Postharvest Biology and Technology 71 (2012) 41–50

Contents lists available at SciVerse ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Influence of maturity and ripening on aroma volatiles and flavor in 'Hass' avocado

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ARTICLE INFO

Article history:
Received 30 September 2011
Accepted 26 March 2012

Keywords:
Dry weight
Minimum maturity
Sensory
Grassy
Carbohydrates

ABSTRACT

Changes in aroma volatiles were determined using solid phase microextraction (SPME) and gas chromatography/mass spectrometry in ripe avocados (*Persea americana* Mill., cv. Hass) throughout an eight-month maturation period and related to the sensory properties of the fruit. As maturation progressed sensory panelists found the likeability of the fruit to increase, coinciding with the fruit becoming creamier and less watery in texture, and the flavor richer and less grassy. During this maturation time the concentration of hexanal, (E)-2-hexenal and 2,4-hexadienal, three of the most abundant volatiles, greatly declined in amount. These volatiles all have a grass-like aroma, and it is likely that the loss in amount was responsible for the decline in grassy flavor during maturation. Acetaldehyde, methyl acetate, pentanal, and β-myrcene were at higher concentrations in mature than non-mature fruit and may also have contributed to the overall flavor. Avocados of an intermediate maturity were ripened at 20 °C and fruit of different ripeness levels (firmnesses) measured for ethylene production, rate of respiration and aroma volatile content. A sharp increase in the rates of respiration and ethylene production marked a rapid increase in softening and the beginning of the climacteric. Twenty-five volatiles were identified in the ripening avocados of which three (pentanol, hexanol, and 2-nonenal) were not detectable in fully-ripe fruit. Of particular interest was an 85% decline in the amount of hexanal in a comparison of firm to fully ripe (4 N firmness) fruit. Aroma volatiles have in the past been little-studied in avocados but appear to have a role in determining the flavor of the fruit.

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1. Introduction

Avocados (*Persea americana*, Mill.) picked prematurely at an immature stage can have an eating quality that has been associated with a green or grassy aftertaste, lack of flavor and a rubbery or watery texture (Harding, 1954). As an early response to this problem, California growers in 1925 established a state maturity standard that required a minimum oil content of 8% before avocados could be harvested in California (Anon., 1925). This was based on the fact that oil accumulates in avocados in large amounts as they mature and was thought to provide a reasonable estimate of maturity (Church and Chance, 1920–1921). Although oil content was shown to relate to flavor and eating quality (Hodgkin, 1939), fruit-to-fruit variability in oil content and varietal differences in accumulation rate (Hatton et al., 1957a; Stahl, 1933) make percent oil an imperfect threshold for maturity. An extensive study in California on the relationship of maturity, eating quality and dry weight accumulation (Lee et al., 1983) led to the adoption of dry matter in conjunction with release dates as the basis for minimum maturity in 1983, replacing oil content. Dry matter and oil accumulation are closely related, but measurement of dry matter is far simpler to perform and is now the standard maturity measurement used throughout most of the world. Nonetheless, it is recognized in the avocado industry that neither dry matter nor percent oil are adequate in themselves to fully explain the differences in the eating quality of avocados and that additional means of assessing eating quality would be desirable.

Volatiles have a well-established role in helping to determine flavor in a wide variety of fruits, but have not been studied extensively in avocado. This may have been largely due to the strong emphasis that other flavor influencing factors in avocado, such as oil content and texture, have received. Also, the high lipid content may make some types of flavor volatile analysis more problematic. In an early study, Yamaguchi et al. (1983) utilized vacuum distillation and solvent extraction to identify 96 compounds, the major constituents being n-hexanol, cis-3-hexanol and trans-2-hexenol which made up over 80% of the extract on a peak area basis. Similar extraction techniques were later used to identify a number of compounds in avocado extracts, with terpenes and aldehydes being some of the most predominant compounds present (Pino et al., 2000, 2004; Sinyinda and Gramshaw, 1998). Extracts that were stored rather than being processed immediately were found to have a greater number of compounds, some being products of lipid

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0925-5214/$ – see front matter. Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.postharvbio.2012.03.006

breakdown such as hexanal and heptanal (Sinyinda and Gramshaw, 1998). The authors of this study also noted that the extracts, regardless of storage, did not have a characteristic aroma of avocado due to the overabundance of terpenoids, and stated that the extraction technique seemed to be unsuitable for the study of avocado aroma. López et al. (2004) and Guzmán-Gerónimo et al. (2008) used solid phase microextraction (SPME) to identify a range of compounds in avocado headspace, but the results may not be very pertinent to understanding the volatile profile of fresh avocados due the very long (24 h) extraction time used. Whitfield et al. (1980) compared 'Sharwil' avocados stored at 5 °C to fresh avocados and identified differences in carbonyls, alcohols and sesquiterpenes that were thought to contribute to the poor flavor characteristic of stored avocados. El-Mageed (2007) noted that ethanol, (Z)-3-hexanol and (E)-2-hexenal, the most abundant volatiles in whole green and ripe 'Fuerte' avocados, declined with ripening, while overripe fruit had the highest ester concentration. In more recent work, Pereira (2010) reported that the most abundant volatiles present in the headspace of unripe, diced 'Simmonds' avocado were sesquiterpenes and hexanal, whereas the amounts of these compounds was greatly reduced by ripening.

