	nd	nd	nd	1.42 ± 0.01	nd	nd	nd	nd	nd
Acids											
2-Methylbutanoic acid	1691	nd	1.52 ± 0.30	1.06 ± 0.14	nd	6.41 ± 0.68	0.36 ± 0.01	0.17^{b}	nd	nd	nd
Hexanoic acid	1862	nd	18.33 ± 3.57	8.22 ± 0.72	10.10 ± 2.35	3.12 ± 0.42	3.05 ± 0.10	2.21 ± 0.04	0.83^{b}	0.04^{b}	3.74 ± 0.60
Furanone											
Mesifurane	1601	0.09^{b}	0.09 ± 0.01	0.04^{b}	nd	0.38 ± 0.09	nd	0.04^{b}	0.01^{a}	1.19 ± 0.01	0.08 ± 0.03

(Continued)

Table 2—Continued

Compound	Retention Index	Concentration (mg/kg)									
		Totem	Puget Reliance	Puget Summer	Hood	Independence	Ventana	Camarosa	San Miguel	Venice	13G97
Terpenoids											
Linalool oxide	1467	nd	0.01[a]	< 0.01	nd	0.01[a]	< 0.01	< 0.01	< 0.01	0.02 ± 0.01	0.02 ± 0.01
Linalool	1565	0.21 ± 0.02	0.19[b]	0.12[b]	0.71 ± 0.04	0.64 ± 0.12	0.06 ± 0.01	0.03[b]	0.03[a]	0.27[b]	0.22 ± 0.15
alpha-Terpineol	1715	0.01a	0.01a	< 0.01	0.33 ± 0.02	0.38 ± 0.03	nd	< 0.01	< 0.01	0.01[b]	0.17 ± 0.08
Linalyl formate	1775	nd	0.49 ± 0.10	0.53 ± 0.02	0.37 ± 0.12	0.13 ± 0.01	0.99 ± 0.04	0.31 ± 0.05	0.15[b]	0.61 ± 0.02	nd
Trans-2-geraniol	1834	nd	0.01[a]	0.03[a]	0.14 ± 0.09	0.02[b]	< 0.01	0.04 ± 0.01	nd	nd	nd
Nerolidol	2063	1.07 ± 0.01	0.97 ± 0.20	0.07[b]	1.69 ± 0.13	3.41 ± 0.81	0.10[b]	0.05[b]	< 0.01	0.03 ± 0.03	0.89 ± 0.77
Lactones											
γ-decalactone	2083	nd	0.30 ± 0.03	nd	0.66 ± 0.06	0.25 ± 0.06	0.11[b]	0.23 ± 0.01	nd	0.14 ± 0.01	0.73 ± 0.55
δ-decalactone	2234	nd	nd	nd	0.66 ± 0.06	0.33 ± 0.03	nd	0.78 ± 0.01	0.03[b]	nd	0.01[b]
Jasmolactone	2249	nd	< 0.01	< 0.01	nd	0.02[b]	< 0.01	< 0.01	< 0.01	nd	nd
γ-dodecalactone	2422	0.20 ± 0.03	0.19[b]	0.17 ± 0.03	0.71 ± 0.04	0.80 ± 0.32	0.09 ± 0.02	0.22 ± 0.02	< 0.01	0.20 ± 0.02	0.20 ± 0.01

[a]SD < 1/1000.
[b]SD < 1/100.
nd = not detected.

correlation coefficients were obtained for most of selected aroma compounds with the standards (Table 1).

