Identification of Volatile Compounds in Cantaloupe at Various Developmental Stages Using Solid Phase Microextraction

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Using an automated rapid headspace solid phase microextraction (SPME) method for volatile extraction in cantaloupes, 86 compounds already reported for muskmelons were recovered and an additional 53 compounds not previously reported were identified or tentatively identified. The SPME method extracted a copious number of volatiles that can be analyzed to clearly differentiate between variety, growth stage, and stage of harvest ripeness. Most of the newly reported compounds in cantaloupe were esters and aldehydes that have already been demonstrated as flavor-related compounds in other products. All esters believed to have flavor impact increased progressively after pollination, and this trend continued with increasing harvest maturity. However, compound recovery often decreased when fruits were harvested over-ripe. Most aldehydes increased during early growth stages and then tapered off with increasing harvest maturity. The SPME method suitably recovered most compounds reported to impart characteristic flavor/aroma in muskmelons. SPME offers experimental flexibility and the ability to discover more compounds and address flavor quality changes in fresh-cut cantaloupe.

Keywords: *Aroma; cantaloupe; Cucumis melo; flavor; fresh-cut; fruit; gas chromatography; mass spectrometry; maturity; melon; quality; solid phase microextraction; SPME; volatiles*

INTRODUCTION

In cantaloupe, development of an abscission layer at the vine is a good indicator of harvest time. Fruit harvested before development of the abscission zone will not develop flavor similar to fruit remaining on the vine until a later developmental stage (*1*). However, fruit harvested at or after development of the abscission will have a shorter storage life, and flavor loss may occur before completion of the marketing process (*2*). Initial horticultural maturity at harvest and rapid developmental changes present a challenge to deliver an optimal cantaloupe or fresh-cut cantaloupe product with both postharvest keeping quality and flavor quality to the consumer. We therefore initiated a study to investigate the flavor profile of two cantaloupe varieties during maturation and at various harvest maturities. Our objectives were to recover as many volatile compounds as possible in cantaloupe of various developmental stages using a simple, rapid automated analysis and ultimately gain the ability to track flavor changes in stored fresh-cut products.

The last comprehensive review of volatile compounds in muskmelons tabulated 219 compounds (*3*). One hundred and seventy-four of the compounds are alcohols, aldehydes, ketones, esters, and sulfur-containing compounds. A recent survey of the literature shows that roughly 240 volatile compounds have been reported in muskmelon. Most typical sample preparations for compound isolation involve steps that are time- and labor-intensive, are prone to volatile loss, and often used solvents that are toxic or potential carcinogens. Furthermore, solvent extractions are generally accomplished at high temperatures or under reduced pressure, conditions that can destroy or alter some volatile flavor compounds and/or produce artifacts. Our long-term objective is to rapidly analyze flavor and aroma compounds in fresh-cut fruits and ultimately correlate chemical analyses with those findings obtained by trained sensory panelists. Therefore, we analyzed for aroma and volatile flavor compounds at approximately the temperature of the human palate, where mastication occurs (~35 °C). Solid phase microextraction (SPME) was chosen because it is rapid, less laborious, and relatively inexpensive and does not require solvents, purge and trap, preconcentration, or vigorous extraction and heating, which may alter endogenous compounds. Also, the absorptive nature of the fibers permits assays at nondestructive temperatures. Flavor and off-flavor aromas have recently been assessed in numerous fruits and juices by SPME (*4*−*9*).

MATERIALS AND METHODS

Plant Material. Cantaloupes (*Cucumis melo* var. *reticulatus* cv. Sol Real) were grown in Kettleman City, CA, on raised beds with standard cultural practices and furrow irrigation. Flowers were anthesis tagged on a single day, and developing fruit were harvested 13, 20, 28, and 35 days after pollination (DAP), hydrocooled in the field, packed carefully with Styrofoam packaging beads, shipped overnight to the Southern Regional Research Center (SRRC) laboratory, and analyzed immediately the following morning. Ripe fruit were harvested 38 DAP at four distinct maturities ($^1/_4$, $^1/_2$, $^3/_4$, and full slip), field hydrocooled, stored over the weekend at ~5 °C, boxed as above, and air freighted to the SRRC and analyzed the following day. Cv. Athena cantaloupes were grown in a high-density planting at the SRRC on raised beds with standard cultural conditions. Flowers were anthesis tagged periodically, and developing fruit were harvested 29 DAP and analyzed the

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same day. Ripe Athena fruit were harvested in Valdosta, GA, at $^3/_4$ to full slip, hydrocooled, boxed as above, and shipped overnight to the SRRC.

Sample Preparation. Whole fruit were sanitized in 100 ppm of bleach and uniformly peeled on a Muro CP-44 melon peeler (Tokyo, Japan), except 13 DAP Sol Real fruit, which were hand peeled with a carrot peeler. The stem and blossom portions (~2−3 cm) were cut off on a cutting board, and then melons were moved to a clean cutting board. Each melon was sliced once longitudinally, then seeds were removed, and the seed cavity was cleaned. Halves were placed face-down, and roughly 2.5 cm equatorial slices were cut, from which all loose endocarp seed cavity tissues (1−2 mm thick) were removed. Approximately 2−3 cm × 2.5 cm cubes were prepared in pie-like wedges cut from the 2.5 cm wide equatorial mesocarp slices. All sanitized melons, subsequent cutting procedures, and fresh-cut tissues were prepared and handled with latex gloves.