In previous studies of avocado maturation there have been overall flavor ratings given to the fruit as it matures prior to harvest, which have shown that the flavor improves as maturation progresses (e.g. Harding, 1954; Lee et al., 1983) but have provided little to no information on what factors are driving the flavor changes during this time. In this research we have attempted to more fully understand the influences of maturation on 'Hass' avocado flavor by detailing changes in individual sensory attributes during maturation and ripening and then relating these changes to alterations in the concentration of aroma volatiles in the fruit.

2. Materials and methods

2.1. Fruit

An initial test (Study 1) was conducted from April 2009 until September 2010 that was used to provide guidance on determining which sensory attributes were most important to determining optimum flavor in avocados. Avocados (cv. Hass) were obtained from two commercial packinghouses in California that market California as well as imported fruit from Mexico, Chile and Peru. Typically, fruit from Mexico are washed with chlorine in the wash water while fruit from Chile and California are brushed but not washed during the packing process. Fruit were obtained within the normal timespan for marketing fruit from each country of origin. Pre-ripened fruit were procured twelve different times throughout the year (Table 1). Two to three grower lots from each country were used on each date. Dry matter was determined for the lot based upon sampling from five individual fruit; this was done by the packinghouses and provided when the fruit was procured. The fruit were pre-ripened by the packinghouse at ~20 °C and 90% RH with ~100 μL L^{-1} ethylene until fruit were 4.4–6.7 N or less in firmness as determined by a penetrometer with a 8-mm head (Imada, Northbrook, IL, USA). After obtaining the fruit from the packinghouse, the fruit was transported at ambient conditions to the University of California Kearney Agricultural Center in Parlier, CA in approximately 3–5 h depending on the packinghouse location. Depending on the test, fruit were held 1–5 d prior to sensory evaluation. If further ripening was required (average flesh firmness > 6.7 N) they were held at 20 °C and 96% RH to finish ripening. If sufficiently ripe the fruit were placed immediately at 5 °C until evaluation.

In the second study (Study 2) 'Hass' avocados were harvested approximately every 5 weeks beginning in January and ending in August 2010 (Table 2), from a commercial grove near Moorpark, CA to enable an evaluation of maturity effects on aroma volatiles and sensory quality. Fruit (120, size 48) were taken from the same 10 trees every harvest. The fruit were then transported (unwashed) to the University of California Kearney Agricultural Center in Parlier, CA under ambient conditions (~4 h). The fruit were held overnight at 5 °C and 90% RH. The following morning the fruit were inspected and any fruit with defects were discarded. One hundred and twenty fruit were selected and all of the fruit were sampled for dry matter content by using a cork borer to remove two tissue plugs from the equatorial region on opposite sides of the fruit and then drying the tissue plugs in a food dehydrator. Holes in the fruit remaining from the sampling were filled with sterile plastic plugs. We have previously evaluated the impact of this approach on 'Hass' avocado ripening behavior and have found that ripening is not affected (Arpaia, unpublished data). Following this the fruit were placed into cold storage at 5 °C for 1 week, followed by ripening at 20 °C and 95% RH in the presence of 50–60 μL L^{-1} ethylene until the fruit were at optimum eating firmness (4.4–6.7 N). The combination of cold storage and ethylene treatment acted to help synchronize the ripening stages of the individual fruit. Avocados are very resistant to typical wound pathogens that cause other types of punctured fruit to decay and we have found that avocados can generally ripen normally without any decay after dry matter sampling by coring. Previous experimentation had determined that there was no effect of the dry matter sampling procedure on either aroma volatiles or on the sensory quality of the fruit (data not shown). Fruit were divided into three different groups based upon the dry weight and fruit were equally taken from each of the groups for both the sensory and volatile evaluation to help ensure an even sampling of the

Table 1
Tasting dates and country of origin for fruit samples used to establish the sensory characteristics most closely linked to likeability in avocados in Study 1.

Tasting date	Country of origin			
	USA[a]	Mexico[b]	Chile[c]	Peru[d]
Apr. 7, 2009	X	X		
May 15	X	X		
June 17	X	X		
July 17	X	X		
Jul. 29	X	X	X	
Aug. 25	X	X	X	
Sept. 29	X	X	X	
Nov. 17		X	X	
Jan. 19, 2010	X	X	X	
Apr. 12	X	X	X	
July 20	X	X		
Sept. 1	X	X	X	X

[a] USA fruit from various growing regions in the state of California.
[b] Mexico fruit from various productions areas in the state of Michoacan.
[c] Chile fruit from various production areas in Region V.
[d] Peru fruit from the state of Libertad.

Table 2
Dates of harvest and corresponding average dry weights for each harvest in Study 2. Current minimum maturity standard for California 'Hass' avocado is 20.6% dry weight.

Harvest number	Date	Dry weight (%)[a]
1	Jan. 14	18.88 (±0.16) g
2	Feb. 8	19.71 (±0.15) f
3	Mar. 23	22.60 (±0.24) e
4	Apr. 19	24.01 (±0.25) d
5	May 25	25.22 (±0.21) c
6	July 16	28.39 (±0.23) b
7	Aug. 10	30.26 (±0.28) a
8	Aug. 31	28.96 (±0.19) b

[a] Numbers in parentheses are standard errors. Mean separation between harvests significant at $P < 0.05$ using LSD.

available dry weights for a given sampling time. Fruit analyzed for volatile quantification were separate from those used for sensory evaluation.