The concentrations of the aroma compounds in the cultivars are presented in Table 2. The results had a high degree of consistency among the replicates with a standard deviation of less than 10% for most of the compounds. Esters accounted for the majority of the aroma compounds in all of the cultivars. Prominent among the esters were ethyl acetate, methyl butanoate, ethyl butanoate, ethyl isovalerate, methyl hexanoate, and ethyl hexanoate. These esters contribute to the fruity notes of the strawberry aroma. Totem and Puget Reliance had very high concentration of ethyl butanoate, but were low in the other esters. Puget Summer followed a similar trend but at slightly lower ester concentrations. The cultivar Venice was unique as it had a very high concentration of ethyl acetate (10 ppm) as well as of methyl butanoate, ethyl pentanoate, and ethyl hexanoate. Surprisingly, it had a relatively low concentration of ethyl butanoate. Ethyl butanoate was highest in Puget Reliance (3.3 ppm) followed by Totem (2.6 ppm), while ethyl isovalerate was present at similar levels in all the cultivars. Hood and Independence had slightly higher levels of methyl hexanoate (0.3 ppm and 0.2 ppm, respectively) than the other cultivars.

Hexanal, trans-2-hexenal, hexanol, and cis-3-hexen-1-ol, which contribute to the fresh, green notes, varied in concentration among cultivars. San Miguel had the highest concentration of hexanal, while Independence and Camarosa had the highest concentration of trans-2-hexenal. Puget Reliance had a high concentration of trans-2-octenal.

Since the SPME fiber has poor extraction efficiency for short chain carboxylic acids, only 2-methylbutanoic and hexanoic acids were quantified. Independence has a high level of 2-methylbutanoic acid, while Puget Reliance, Hood, and Puget Summer all had very high concentrations of hexanoic acid.

Lactones contribute to fruity, coconut aromas. Although the concentration of individual lactones varied based on cultivar, in general, Hood, Independence, and Camarosa had higher levels of lactones. Terpenoids are responsible for the fruity, citrus aromas. Hood and Independence also had higher levels of terpenoids. Mesifurane is partially responsible for the sweet, caramel, strawberry-like aromas, and it ranged in concentration from 0.01 to 1.19 ppm, with Venice having the highest concentration.

Since some compounds have greater impacts on the overall aroma than others due to the odor sensory threshold difference, OAVs (=ratio of concentration of compound to its threshold value) were used to better understand the contribution of each compound to the overall aroma. Table 3 summarizes the OAVs calculated based on the threshold concentrations from the literature. Although OAV varied based on the cultivars, in general, ethyl butanoate, mesifurane, ethyl hexanoate, ethyl 3-methylbutanoate, hexyl acetate, and γ-dodecalactone had the highest OAVs. Venice cultivar had the highest combined total OAVs for all the compounds while Hood had the lowest. Totem, Puget Reliance, Puget Summer, and Venice had high total OAV for esters, while Hood, Independence, and Ventana had high total OAV for lactones. In addition, Hood and Independence had high total OAVs for terpenoids.

Correlation of analytical data with sensory analysis

Quantitative flavor profiling (Stampanoni 1993) is a sensory descriptive that involves the detection (discrimination) and the description of a product by a trained panel. The panel leader acts as a facilitator and assists the panel in developing a consistent terminology. Reference standards as well as the samples to be tested are presented to the panel during training sessions. The panelists evaluate products one at a time in separate booths to reduce distraction

Table 3—Odor activity values (OAVs) of selected aroma-active compounds in 10 strawberry genotypes from Oregon and California