Volatile samples were prepared in triplicate, each from 300 g of randomized cubes from a representative pool of five immature fruits or 300 g of randomized fresh-cut cubes from a minimum of five fruits per maturity. Tissue was rapidly juiced (~15 s) into a slurry with a Braun MP80 juicer. A 3 mL slurry (without foam) was immediately pipetted into 10 mL glass vials containing 1.1 g of NaCl, and then a 10 μg kg^{-1} (final concentration) mixed 3-hexanol and 2-methylbutyl isovalerate (2-methylbutyl 3-methylbutanoate) internal standard (IS) was added. Vials were sealed with a steel crimp cap fitted with a Teflon/silicon septum and placed on a Combi-Pal autosampler (Leap Technologies, Carrboro, NC) cooling rack at 4 °C.

SPME Analysis. Because variability in analyte recovery with SPME was observed with various sampling regimes (*5*, *7*, *9*), we minimized variation by saturating slurries with sodium chloride and keeping the heating time, sample volume, and temperature (40 °C, slightly higher than the human palate) constant. Preliminary data indicated that a 1-cm 100 μm automated poly(dimethylsiloxane) (PDMS) SPME fiber (Supelco, Inc., Bellefonte, PA) delivered favorable automated results with 3 mL samples in 10 mL vials (*10*). Sample vials were removed from the 4 °C holding tray of the autosampler and equilibrated for 10 min via oscillation in a 40 °C chamber, followed immediately by a 12.5 min SPME exposure to the headspace above the slurry at 40 °C. Vials were continuously swirled during SPME exposure with an agitation speed of 100 rpm.

GC-MS Parameters and Analyses. SPME fibers were desorbed at 250 °C for 1 min in the injection port of an HP6890/5973 GC-MS (Hewlett-Packard, Palo Alto, CA) with a DB-5 (cross-linked 5% phenyl methyl silicone, J&W Scientific, Folsom, CA) column (30 m, 0.25 mm i.d., 25 μm film thickness) for 35 min runs (although no peaks of interest were collected after 20 min). Fibers remained in the heated injection port for 5 min as a bake-out step. The injection port was operated in splitless mode and subjected to a pressure of 25 psi of ultrahigh-purity He (99.9995%) for the first minute and then set at a constant velocity of 40 cm s^{-1} for the remainder of the GC run. The initial oven temperature was 50 °C, held for 1 min, ramped at 5 °C min^{-1} to 100 °C and then at 10 °C min^{-1} to 250 °C, and held for 9 min. The HP5973 quadrupole mass spectrometer was operated in the electron ionization mode at 70 eV, a source temperature of 200 °C, quadrupole at 106 °C, with a continuous scan from *m*/*z* 33 to 300.

Data were collected with HP ChemStation software (A.03.00) and searched against the NIST (v. 1.5) and Wiley (v. 7 NIST98) libraries (Palisade Corp., Newfield, NY). Compounds were preliminarily identified by library search, and then the identities of most were confirmed by GC retention time (RT), MS ion spectra, authentic compounds or a homologous series, and a retention index (RI). The RTs from a series of straight-chain alkanes (C_5−C_{20}) were used to calculate RIs for all identified compounds. The MS library generally delivers a high-quality hit and matching spectra for the *E*,*Z* isomer on some *E*,*E* and *E*,*Z* isomer pairs occurring at different RTs. Because some standards were not available, we relied upon the fact that *Z* isomers generally elute first to deduce their identity and present RIs. An interfering peak intermittently coeluted with the 10 ppb 2-methylbutyl isovalerate IS with similar ion fragments. The presence of the interfering compound was detected by the change in target ion/qualifier ion ratio. It was determined that only a single compound was coeluting with the internal standard, and its target ion/qualifier ion ratio was measured. Employing the two known ratios, the contribution from each compound was calculated on the basis of the *m*/*z* 85 ion, to yield a corrected value for the pure internal standard. The corrected IS value was used to normalize only data within ±20% (0.80−1.20) of a theoretically perfect IS ratio (1.00) per triplicate. All integrated responses were examined carefully, and relative recovery (integrated area count) for specified compounds, based on selected unique qualifying target ions, was presented ($n = 3$).

RESULTS AND DISCUSSION

Complete volatile analysis of whole apple fruit with SPME has been reported to be challenging (*9*, *11*). The length of the SPME equilibration time in the sample container and the overall SPME exposure time were excessive for an acceptable rapid automated analysis (*11*). High molecular weight compounds did not equilibrate in the vapor phase due to their partition coefficients, and there was a strong dependency of volatile uptake on the rate of air movement through the system (*9*). On the other hand, rapid SPME analysis of headspace orange and strawberry juices has proven to be very effective (*5*, *6*, *12*). We therefore performed a preliminary analysis of SPME volatile extraction and recovery from the headspace above cantaloupe cubes, Braun slurries, and hand-expressed (through Miracloth) juice. Slurry and juice gave similar ion traces that varied minutely compared to freshly cut cubes, but SPME exposure time for cubes was excessively long (30−60 min, depending on sample to free space ratio) to attain similar ion profiles (data not shown).

Using SPME with two varieties of rapidly juiced cantaloupe samples, we recovered 86 of the 240 reported muskmelon volatile compounds in the literature (Table 1). We also recovered 53 compounds not previously reported. Twenty-five compounds were confirmed and designated "first known observation", whereas the remainder are tentative because not all standards were available (Table 1). Fifteen of the new compounds were esters and acetates that are considered to be flavor volatiles in other commodities. Some of the compounds we isolated in cantaloupe were only previously reported in other Cucurbitaceae (watermelon or bittermelon) (*13*, *14*). Additionally, we recovered two new alcohols, 1-heptanol and (*Z*)-3-octen-1-ol, even though the 100 μm PDMS fiber did not effectively recover most alcohol compounds previously reported in muskmelon. Sample preparation time was minimal, and the automated SPME method can be used to effectively assess cantaloupe varieties as well as optimum maturity levels necessary to deliver high quality fresh-cut cantaloupe products.