Fruit for the ripening portion of the experimentation (Study 3) consisted of additional avocados that had been picked during the April harvest (harvest date 4) of the maturity test. After harvest the unwashed fruit were placed into cold storage at 5 °C (95% RH) for a week to lessen the variability in stage of ripening among the fruit lot. Following cold storage the fruit were moved to 20 °C (95% RH) and allowed to ripen without the aid of exogenous ethylene. Avocados used for volatile quantification were separate from those used for sensory evaluation.

2.2. Sensory evaluation

Panelists were employees of the Kearney Agricultural Center in Parlier, CA and could be considered as semi-expert due to their prior experience with taste panel evaluation of avocados. It is recognized that our panel was not a consumer panel due its small size and experience and that care must be taken not to interpret the data as being completely representative of consumers. Generally 15–20 panelists participated in each evaluation test, the testing being conducted over a 2–3 d period. Females composed the largest proportion of the panel (55%) and the ages of both sexes ranged from 21 to over 60. Just prior to tasting fruit preparation was initiated by cutting each avocado vertically from stem to the stylar end, separating the halves and removing the seed. The flesh above and below the seed cavity for each half was then cut away, the remaining portions peeled and then the flesh cut into small square pieces approximately 15 mm in size. Areas of the fruit that had been disrupted by prior dry weight sampling (if present) were avoided. Individual pieces were placed into 30-mL soufflé cups that were identified using three-digit random numbers. The tasting was conducted in individual booths with small doorways through which trays containing samples were passed. Samples were presented with random 3-digit codes to avoid order and carryover effects.

For the initial work on determining attributes of most importance to avocado likeability (Study 1) 12 fruit (4 for each grower lot) were tasted for each country of origin on each of the evaluation dates, with an average of seven panelists tasting each fruit. Instructions were given to take a drink of water before tasting and to utilize a small bite of carrot followed by a drink of water to cleanse the pallet between samples. It had been previously determined that carrots were preferred for palette cleansing for avocado. Panelists gave each sample a hedonic rating ranging from 1 to 9, with 1 being dislike extremely and 9 being like extremely. The panelists then examined a list of attributes and placed a check mark next to each one that they believed best described that particular avocado sample. Textural descriptors included: mushy, firm, stringy, gritty, creamy, smooth, dry, watery and oily. Flavor descriptors included: bland, grassy, woody-pine, sweet, nutty, buttery, savory, oily, rancid, canned pea, with sharp, astringent, metallic and bitter referring to aftertaste. The choice of these attributes for evaluation was based on prior work in New Zealand indicating that they were of potential importance in determining flavor in 'Hass' avocados grown under the conditions there (Yahia and Woolf, 2011). A reference card with descriptions of each attribute was provided to assist the panelists during the sensory evaluations.

During sensory evaluation for the maturity test (Study 2) between 50 and 60 fruit were tasted for each maturity stage with an average of five different panelists (15–20 panelists total) tasting each fruit. In an attempt to ensure an equal distribution of the range of dry weights available at that harvest date, an equal number of upper, middle and lower dry weight fruit for that harvest date were tasted. Cleansing of the palette between samples was as previously mentioned. Panelists gave each sample a hedonic rating ranging from 1 to 9, with 1 being dislike extremely and 9 being like extremely. The prior work in this study on avocado sensory attributes (Study 1) was used to choose those attributes that were evaluated in this portion of the project. Ratings for richness, creaminess, and grassiness were given by placing marks on 150-mm line scales that corresponded to the amount of the attribute present.

2.3. Volatile analysis

Avocado mesocarp samples for the testing of maturity effects (Study 2) were prepared immediately prior to measurement using fresh fruit. Fruit were sectioned in the same manner as described for sensory analysis, using only tissue from the equatorial region and avoiding areas near to those that had been sampled prior to storage for dry weight determination. Tissue (20 g) was diced and homogenized with 40 mL saturated NaCL at high speed for 1 min in a home blender. The addition of NaCl was to limit the formation of volatiles after homogenization to try to better approximate the volatile composition present at the time of eating. The resulting purée was poured into 12 mm × 32 mm glass vials to a volume of 10 mL. Linalool was then added as an internal standard and the vial capped with a Teflon-coated septum. Due to the inability to measure large numbers of samples within a short time, samples from the ripening part of the study were frozen at −20 °C and stored until the time of analysis. Storage time was from 6 to 8 weeks. These samples were thawed for 45 min at 5 °C and then warmed to 40 °C in a water bath for 15 min prior to headspace analysis. Volatile content of the purée headspace was measured by the use of solid phase microextraction (SPME) using a 75-μm carboxen/polydimethylsiloxane phase (Supelco, St. Louis, MO). Temperature was maintained at 40 °C during the 30 min volatile extraction by immersing the vial into a water bath with the level of the water being just over that of the purée in the vial. At the completion of the extraction the SPME device was removed from the vial and desorbed for 2 min into the 300 °C inlet of an Agilent (Agilent, Palo Alto, CA) 6890 gas chromatograph equipped with a DB-5 column (30 m × 0.32 mm ID, 1 μm thickness; J&W Scientific, Folsom, CA) and an FID detector. Helium carrier gas flow was 0.03 mL s^{-1} and hydrogen, air and nitrogen make-up flows were 0.7, 7.5 and 0.8 mL s^{-1}, respectively. Oven temperature was 32 °C for 3 min and ramping up to 200 °C at 0.1 °C s^{-1}. Peaks were identified by the use of retention times of standards, retention indices and mass spectrometry. The system used for mass spectrometric determination was an Agilent 7890 GC coupled to an Agilent 5975 mass selective detector. The column was an Agilent HP-5 ms ultra-inert (30 m × 0.250 mm I.D., 0.25 μm film thickness). Helium flow was 0.02 mL s^{-1} and the temperature programming of the oven and conditions used for the SPME analysis were as previously described for the FID work. Spectra of unknown compounds were compared to spectra from the Wiley/NBS library to aid in identification. Quantification was accomplished by the use of standard curves for each of the compounds of interest and adjusting for the response of the internal standard (linalool). For the maturity testing (Study 2) 15 fruit were individually measured for each maturity stage. In the ripening portion of the test (Study 3)10 fruit were individually measured for each firmness group.