		OAV									
Compound	Threshold	Totem	Puget Reliance	Puget Summer	Hood	Independence	Ventana	Camarosa	San Miguel	Venice	13G97
Esters											
Ethyl acetate	0.025	17.9	24.1	2.5	nd	0.7	1.9	1.2	6.1	411.6	1.2
Methyl butanoate	0.06	16	16	14	3	9	4	6	8	31	7
Ethyl butanoate	0.001	2610	3331	1956	324	16	273	14	280	550	70
Ethyl isovalerate	0.002	72.5	85.7	8.7	nd	21.2	7.7	20.5	8.4	36.4	17.7
Butyl acetate	0.066	2.3	2.2	0.9	nd	nd	0.1	0.8	nd	1.5	0.2
Isoamyl acetate	0.02	5.0	5.0	1.5	0.8	1.6	0.3	0.8	0.2	3.9	0.5
Ethyl hexanoate	0.001	400	685	64	75	67	87	65	49	2080	257
Hexyl acetate	0.002	33	32	26	42	32	2	10	2	28	20
Hexyl butanoate	0.25	0.5	0.6	0.6	0.2	0.1	0.1	0.9	0.1	nd	0.1
Octyl acetate	0.001	nd	0.1	0.2	10.7	2.6	0.0	0.5	0.1	35.3	2.6
Benzyl acetate	0.75	1.8	23.5	7.2	0.7	9.2	3.2	1.5	0.7	9.5	5.5
Aldehydes											
Hexanal	0.054	nd	1.2	1.5	2.0	2.0	0.8	nd	1.2	nd	0.8
Trans-2-hexenal	0.017	nd	9.6	9.2	6.8	16.7	1.0	15.4	1.3	3.4	5.4
Octanal	0.001	nd	14.7	nd	36.1	nd	nd	nd	4.9	nd	nd
Nonanal	0.001	nd	nd	nd	13.8	10.6	nd	nd	nd	nd	12.1
t-2-Octenal	0.436	nd	nd	16.8	7.1	2.8	4.8	5.0	1.4	48.7	3.7
Benzaldehyde	0.35	0.3	0.5	0.2	0.1	nd	0.3	0.2	0.2	0.5	0.2
Alcohols											
1-Hexanol	2.5	nd	< 0.01	2.5	nd	nd	< 0.01	nd	< 0.01	< 0.01	nd
Cis—Hexen-1-ol	0.07	nd	0.2	0.1	nd	nd	0.0	0.1	0.0	nd	nd
1-Octanol	0.11	0.2	0.1	0.2	1.9	nd	0.2	0.4	0.2	0.7	0.7
Acids											
2-Methyl butyric acid	0.25	nd	nd	4.3	nd	25.7	nd	nd	nd	nd	nd
Hexanoic acid	3	nd	6.1	2.7	3.4	1.0	1.0	0.7	0.3	0.0	1.3
Furanone											
Mesifurane	0.00003	3000	3000	1333	nd	12670	nd	1333	333	39670	2666
Terpenoids											
Linalool	0.01	21	19	12	72	65	6	4	3	27	23
Trans 2 geraniol	0.009	nd	1.5	3.4	15.6	1.7	0.6	5.0	nd	nd	nd
Lactones											
γ-decalactone	0.011	nd	27	nd	60	23	187	22	4	13	66
δ-decalactone	0.336	nd	nd	nd	nd	3.4	nd	7.8	0.3	nd	0.2
Jasmolactone	0.006	nd	< 0.01	< 0.01	nd	0.0	< 0.01	< 0.01	< 0.01	nd	nd
γ-dodecalactone	0.007	28	28	25	102	115	13	32	1	29	29

nd = not detected.
Sensory thresholds were obtained from the flavor base of Leffingwell & Associates.