Striking differences were observed in volatiles recovered from immature versus mature fruit (Figures 1 and 2). In general, there were many more esters and acetates recovered from mature fruit and the relative recovery of compound classes differed depending on whether the fruit were western- or eastern-grown varieties. Cv. Sol Real (western) had more aromatic structure compounds and greater relative recovery for most esters common to both varieties. Cv. Athena fruit

Table 1. Volatile and Semivolatile Cantaloupe Flavor Compounds Reported in the Literature and Recovered by SPME

chemical name[a]	ID[b]	RI[c]	refs[d]
acetaldehyde	1	528	*21, 35−38*
ethanol	2	537	*21, 35−40*
propanal	3	554	*35*
methyl acetate	4	559	*21−23, 35−37, 39, 41, 54*
carbon disulfide	a	568	probable contaminant
ethyl acetate	5	605	*21−24, 35−47, 54*
methyl propanoate	6	621	*36, 37*
isopropyl acetate	7	648	*24, 36, 37, 41, 45, 46*
methyl isobutyrate	8	690	*24, 36, 37, 41, 46, 47*
valeraldehyde	9	699	first known observation
S-methyl ethanethioate	10	701	*46*
propyl acetate	11	707	*21−24, 35−39, 42, 44−48, 54*
ethyl propanoate	12	708	*23, 24, 35−43, 45−47*
methyl butanoate	13	717	*22−24, 36−38, 44−47*
3-methylbutanol[e]	14	733	*35*
2-methylbutanol[e]	15	733	*36, 37, 45, 46, 48, 49*
ethyl isobutyrate	16	751	*20, 35−38, 41, 45, 46*
1-pentanol	17	761	*35−37, 42*
isobutyl acetate	18	768	*21−24, 35−39, 42−48, 50*
methyl 2-methylbutanoate	19	772	*20, 23, 35−37, 41, 44−46*
(*Z*)-3-hexenal	20	796	*20*
3-hexanol	IS	797	*23*
hexanal	21	801	*20, 23*
ethyl butanoate	22	803	*20, 22−24, 35−42, 45−48, 50, 54*
propyl propanoate	23	807	*24, 41, 46*
butyl acetate	24	812	*21−24, 36−48, 50, 54*
methyl pentanoate	25	821	*24, 36, 37*
isopropyl butanoate	26	837	first known observation
ethyl 2-methylbutyrate	27	846	*21−24, 36−39, 41, 45, 46, 48, 50, 43*
(*E*)-2-hexenal	28	850	*20*
(*Z*)-3-hexenol	29	851	*23, 36, 37, 48*
isobutyl propionate	30	863	*24, 35−37, 46*
1-hexanol	31	865	*23, 35−37, 45−50*
isoamyl acetate[f]	32	876	*22, 41, 42, 44, 46, 48*
2-methyl-1-butyl acetate[f]	33	877	*37−41, 46, 50, 54*
propyl butanoate	34	897	*24, 36, 37, 46, 54*
ethyl valerate	35	900	*24, 36, 37, 46*
heptanal	36	902	first known observation
butyl propanoate	37	907	*24, 35−37, 41, 44, 46*
amyl acetate	38	912	*22−24, 35−37, 44−47, 54*
3-methyl-2-butenyl acetate	39	918	*22, 23, 46*
methyl hexanoate	40	922	*24, 36, 37, 46*
S-methyl 3-methylbutanethioate	41	938	tentative
propyl 2-methylbutanoate	42	943	*36, 37, 46*
2-methylpropyl butanoate	43	953	*24, 36, 37*
(*E*)-2-heptenal	44	955	tentative
benzaldehyde	45	962	*17, 21−23, 48*
pentyl propanoate	46	968	*24*
1-heptanol	47	969	first known observation
unknown alkyl acetate	48	975	
1-octen-3-one	49	975	tentative
1-octen-3-ol	50	978	*23, 48, 50*
ethyl 2-(methylthio)acetate	51	981	*21−24, 45, 46, 49, 51*
2,5-octanedione	52	983	tentative
2-pentylfuran	53	989	tentative
2-furanmethanol acetate	54	991	first known observation
butyl butanoate	55	994	*24, 36, 37, 41, 45*
(*E*,*Z*)-2,4-heptadienal	56	996	tentative
ethyl hexanoate	57	999	*17, 22, 24, 35−38, 41, 42, 45, 46,48, 50*
octanal	58	1003	*35*
(*Z*)-3-hexenyl acetate	59	1004	*21−24, 36−38, 44−46, 48*
(*E*,*E*)-2,4-heptadienal	60	1011	first known observation
hexyl acetate	61	1011	*21, 22, 24, 36−38, 41, 42, 44−50, 54*
2-methylbutyl 2-methylpropanoate	62	1014	tentative
(*E*)-3-hexenyl acetate	63	1018	tentative
p-methylanisole	64	1020	first known observation
methyl heptanoate	65	1021	tentative
methyl 3-(methylthio)propanoate	66	1023	*23, 40, 46, 51*
limonene	67	1029	*23, 46*
3-ethyl-2-methyl-1,3-hexadiene	68	1030	tentative
1,8-cineole	69	1032	*20, 23*
benzyl alcohol	70	1033	*21, 23, 44, 48, 49*
phenylacetaldehyde	71	1043	*23*
(*Z*)-3-octen-1-ol	72	1054	first known observation
isoamyl butyrate[g]	73	1056	*41*
2-methylbutyl butanoate[g]	74	1056	*35−37*
(*E*)-2-octenal	75	1057	first known observation
butane-2,3-diol diacetate	76	1064	*23, 44−46, 49*
(*E*,*E*)-3,5-octadien-2-one	77	1068	tentative
1-octanol	78	1070	*17, 23, 35, 46, 50, 52*
ethyl (*E*)-4-heptenoate	79	1090	tentative
propyl hexanoate	80	1094	*35*
ethyl 3-(methylthio)propanoate	81	1098	*23, 36, 45, 51*
ethyl heptanoate	82	1099	first known observation
(*Z*)-6-nonenal	83	1101	*16, 53, 54*
2-acetyl furan	84	1101	tentative
nonanal	85	1104	*16, 17, 22, 23*
2-methylbutyl isovalerate	IS	1107	
heptyl acetate	86	1111	*21, 22, 24, 42, 54*
phenyl ethyl alcohol	87	1113	*23, 44, 48, 49*
3-(methylthio)propyl acetate	88	1123	*23, 45, 46, 51*
1,10-undecadiene	89	1146	tentative
(*E*,*Z*)-2,6-nonadienal	90	1155	*16, 20, 54*
(*E*)-2-nonenal	91	1162	*16, 17, 20, 21*
benzyl acetate	92	1164	*21−24, 41, 42, 44−46, 49, 50, 54*
(*Z*)-6-nonenol	93	1171	*16, 17, 22, 23, 50*
ethyl benzoate	94	1172	*23*
1-nonanol	95	1172	*16, 17, 21, 23, 48, 50*
(*E*,*E*,*Z*)-1,3,5,8-undecatetraene	96	1177	tentative
ethyl (*Z*)-4-octenoate	97	1187	tentative
butyl hexanoate	98	1190	*46*
ethyl octanoate	99	1194	*21, 46*
(*Z*)-3-octenyl acetate	100	1194	tentative
(*E*,*Z*)-2,4-nonadienal	101	1196	tentative
dodecane	102	1200	first known observation
decanal	103	1205	*35*
octyl acetate	104	1213	*17, 21, 24, 35, 45, 46, 52*
(*E*,*E*)-2,4-nonadienal	105	1216	first known observation
β-cyclocitral	106	1220	*23*
3-phenylpropyl alcohol	107	1232	*23, 49*
(*Z*)-citral	108	1240	first known observation
ethyl phenylacetate	109	1243	tentative
isoamyl hexanoate	110	1254	first known observation
phenethyl acetate	111	1255	*23, 24, 44, 45, 50*
(*E*)-2-decanal	112	1265	tentative
ethyl 3-acetoxyhexanoate	113	1266	tentative
(*E*)-citral	114	1270	first known observation
pentyl hexanoate	115	1282	first known observation
(*E*,*Z*)-2,4-decadienal	116	1294	tentative
undecanal	117	1306	first known observance
(*E*,*E*)-2,4-decadienal	118	1318	first known observation
(*E*)-2-undecanal	119	1364	tentative
3-phenylpropyl acetate	120	1373	*23, 45, 50*
methyl diethyl carbamodithioic acid	121	1381	tentative
ethyl decanoate	122	1392	*17, 21*
tetradecane	123	1400	first known observation
geranylacetone	124	1448	*23*
isoamyl octanoate	125	1450	first known observation
β-ionone	126	1484	*17, 21, 23, 50, 52*
α-farnesene	127	1496	*21, 54*
pentadecane	128	1500	first known observation
pentadecanal	129	1513	tentative
ethyl dodecanoate	130	1554	*17, 45*
hexadecane	131	1600	first known observation
hexadecanal	132	1614	tentative
heptadecane	133	1700	first known observation
heptadecanal	134	1716	tentative
octadecane	135	1800	first known observation
otadecanal	136	1818	tentative
nonadecane	137	1900	first known observation
hexadecanoic acid	138	2010	*23, 44*
octadecanoic acid	139	2200	*23*