2.4. Respiration and ethylene during the fruit ripening

In the initial portion of the ripening study (Study 3) 10 fruit were randomly selected each day for measurement and sampling, whereas when the fruit began to rapidly soften, greater numbers of fruit were selected daily in an attempt to build a sample database consisting of a wide range of fruit firmnesses. All of the measurements and samplings were conducted over a 3-week period. On each measurement day respiration and ethylene production were both determined in a flow-through system using fruit placed separately into 825 mL plastic containers with the flow rate through all of the containers being 3.8 mL s^{-1}. After a 15-min equilibration period the outflow from the containers was sampled using a syringe and then injected into a Carle Series 400 gas chromatograph (E.G. & G. Chandler, Broken Arrow, OK, USA) equipped with a 2-mL sample loop, a 3.18 mm × 1.22 m column with 8% NaCl on alumina F-1 (80/100 mesh) and a FID detector. The column was maintained at 70 °C throughout the run. Helium flow through the column was 0.5 mL s^{-1}. A gas sample was taken also taken at the same time to measure respiration and was injected into a Carle Series 400 gas chromatograph with a 1 mL sample loop, a 3.18 mm × 1.52 m HayeSepA column (60/80 mesh) and a TCD detector. The oven temperature was maintained at 70 °C throughout the run. Helium flow through the column was 0.5 mL s^{-1}. Quantification of ethylene and carbon dioxide were done by comparing sample and standard peak heights for each component. Following the gas chromatography measurements the fruit were measured for peel color using a colorimeter (Minolta, Ramsey, NJ) set to collect data expressed in $L^*C^*h^*$ color coordinates. Firmness was determined on each fruit by using an Imada or an Ametek (Landsdale, PA, USA) penetrometer with a 8-mm tip.

2.5. Analysis of carbohydrates

The extraction and assay of soluble carbohydrates was performed as previously detailed (Liu et al., 1999). Ten fruit were individually measured for each of the firmness levels. A 2–3 g mesocarp sample was taken from the equatorial region of the fruit avoiding any areas where penetrometer measurements had been taken. The carbohydrates were analyzed using a Beckman 110B pump (Brea, California), a Rezex RCM-monosaccharide column (Phenomenex, Torrance, CA) and an Altex 156 refractive index detector (Fullerton, CA).

2.6. Statistics

For the portion of this experimentation to determine which sensory attributes were most important to avocado flavor (Study 1) the proportion of panelists noting the occurrence of a given attribute for each hedonic score category was determined using the pooled data across countries and grower lots. PROC Logistic (SAS Institute, Cary, NC, USA) was then used to determine the significance of the slopes that were generated and to estimate 95% confidence intervals. Total panelist responses ranged from 183 for hedonic category 3 to 1503 for hedonic category 6.

Significance among harvest dates for each of the sensory attributes and aroma volatiles was determined by analysis of variance (SPSS, Chicago, IL, USA) using the Bonferroni test to perform the multiple mean comparisons. The sensory data were means from 50 to 60 fruit with each fruit being tasted by five or more panelists. Aroma volatile data was separately collected from 15 individual fruit. A principal component analysis (PCA) based upon Pearson correlations was performed on the aroma volatiles using XLStat (New York, NY, USA). Kendall's tau-b correlation coefficients were determined among the sensory attributes and aroma volatiles using SPSS. Statistical significance among firmness categories in the ripening portion of the experiment (Study 3) for respiration, ethylene, carbohydrates, lipids and aroma volatiles utilized SPSS and the Bonferroni test for multiple mean comparisons. Ten fruit were individually measured for each of the firmness category means.