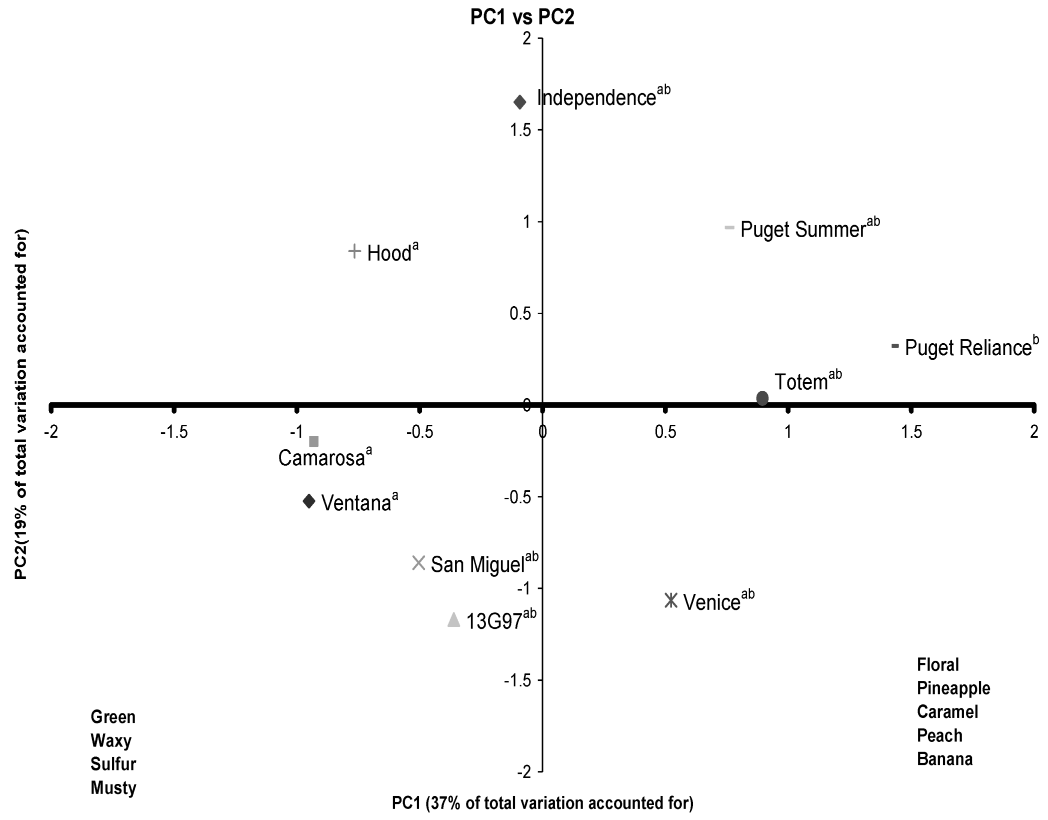

Figure 1—Principal component 1 and 2 showing the descriptive profile for the attributes-floral, pineapple, caramel, peach, banana, green, waxy, sulfur, and musty in 10 strawberry genotypes