[a] Chemicals are ordered by our retention index. Common or alternate names are in parentheses. [b] ID = identification, used for labels in Figures 1−3. IS = internal standard. [c] RI = retention index based on identified compound RTs, calculated from a linear equation between each pair of straight chain alkanes (C_5−C_{20}). [d] Compounds we recovered that are reported in the literature were compared to library ion spectra or standards or compounds in a homologous series. Compounds, verified with standards, apparently recovered for the first time in cantaloupe are denoted. Compounds reported for the first time in cantaloupe, which are considered to be *tentative* (based on the MS library), are also denoted. Reference 54 corresponds with compounds recovered in honeydew (Saftner, 1998, personal communication). [e] GC peaks coeluted and the MS could not differentiate the two isomers. [f] GC peaks coeluted but the MS library differentiated two isomers. [g] This compound (106-27-4, recovered only in cv. Athena) has the same RI as the isomer (2-methylbutyl butanoate, 51115-64-1), but the two were recovered in different varieties.

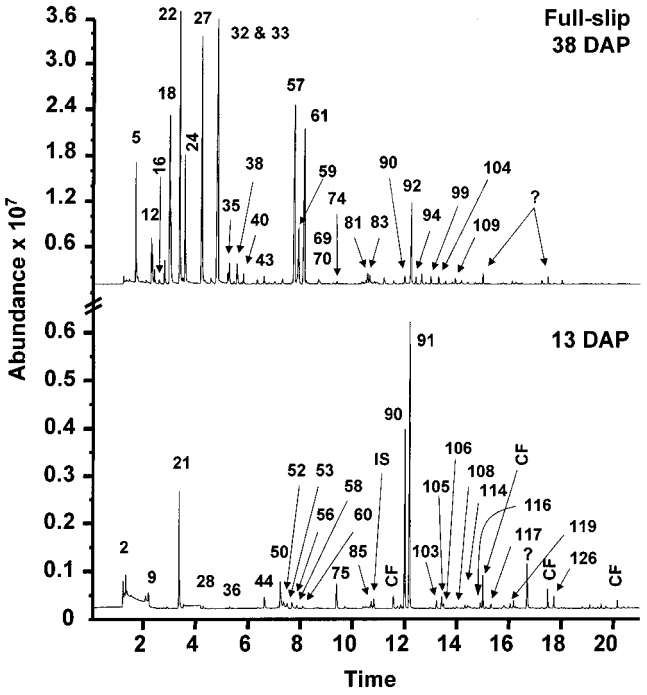

Figure 1. Total ion spectra from immature (13 DAP) and mature (full slip, harvested 38 DAP) cv. Sol Real cantaloupe fruit. One of the three replicate runs, which were virtually identical, is presented. Peak numbering corresponds with Table 1. For clarity, and because many compounds were confidently recovered at quantifiable levels with low relative abundance, not all are labeled. CF indicates peaks due to column or fiber impurities.