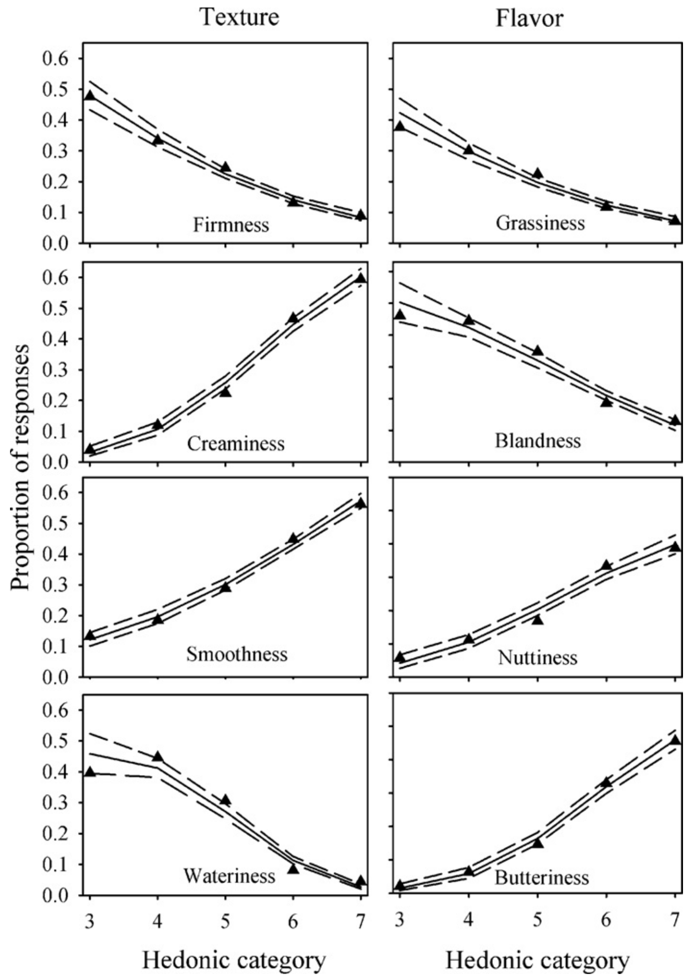

Fig. 1. The proportion of times that panelists noted the presence of specific textural and flavor attributes of 'Hass' avocado as associated with hedonic category ranking in Study 1. Means values for each category for all countries and evaluation dates are indicated by triangles while solid lines represent predicted values and dotted lines the confidence intervals around the predicted values ($P < 0.05$).

3. Results

3.1. Determination of attributes important to avocado likeability (Study 1)

An analysis was conducted across all countries of origin and all evaluation dates to identify the sensory attributes that were most closely associated with likeability. This was initially done by calculating the proportion of times that the panelists found each of the 25 sensory attributes that were listed on the score sheets to be present for each hedonic class. Seventeen of the attributes were found to be present less that 20% of the time for any of the hedonic classes and so were considered of lesser importance and not dealt with further (data not shown). Limiting the number of attributes being evaluated was also important to prevent fatigue of the panelists and maintain sensory data quality. Four textural attributes (firm, creamy, smooth, watery) and four flavor attributes (grassy, bland, nutty, buttery) were commonly identified to be important by sensory panelists and were subjected to further analysis. Logit curves of the proportion of panelists that responded versus the hedonic score given that fruit were generated (Fig. 1). Firm, watery, grassy, and bland characteristics were attributes found to be present to a larger extent in fruit with low hedonic scores, while creamy, smooth, nutty and buttery were attributes more associated with high hedonic scores. Based upon these results, creamy (watery to

Table 3
'Hass' avocado volatile concentrations from homogenized mesocarp tissue as influenced by harvest date in Study 2. Volatiles are grouped depending on whether concentrations were decreasing, increasing or had no clear pattern of change (mixed) in relation to advancing harvest date. Only volatiles that were significantly different among the harvest dates are shown.

Compound	Descriptors[a]	AT[b] ($\mu g L^{-1}$)	Concentration ($\mu g L^{-1}$) harvest date 1	2	3	4	5	6	7	8
Decreasing										
1-Penten-3-one	Earthy, green	1.25[e]	10.0a	5.7b	4.4bc	3.3cd	2.7de	2.9cd	1.1e	2.7de
Hexanal	Fresh, green	4.5–5[c]	2293.9a	783.8bc	844.9b	589.0cd	402.0d	268.0d	200.5d	179.2d
(E)-2-Hexenal	Green, fruity	17[c]	10,342.1a	5453.5bc	6028.2b	5410.8cd	4371.0de	3892.4e	3122.9e	1089.7e
2,4-Hexadienal	Green	60[f]	219.8a	95.5bc	104.1b	74.9bcd	66.5c	59.4d	49.3d	75.0bcd
Benzaldehyde	Cherry	350[g]	30.7a	10.9b	10.8b	6.5bcd	5.6bcd	3.0cd	0.5d	8.6bc
Increasing										
Acetaldehyde	Pungent, solvent	15[g]	55.0d	953.7c	1132.2c	1234.2c	1273.1c	2332.3b	4680.5a	2933.0b
Methyl acetate	Ether, sweet		15.9c	17.5c	20.3c	20.4c	14.5c	63.0b	101.0b	230.5a
Pentanal	Fruity, nutty	12[g]	ND	7.8c	5.7c	6.5c	9.8c	30.6b	15.2bc	68.6a
β-Myrcene	Musty, wet soil	13–15[c]	0.0c	74.4b	99.8b	97.1b	107.8b	101.1b	73.9b	172.8a
2,4-Heptadienal	Fatty, green	360[h]	2.0c	3.0c	3.8bc	3.3c	3.5c	3.9c	7.0b	11.2a
Nonanal	Fatty, citrus	1[g]	252.0c	265.7c	350.4bc	453.9bc	449.8bc	1106.3b	3202.7a	1050.0b
Mixed response										
Limonene	Citrus	10–200[d]	7.5cd	7.0cd	9.8b	7.9bcd	8.4bc	5.8d	0.5e	13.1a