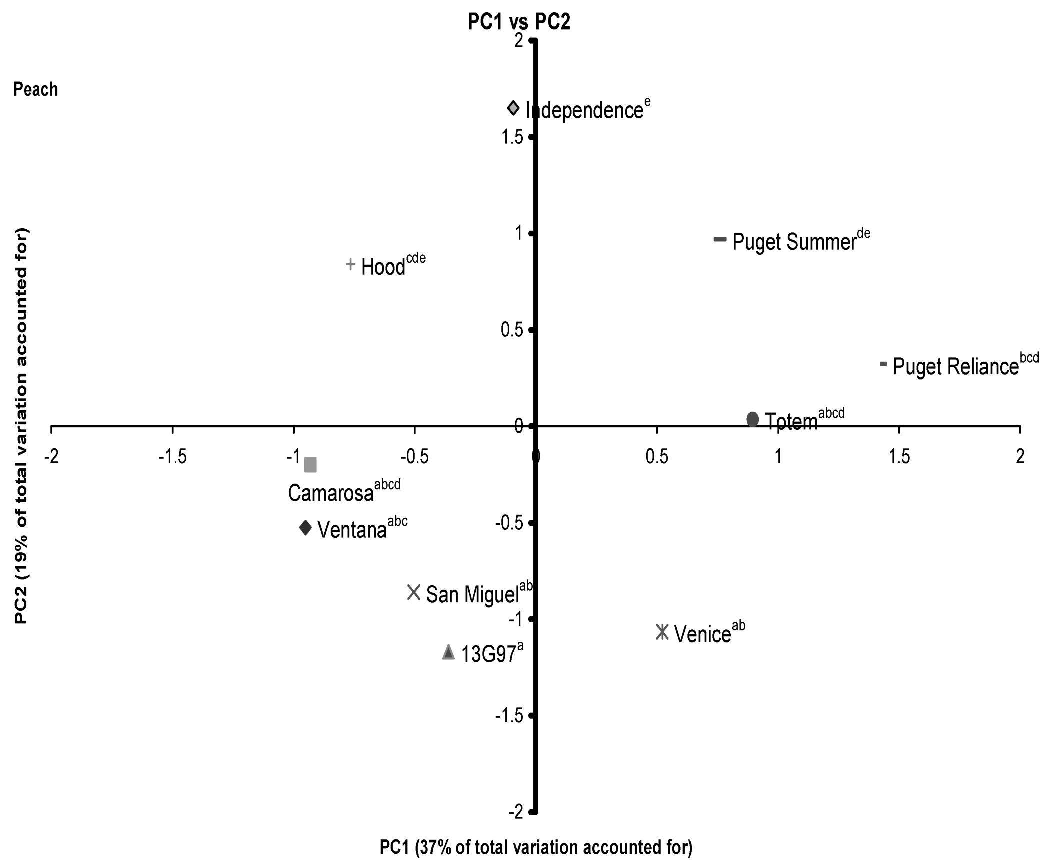

Figure 2—Principal component 2 showing the descriptive profile of strawberries for the attribute peach

and panelist interaction. The results of the test are analyzed statistically.

ANOVA and PCA were used to describe the sensory profile of the 10 strawberry cultivars. In the PCA plot, principal component one (PC1) accounted for 37% of total variation, while principal component two (PC2), three (PC3), and four (PC4) accounted for 19%, 11%, and 9% respectively. PC1 differentiated the samples based on the floral, pineapple, caramel, peach, banana, green, waxy, sulfur, and musty notes, while PC2 grouped the samples based on the peach note (Figure 1 and 2). Totem, Puget Reliance, Puget Summer, and Venice had higher amounts of floral, caramel, pineapple, peach, and banana notes. Hood, Independence, Ventana, Camarosa, San Miguel, and "13G97" had higher amounts of green, sulfur, musty, and waxy notes (Figure 1). Puget Reliance was significantly different from Hood, Camarosa, and Ventana on PC1. 13G97 was significantly different from Independence, Puget