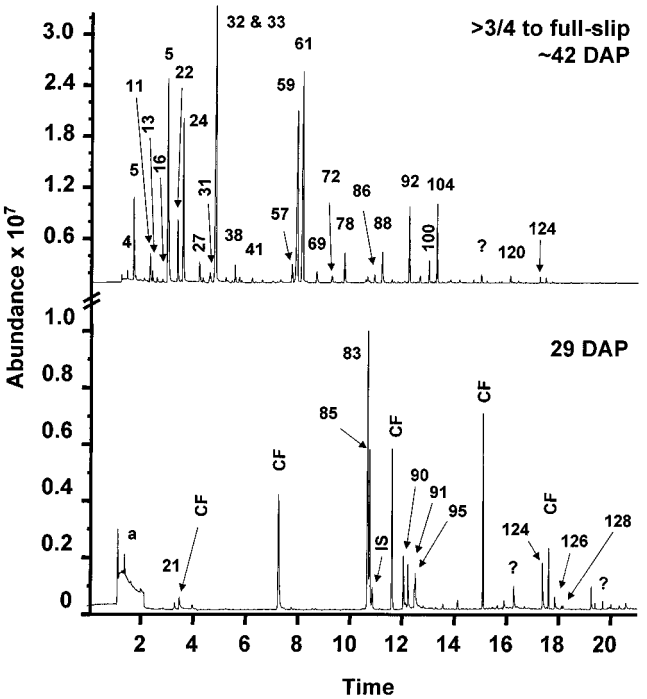

Figure 2. Total ion spectra from immature (29 DAP) and mature ($^3/_4$ to full slip, harvested ~42 DAP) cv. Athena cantaloupe fruit. One of the three replicate runs, which were virtually identical, is presented. Peak numbering corresponds with Table 1. CF indicates peaks due to column or fiber impurities.

(eastern) had generally more acetates, including unsaturated alkenyls of higher molecular weight.

Both varieties had only a few predominant peaks in immature fruit and a wider range of many volatiles, mainly esters and acetates, once mature. Immature Sol Real (Figure 1) contained predominantly aldehydes [valeraldehyde, hexanal, (*E*)-2-hexenal, heptanal, (*E*)-2-heptenal, (*E*,*Z*)-2,4-heptadienal, octanal, (*E*)-2-octenal, nonanal, (*E*)-2-nonenal, (*E*,*Z*)-2,6-nonadienal, (*E*,*E*)-2,4-nonadienal, decanal, (*E*,*Z*)-2,4-decadienal, and (*E*,*E*)-2,4-decadienal] and ketones (2,5-octanedione, 3,5-octadien-2-one, and β-ionone). Similarly, immature Athena (Figure 2) contained predominately aldehydes [acetaldehyde, valeraldehyde, hexanal, (*E*)-2-hexenal, heptanal, octanal, nonanal, (*E*)-2-nonenal, and (*E*,*Z*)-2,6-nonadienal], ketones [2,5-octanedione and (*E*)-6,10-dimethyl-5,9-undecadien-2-one], and alcohols [1-pentanol, (*Z*)-6-nonenol, and 1-nonanol] in addition.

Many C_9 aldehydes, alcohols, and esters [(*Z*)-6-nonenyl acetate, (*Z*)-6-nonenol, (*Z*,*Z*)-3,6-nonadienol, (*Z*)-6-nonenal, 3-nonenal, and 3,6-nonadienal] recovered in the Cucurbitaceae family have been reported to be characteristic flavor/aroma compounds (*15*, *17*). Cucumber (*Cucumis sativus*) flavor has been attributed mainly to aldehydes and to a lesser extent to certain corresponding alcohols. The pleasant odor was attributed to (*E*,*Z*)-2,6-nonadienal, and two unsaturated aldehydes [(*E*)-2-hexenal and (*E*)-2-nonenal] and three saturated aldehydes (acetaldehyde, propanal, and hexanal) were considered to contribute secondarily to overall flavor (*18*). However, Fleming et al. demonstrated that some of the characteristic flavor compounds in cucumber fruit such as (*E*,*Z*)-2,6-nonadienal, (*E*)-2-nonenal, and 2-hexenal were generated enzymatically as a consequence of cutting or mechanical rupturing (*19*). Only two aldehydes attributed to cucumber flavor are included among those compounds [ethyl 2-methylpropanoate, methyl 2-methylbutanoate, ethyl 2-methylbutanoate, ethyl butyrate, ethyl hexanoate, hexyl acetate, 3-methyl-1-butyl acetate, (*Z*)-3-hexenal, (*E*)-2-hexenal, benzyl acetate, (*Z*)-6-nonenyl acetate, (*Z*)-6-nonenol, (*E*)-2-nonenal, (*E*,*Z*)-2,6-nonadienal, (*Z*,*Z*)-3,6-nonadienol, (*Z*)-6-nonenal, 1,8-cineole (eucalyptol), and (*Z*)-1,5-octadien-3-one], which are suspected of contributing to the characteristic aroma of muskmelon (*17*, *20*−*22*).