Each concentration value is the mean of 15 fruit that were individually measured. Volatile concentration values followed by a different letter are statistically significant at $P \leq 0.05$ using the Bonferroni correction to adjust for the number of comparisons made. ND = not determined.
[a] Flavor descriptors from the University of Florida Citrus Flavor and Color Database (Rouseff, 2010), Cornell University Flavornet (http://www.flavornet.org/flavornet.html), and the Good Scents Company (http://www.thegoodscentscompany.com/index.html).
[b] Aroma threshold.
[c] Rouseff (2010), in water.
[d] Buettner and Schieberle (2001), in water.
[e] Buttery et al. (1971), in water.
[f] Teranishi et al. (1974), in water.
[g] Buttery et al. (1988), in water.
[h] Morales et al. (2005), in oil.

creamy), rich (bland to rich) and grassy (grassy to not grassy) were the attributes chosen in addition to likeability (hedonic score) for evaluation in all later sensory testing in Study 2.

3.2. Maturity influence on sensory characteristics and aroma volatiles (Study 2)

Sensory panelists reported that the likeability of the fruit (hedonic rating) declined from harvest 1 to harvest 2, the decline being associated with a lesser degree of creaminess and an increased amount of grassiness (Fig. 2). Fruit harvested from the first 2 harvest dates, although legal to pick due to the release date provisions of the California Agricultural Code, were well below the 20.6% minimum dry weight standard for 'Hass' (Table 2). From harvest 2 to harvest 3 there was a large increase in likeability ($P \leq 0.001$) as fruit from harvest 3 were judged to be richer in flavor, creamier, and with a flavor that was less grassy than those from harvest 2. Dry weight had increased between the two harvests from below 20% dry weight to above 22% dry weight. In subsequent harvests the likeability of the fruit slowly increased until harvest 6 after which point likeability did not significantly change ($P > 0.05$). Fruit from the final 3 harvests had an average hedonic score of 7 indicating that the fruit were "liked moderately". Paralleling the increases in likeability were changes in the sensory ratings that indicated that the fruit were becoming creamier, richer and less grassy in flavor.

Twenty-five aroma volatiles, including aldehydes, alcohols, esters, ketones and terpenes were identified in the avocado fruit, although only 12 of these aroma volatiles significantly changed in concentration during maturation (Table 3). Of these volatiles, five decreased and six increased in amount as the season progressed, while the pattern for limonene was variable. Five of the eleven volatiles had green or grassy as part of the odor descriptor. The sum of volatiles with a grassy aroma declined markedly during maturation (Fig. 2). PCA analysis indicated that 1-penten-3-one, hexanal, 2-hexenal, 2,4-hexadienal and benzaldehyde were most closely associated with the first three maturity dates and acetaldehyde, nonanal, methyl acetate, β-myrcene and 2,4-heptadienal with the later harvest dates (Fig. 3). Pentanal was not included in the analysis due to missing data for the first harvest date.

Relationships between hedonic score, sensory attributes, dry weight and aroma volatiles were further explored by correlation analysis (Table 4). As was indicated in the graphs in Fig. 2, likeability (hedonic score) increased concurrently with increases in creaminess, richness and a loss of grassiness and, accordingly, all of these components were significantly correlated to each other. Hedonic score was significantly correlated in a positive manner with acetaldehyde, methyl acetate, 2,4-heptadienal and nonanal

Table 4
Kendall's tau-b correlation coefficients among sensory attributes and volatile concentrations of 'Hass' avocados harvested at different dates over a period of 8 months in Study 2.

	Hedonic[a]	Creaminess[b]	Richness[b]	Grassiness[b]
Creaminess	0.714[*,d]			
Richness	0.857[**]	0.857[**]		
Grassiness	−0.786[**]	−0.929[**]	−0.929[**]	
1-Penten-3-one	−0.571[*]	−0.571[*]	−0.571[*]	0.643[*]
Hexanal[c]	−0.714[*]	−0.714[*]	−0.714[*]	0.786[**]
(E)-2-Hexenal[c]	−0.714[*]	−0.714[*]	−0.714[*]	0.786[**]
2,4-Hexadienal[c]	−0.429[ns]	−0.571[*]	−0.429[ns]	0.500[ns]
Benzaldehyde	−0.500[ns]	−0.643[*]	−0.500[ns]	0.571[*]
Acetaldehyde	0.714[*]	0.714[*]	0.714[*]	−0.786[**]
Methyl acetate	0.714[*]	0.429[ns]	0.571[*]	0.571[*]
Pentanal	0.524[ns]	0.714[*]	0.714[*]	−0.810[*]
β-Myrcene	0.214[ns]	0.357[ns]	0.357[ns]	−0.429[ns]
2,4-Heptadienal[c]	0.714[*]	0.571[*]	0.571[*]	−0.643[*]
Nonanal	0.714[*]	0.714[*]	0.714[*]	−0.643[*]
Limonene	0.071[ns]	0.071[ns]	0.071[ns]	−0.143[ns]

[a] Hedonic score indicating degree of liking.
[b] Ratings that used a 150-mm scale where a higher number indicated more creaminess and richness but less grassiness.
[c] Volatiles with a grassy odor.
[d] ns = not significant.
[*] Significant at $P \leq 0.05$.
[**] Significant at $P \leq 0.01$.