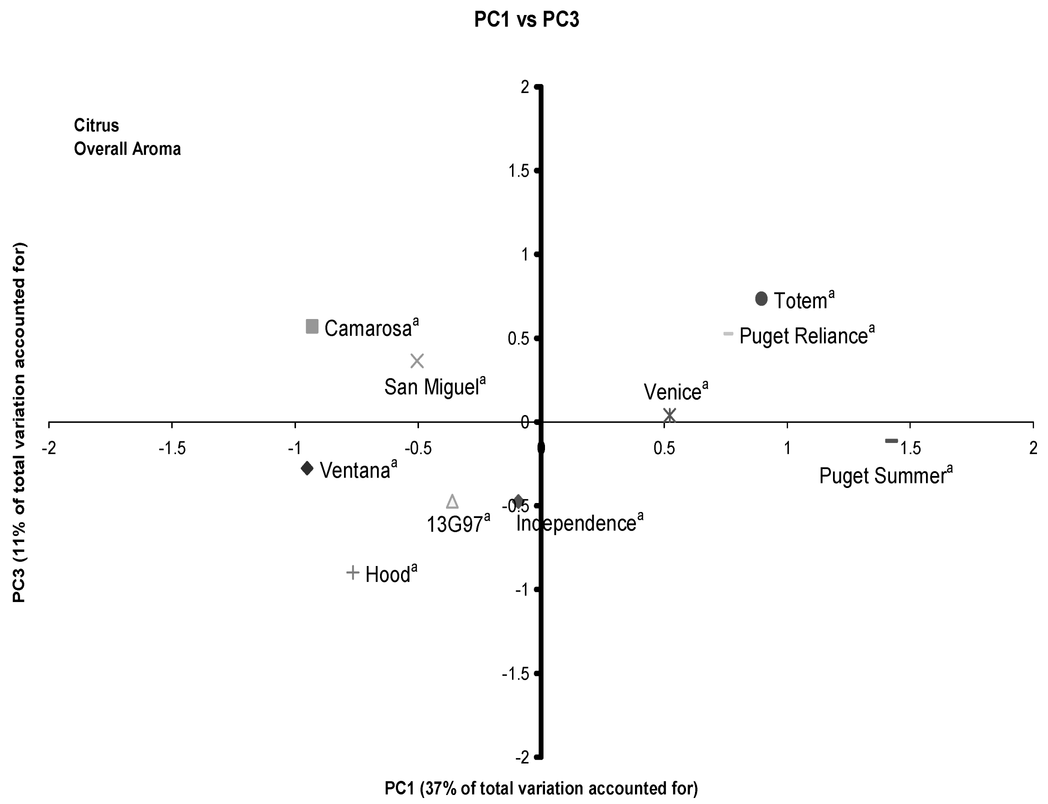

Figure 3—Principal component 3 showing the descriptive profile of 10 strawberry genotypes for the attributes citrus and overall aroma

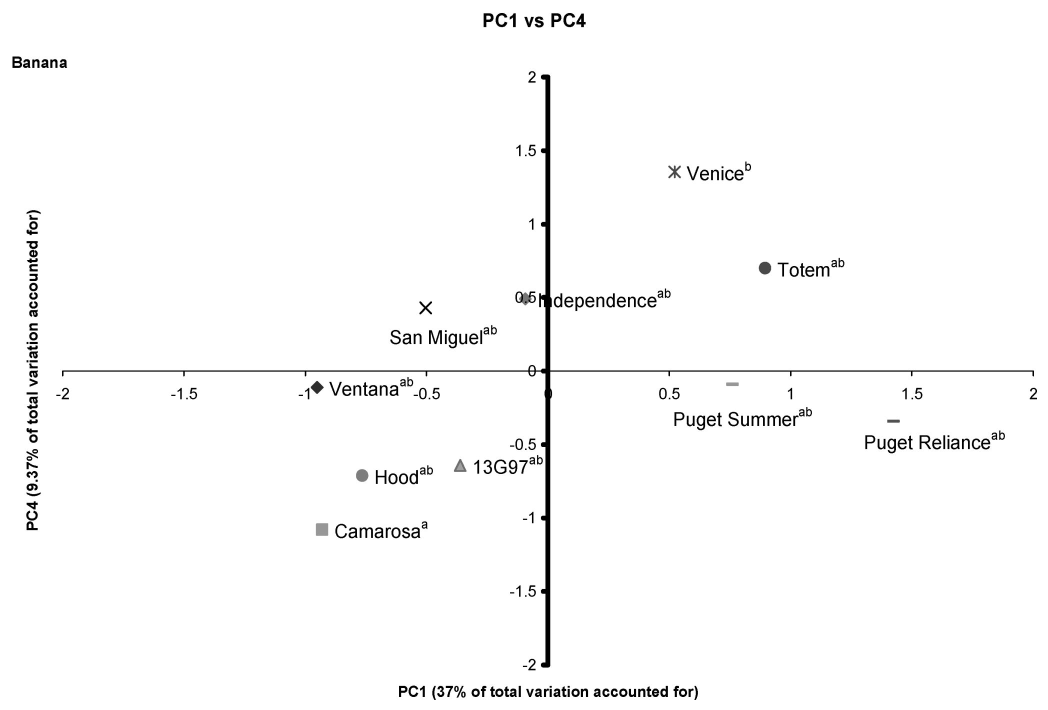

Figure 4—Principal component 4 showing the descriptive profile of 10 strawberry genotypes for the attribute banana

Summer, Hood, and Puget Reliance on PC2 (Figure 2) due to the variation in the peach note. Totem, Camarosa, Puget Reliance, San Miguel, and Venice had slightly higher citrus aroma (Figure 3). Independence, San Miguel, Totem, and Venice had higher amounts of banana notes than the rest of the cultivars on PC4 (Figure 4). Venice and Camarosa differed significantly from one another in terms of the amount of banana note in them.