We recovered most of the above aldehydes in immature cantaloupe (13, 20, and 28 DAP) samples. Many flavor aldehydes, such as acetaldehyde, propanal, (*E*)-2-butenal (crotonaldehyde), valeraldehyde, 2-pentenal, hexanal, 2-hexenal, 2-heptenal, 2-octenal, nonanal, 2-nonenal, 2,6-nonadienal, and 2,4-decadienal, were isolated from cucumbers (*15*). Many of the lower molecular weight aldehydes we recovered in immature cantaloupe fruit [i.e., acetaldehyde, valeraldehyde, hexanal, (*E*)-2-hexenal, heptanal, (*E*)-2-heptenal, and (*E*)-2-octenal] were also reported as flavor aldehydes in cucumber. However, only hexanal, nonanal, (*E*)-2-nonenal, (*Z*)-6-nonenal, (*E*,*Z*)-2,6-nonadienal (Table 3), and the aromatic aldehydes benzaldehyde (phenylmethanal) and benzeneacetaldehyde (phenylethanal) were recovered from mature fruit in relatively high concentrations. Most muskmelon volatiles reported in the literature have been extracted from mature fruit, and typically "green-grassy" (aldehyde) compounds have only been reported in squash or cucumber. A number of our previously unreported compounds were aldehydes that may have been formed as a result of oxidation during sample preparation. However, (*Z*)-3-hexenal and (*E*)-2-hexenal were attributed to the "green notes" in muskmelon (*20*). Furthermore, four scientists trained in sensory analysis determined that only our immature cantaloupe (13 and 20 DAP) smelled and tasted like

Table 2. Change in Integrated Area Counts for Selected Compounds Recovered in Immature Anthesis Tagged Sol Real Cantaloupe ($n = 3$)[a]

compound	days after pollination				compound	days after pollination			
	13	20	28	35		13	20	28	35
methyl 2-methylbutanoate (71)	404	54	45	50 744	hexyl acetate (56)	446	313	176	175 763
ethyl 2-methylpropanoate (88)	248	89	1 199	1 824	(*E*)-2-octenal (83)	87 587	59 770	4 417	7 227
hexanal (82)	142 180	124 044	6 843	13 464	(*Z*)-6-nonenal (55)	952 754	1 282 636	1 335 857	581 919
ethyl butanoate (88)	120	210	73	6 131	1-nonanal (57)	41 190	146 755	220 535	265 623
ethyl 2-methylbutanoate (102)	644	137	59	10 641	(*E,Z*)-2,6-nonadienal (70)	1 348 210	2 072 741	2 272 967	1 596 385
3-methyl-1-butyl acetate (87)	168	60	51	234	(*E*)-2-nonenal (70)	952 754	1 282 636	1 335 857	581 919
ethyl hexanoate (88)	627	1 018	119	13 500	benzyl acetate (108)	154	4 757	2 982	1 433 874
(*Z*)-3-hexenyl acetate (67)	3 445	4 378	315	103 679					

[a] Recovery of each compound is based on specific MS target ions (*m/z*) used for quantification.

Table 3. Change in Integrated Area Counts for Selected Compounds Recovered in Cv. Sol Real Cantaloupe Harvested at Five Distinct Maturities ($n = 3$)[a]

compound	harvest maturity				
	1/4 slip	1/2 slip	3/4 slip	full slip	over-ripe
methyl 2-methylbutanoate	570 083	1 036 155	1 682 531	2 815 737	2 537 981
ethyl 2-methylpropanoate	188 330	265 380	628 413	1 093 323	1 057 498
hexanal	11 619	25 302	40 066	116 181	298 459
ethyl butanoate	767 906	1 981 374	4 779 025	11 220 840	9 953 456
ethyl 2-methylbutanoate	2 166 833	4 063 634	9 446 885	13 975 977	9 653 297
3-methyl-1-butyl acetate	339	324	333	563	52 648
ethyl hexanoate	495 901	1 287 866	3 864 2151	1 209 671	12 701 858
(*Z*)-3-hexenyl acetate	709 749	1 164 184	1 978 152	3 334 976	1 262 064
hexyl acetate	843 992	1 606 233	3 843 504	8 136 099	6 767 782
(*E*)-2-octenal	3 669	2 008	1 680	2 088	3 158
(*Z*)-6-nonenal	149 578	65 075	37 268	49 172	22 067
1-nonanal	53 716	92 462	83 618	61 102	110 941
(*E,Z*)-2,6-nonadienal	574 500	377 927	234 355	331 026	161 649
(*E*)-2-nonenal	149 578	65 075	37 268	49 172	22 067
benzyl acetate	2 420 494	2 113 873	5 521 323	5 384 884	1 691 739

[a] Recovery of each compound is based on MS target ions listed in Table 2.

cucumber. Enal aldehydes normally decreased appreciably in fruit harvested over-ripe, whereas some aldehydes, such as hexanal and nonanal, increased with increasing harvest maturity, and this could lead to development of off-flavors in stored fresh-cut products. It is therefore possible that some of the green-grassy and C_9 compounds, previously attributed as Cucurbitaceae flavor notes, have significance in under- and over-ripe cantaloupe fruit, and they may vary per cultivar.

Mature fruit have many more volatile compounds compared to immature fruit, and an amplified view of their ion traces is therefore presented (Figure 3). Most aldehydes (especially less than C_8) and ketones that were dominant in immature samples were not detected or recovered in only trace levels from mature fruit. Numerous compounds recovered and illustrated in Figures 1−3 have been labeled according to the IDs in Table 1. Upon careful examination of the ion traces, one can identify numerous compounds in Table 1 that changed during growth, development, and maturation. Several compounds were quantified and are discussed below (Tables 2 and 3).