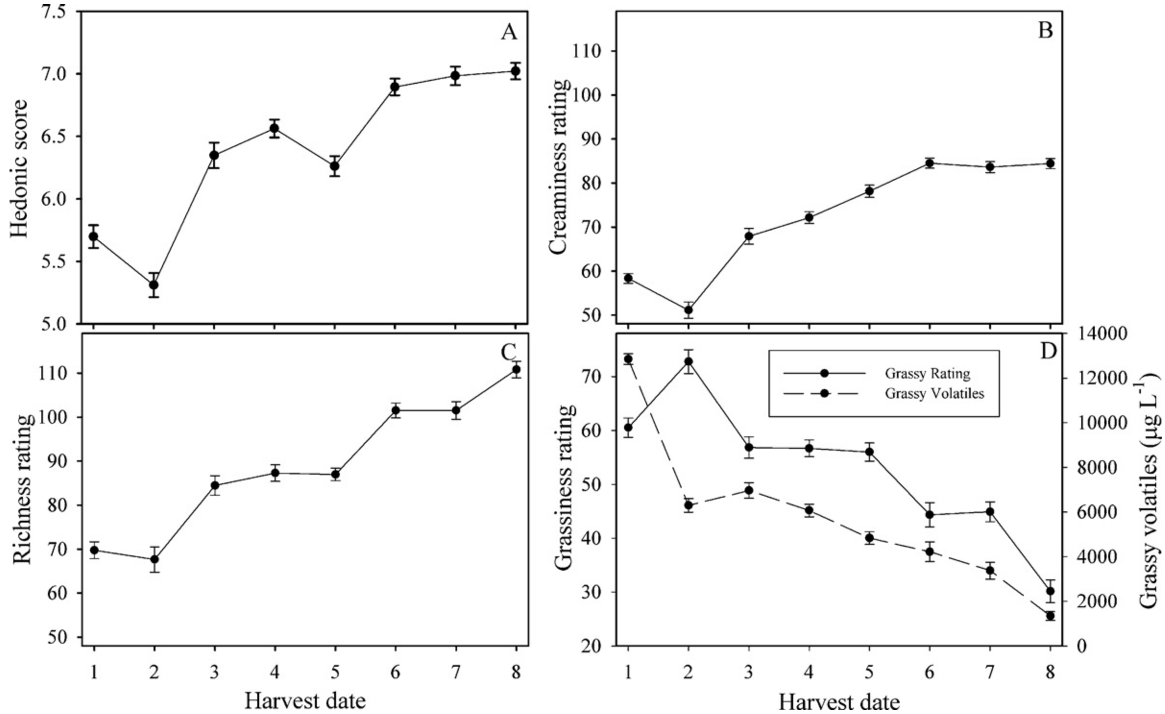

Fig. 2. Changes in 'Hass' avocado sensory attributes as influenced by harvest date in Study 2. Hedonic score (A), creaminess (B), richness (C) and grassiness (D) were evaluated in 50–60 fruit for each harvest date. The total concentration of major volatiles with a grassy aroma for each date is also shown (D). Vertical bars indicate SE.