The sensory characteristics of a product generally result from many chemical compounds acting in concert (Chien and Peppard 1993). Totem, Puget Reliance, Puget Summer, and Venice had higher amounts of fruity aroma (pineapple, banana, peach). The combined OAVs of the esters responsible for fruity aroma notes for Totem, Puget Reliance, Puget Summer, and Venice were in the range of 3000 to 4000 while they were in the range of 100 to 400 for Ventana, Camarosa, San Miguel, 13G97, Hood, and Independence, which were perceived as having lower fruity notes by the panelists. The high total OAVs of esters for Totem, Puget Reliance, Puget Summer, and Venice correlated well with their higher intensity of floral, pineapple, and banana notes.

The peach note is primarily contributed by lactones. Hood and Independence had higher total OAVs for lactones, which correlated well with perceived high peach note levels in the sensory study. Ventana also had high total OAVs for lactones, but its peach note seemed to be suppressed.

In general, hexanal, trans-2-hexenal, cis-3-hexen-1-ol, octanal, nonanal, and trans-2-otenal are responsible for the green notes in strawberry. Hood, Independence, Ventana, Camarosa, San Miguel, and 13G97 had higher amounts of green notes. But the OAVs do not convey the same information. The sensory impact of some compounds may be masked or enhanced by other compounds (McBride 1990; Grosch 2001). The OAVs of the fruity notes were significantly higher than the green notes. The OAVs of all the fruity notes ranged from 100 to 3000, while the combined green notes ranged from 5 to 20. Hence it is possible that the very strong fruity notes are able to suppress the green notes. The higher amounts of green note in Hood, Independence, Ventana, Camarosa, San Miguel, and 13G97 were probably due to the lack of fruity notes.

The caramel note in strawberries is contributed mainly by mesifurane and furaneol. Furaneol cannot be reliably quantified by the SPME method, and correlation of this attribute is only possible when a more accurate method is deployed to quantify the actual concentration of furaneol.

Musty and sulfur notes could not be correlated with any of the compounds identified in strawberry. All the samples tended to lose the musty note within 30 to 45 min after the samples were blended. Many sulfur compounds like hydrogen sulfide, methanethiol, dimethyl sulfide, dimethyl disulfide, methyl thioacetate, and methyl thiobutanoate have been identified in strawberry (Dirinck and others 1981; Schulbach and others 2004) using different extraction techniques and a sulfur specific detector. Sulfur compounds were not quantified in this study, and there were no other compounds identified to be responsible for the perceived sulfur odor by the panelists. Quantitative analyses of sulfur compounds are needed to correlate with the sulfury attributes of strawberry.

Conclusions

The aroma profiles of some strawberry cultivar grown in California and Oregon were analyzed using HS-SPME. Quantification of the aroma compounds was achieved using multiple internal standards and calibration curves of the standard. Correlation of the sensory data with the instrumental data gives interpretation of the importance of individual compounds in the overall aroma of strawberry. The stronger fruity notes in some of the cultivars may mask the green notes due to their higher OAVs. Both sensory and instrumental analysis demonstrated that Totem, Puget Summer, Puget Reliance, Independence, and Hood from the Pacific Northwest and Venice from California had higher fruity and peach aroma. These data can be used in the breeding programs to develop cultivars that have a desirable aroma in addition to other quality parameters.

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