Because our method recovered numerous C_8−C_{10} compounds, we believe that some of the C_9 compounds formerly designated as flavor active in the Cucurbitaceae family (e.g., cucumber and honeydew) may not be present in all netted cantaloupe. For example, only three C_9 aliphatic acetates [nonyl acetate, (*Z*)-6-nonenyl acetate, and (*Z,Z*)-3,6-nonadienyl acetate] were reported for cantaloupe (*C. melo* var. *reticulatus* and var. *cantaloupensis*) (*21*, *23*). Using Tenax trapping in Charentais melons, Bauchot et al. did not report finding many (quantifiable) C_6 and C_9 alcohol and aldehyde compounds, formerly reported to be flavor significant in muskmelons (*25*). Nonyl acetate, (*Z,Z*)-3,6-nonadienyl acetate, nonanol, (*Z*)-6-nonenol, and (*Z,Z*)-3,6-nonadienol were recovered in Charentais with Freon extraction (*23*).

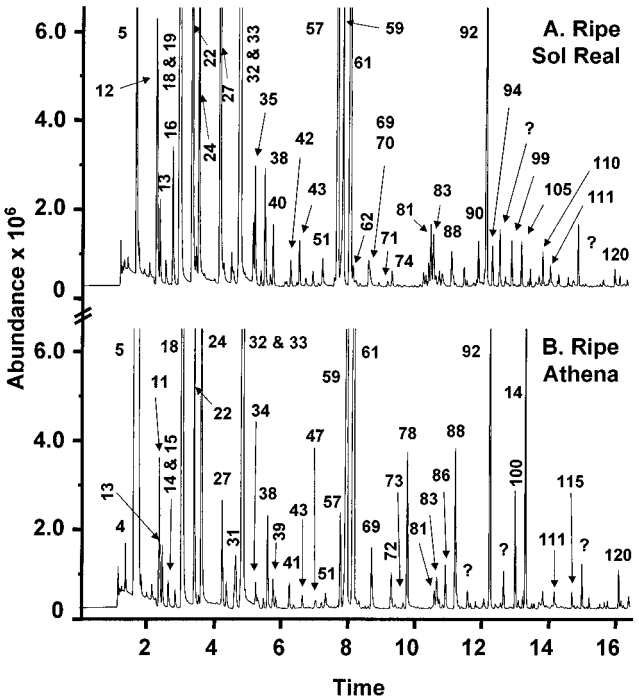

Figure 3. Enlarged total ion spectra from full-slip cv. Sol Real (A) and 3/4-slip cv. Athena (B) cantaloupe fruit. Peak numbering corresponds with Table 1. CF indicates peaks due to column or fiber impurities.

Aroma extract dilution analysis was used to determine that the primary aroma compounds of muskmelon

(*C. melo*) were ethyl 2-methylpropanoate, methyl 2-methylbutanoate, (*Z*)-3-hexenal, (*E*)-2-hexenal, 1,8-cineole, and (*Z*)-1,5-octadien-3-one (*20*). In our analyses, methyl 2-methylbutanoate, ethyl 2-methylpropanoate, and ethyl 2-methylbutanoate increased markedly with fruit maturity (Table 3), and other esters such as ethyl butanoate and hexyl acetate reported in Tables 2 and 3 had similar trends. Although 3-methyl-1-butyl acetate is reported to be flavor related in muskmelon, our recovery was rather low, and the 2-methyl-1-butyl acetate stereoisomer was the predominant coeluting peak recovered (Figures 1 and 2). Also, (*E*)-2-hexenal varied insignificantly and eucalyptol recovery was highest in $^1/_4$ slip and over-ripe fruit (data not shown). Variable compound recovery can be expected because significant genetic and biochemical differences exist between different varieties of cantaloupe, honeydew, and Charentais melons. Other compounds, possibly including alkenyl acetates, may be significant with regard to cantaloupe flavor, and varietal/genetic effects are highly important.

Certain C_9 compounds, such as (*Z*)-6-nonenyl acetate, (*Z*)-6-nonenal, (*Z*)-6-nonenol, (*Z*,*Z*)-3,6-nonadienal, and (*Z*,*Z*)-3,6-nonadienol, are flavor related in honeydew (*22*). Utilizing our method with honeydews, we have recovered nonanal, (*E*)-2-nonenal, (*E*)-2-nonen-1-ol, (*Z*)-6-nonen-1-ol, (*Z*)-6-nonanal, (*E*,*Z*)-2,6-decadienal, and benzyl acetate (data not shown). We did not recover nonyl acetate or (*Z*,*Z*)-3,6-nonadienal in cantaloupe, but many aldehyde stereoisomers, such as (*E*,*E*)-2,4-heptadienal, (*Z*)-3,7-dimethyl-2,6-octadienal, (*E*)-3,7-dimethyl-2,6-octadienal, (*E*,*E*)-2,4-nonadienal, (*E*,*Z*)-2,4-nonadienal, (*E*,*Z*)-2,6-decadienal, and (*E*,*E*)-2,4-decadienal, were recovered. *Z*,*Z* isomers are highly unstable, and our method may have resulted in recovery of both *E*,*Z* and *E*,*E* dienals via isomerization. We also recovered other C_9 aliphatic compounds in cantaloupe, including nonanal, (*Z*)-6-nonenal, (*E*)-2-nonenal, 1-nonanol, and (*Z*)-6-nonen-1-ol. Our technique also routinely recovered other flavor-related esters such as ethyl hexanoate and (*Z*)-hexenyl acetate and aromatic esters such as benzyl acetate (Tables 2 and 3).

Change in relative abundance for compounds reported to impart characteristic flavor to muskmelon, as well as some compounds we believe could be flavor related, are presented in Tables 2 and 3. Recovery of all esters believed to be flavor important increased progressively after pollination (Table 2). By 35 DAP many esters had pronounced levels that increased until harvest, and the concentration of most esters progressively increased with increasing harvest maturity (Table 3). In contrast, most aldehydes had initially high levels or increased markedly during early growth stages (Table 2) and then decreased with increasing harvest maturity (Table 3). Nonanal, (*E*)-2-nonenal, and (*E*)-2-octenal concentrations were highest through growth and development and then tapered off significantly by harvest. However, hexanal and (*Z*)-6-nonenal remained relatively high for all maturity harvest stages. (*E*,*Z*)-2,6-Nonadienal was highest in immature samples ($^1/_4$ slip), and the level generally decreased with increasing harvest maturity. Esters, namely, ethyl 2-methylbutanoate, (*Z*)-3-hexenyl acetate, hexyl acetate, and benzyl acetate, decreased appreciably in fruit harvested over-ripe.

The probable long-chain C_{18} fatty acid precursors, octadecanoic, octadecadienoic, and octadecatrienoic acid, that give rise to C_9 aldehyde, alcohol, and ester intermediates (*16*) were generally only recovered by our SPME method in immature cantaloupe (13, 20, or 29 DAP). Precursors of $C_{12}-C_{16}$ long-chain and branched volatiles (aldehydes, ketones, aromatics, and fatty acids) were recovered by 28 or 29 DAP. Free amino acids (e.g., alanine, isoleucine, leucine, methionine, and valine), which are known to be precursors of many volatile compounds, increase significantly during *C. melo* ripening, providing the fruit was not harvested immature (*25*). Recovery of 2-methylbutyl, 2-methylpropyl, and thioether esters in mature fruit indicates an abundance of free isoleucine, valine, and methionine, respectively.

Fatty acids are oxidized in the presence of lipoxygenase (LOX), and the intermediary substrates are converted into various organoleptic compounds via hydroperoxide lyase (HPL). HPL has been found in many fruits (*26*−*31*) but not for cantaloupe. In bell peppers, both HPL and LOX activities decreased with maturation, and the amounts of C_6 aldehydes and alcohols formed from homogenization of mature fruit also decreased, suggesting that the limiting factor contributing to changes in the composition of volatile compounds were these activities (*27*).

During cantaloupe maturation, fatty acids declined and LOX activity was highest in hypodermal tissue, but there was little to no LOX activity present in mesocarp tissue of mature fruit; the highest relative level of antioxidant activity was found in immature fruit (*32*). Lipid degradation via LOX activity was greatest in the plasma membranes of mature and postharvest cantaloupe and honeydew fruit (*32*−*34*). However, both lipid peroxidation and LOX activity were highest in stored non-netted ripe muskmelon fruit, considered to be a short storage life variety, but remained very low in a long storage life variety. Loss of membrane integrity and softening were not observed in the long shelf life variety, and this correlated well with high levels of the antioxidant enzymes superoxide dismutase and catalase (*33*).

Depending upon variety, one might expect the formation of oxidized compounds in mature cantaloupe extracts and minimal amounts in immature extracts. However, we recovered numerous aldehydes in immature fruit, and the relative recovery for a select, small group of aldehydes increased with harvest maturation (Table 3). In immature cantaloupe mesocarp tissue, LOX is absent or present in minute quantities but LOX substrates are present (*32*), and the free amino acid concentration is low (*25*). However, the absence of LOX inhibition, measured as percent inhibition of LOX activity, in the mesocarp (*32*) and the probable fact that the rate-limiting step is the amount of LOX present may lead to a large amount of aldehyde production in immature mesocarp tissue. Alternatively, because immature fruit have little or no free amino acids, the only volatile compounds recoverable are lipid oxidation products, which will appear high compared to mature extracts that have numerous compounds.

Many off-flavor compounds are aldehydes, and sensory evaluation detected green-grassy notes, reminiscent of cucumbers, in immature fruit but not in any mature fully ripe samples. Because numerous aldehydes remained relatively high in most immature and mature samples, their presence could be either endogenous or a result of maceration. Nonetheless, some of the unique aldehydes we reported were found only in immature fruit, and few of the same aldehydes were recovered in mature samples. We have not determined if recovered

aldehydes are endogenous or created during tissue homogenization, and their relevance to maturity-dependent cantaloupe flavor quality remains unknown.

Our simple, rapid extraction and automated SPME headspace method recovered >80 compounds that were previously reported for muskmelons and 54 additional compounds in cantaloupe. Many of these compounds were authenticated, and we are continuing efforts to discover and validate additional compounds. The SPME method can be used as a tool for rapidly assessing flavor compounds in different cantaloupe varieties as well as determining relative maturity levels for fresh-cut cantaloupe products. The results from immature cantaloupe fruit compared to mature fruit indicate that particular aldehydes and esters could be used as flavor quality markers. The method can be used to clearly differentiate muskmelon maturity from the marked shift from toward characteristic flavor-related aldehydes, esters, and acetates. Our ultimate goal is to correlate fresh-cut fruit flavor compounds with trained sensory panelists who smell cut cubes (~20 °C), then macerate the samples in their mouths (~35 °C) as they analyze flavor and texture, and to identify the critical flavor compounds in cantaloupe and fresh-cut cantaloupe products and assess their impact on sensory flavor. Determination of these compounds, flavor quality, and relative abundance of substrates and enzymes may help breeders deliver varieties better suited for the fresh-cut industry.

ABBREVIATIONS USED

DAP, days after pollination; HPL, hydroperoxide lyase; ID, identification; IS, internal standard; LOX, lipoxygenase; PDMS, poly(dimethylsiloxane); SPME solid phase microextraction; RI, retention index; RT, retention time.

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