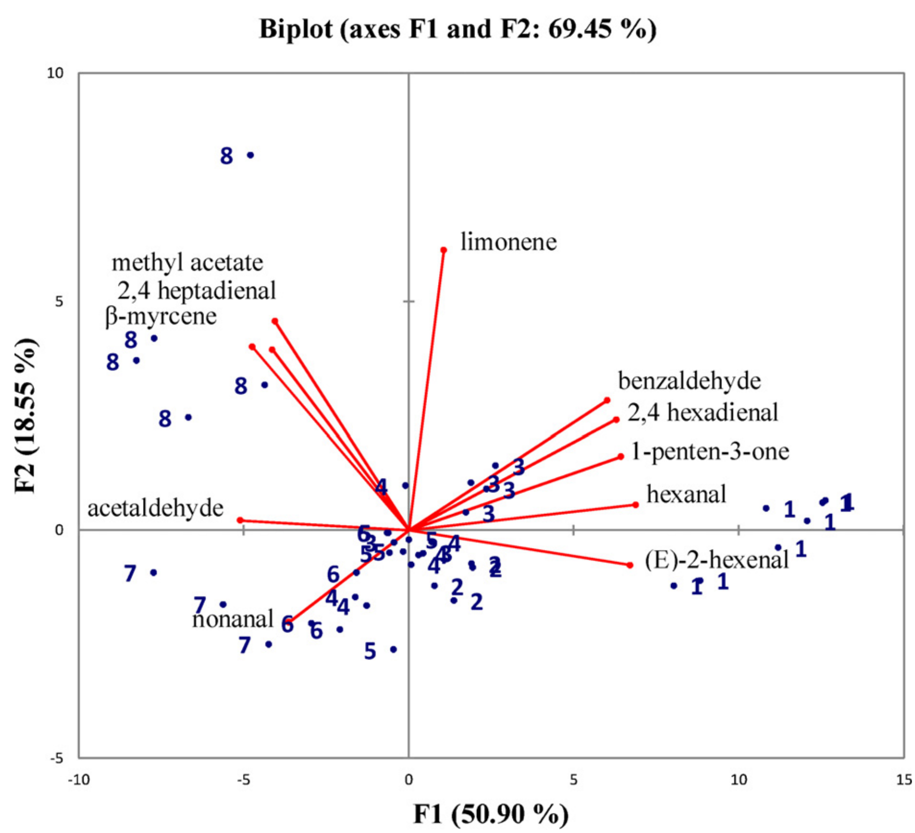

Fig. 3. Principal component analysis biplot of aroma volatiles present in 'Hass' avocados from eight different harvest dates (Study 2). Numbers within plot in bold refer to harvest dates.

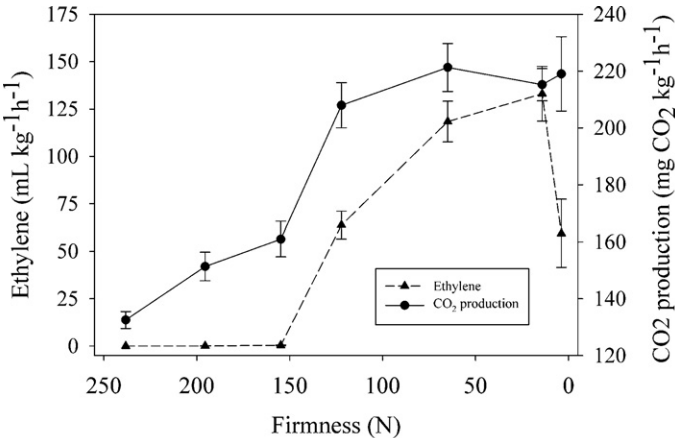

Fig. 4. Rates of CO_2 production and ethylene production measured at different firmness levels from 'Hass' avocados ripening at 20 °C in Study 3. Concentrations are expressed on a kg FW basis. Vertical bars indicate SE.

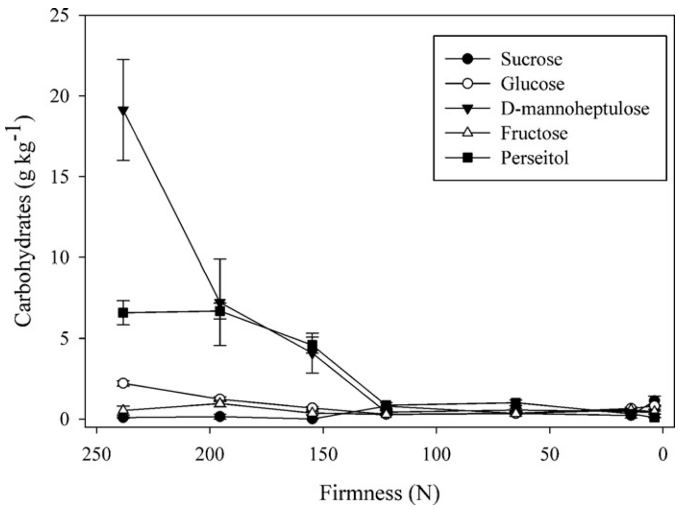

Fig. 5. Carbohydrate concentrations at different firmness levels in the flesh of 'Hass' avocados ripening at 20 °C in Study 3. Concentrations are expressed per kg FW. Vertical bars indicate SE.

and negatively correlated with the concentrations of 1-penten-3-one, hexanal, and 2-hexenal. The pattern of correlations between sensory attributes and aroma volatiles was similar to that between the volatiles and hedonic score.

3.3. Ripening influence on respiration, carbohydrates, and aroma volatiles (Study 3)

Avocados are eaten following ripening, yet the influence of this ripening period on aroma volatile concentration has not previously been well investigated. To better understand the influence of ripening on these compounds as well as on soluble carbohydrates, which also potentially impact flavor, avocados from the fourth harvest in April were allowed to soften at 20 °C and samples of various firmnesses ranging from 257 N to 3 N were collected for analysis. For comparisons the fruit samples were separated into seven classes that were separated from each other by 45 N.

Respiration slowly increased during ripening until the fruit reached an average firmness of 155 N, following which the respiration rate sharply increased and then reached a plateau Fig. 4. Corresponding to the sharp increase in respiratory rate was the initiation of rapid ethylene production. The rate of ethylene production increased with further softening and then leveled off by 14 N, followed by a steep decline as the fruit became fully soft.

D-Mannoheptulose and its sugar alcohol derivative perseitol composed the bulk of the soluble carbohydrates present in the unripe avocado flesh, with sucrose, glucose, and fructose being relatively minor components (Fig. 5). The concentrations of D-mannoheptulose and perseitol both rapidly declined to near-zero levels during ripening, while the other minor carbohydrates also declined or remained unchanged.

Of the twenty-five volatiles that were identified, twelve aroma volatiles, consisting of six aldehydes, two esters, one ketone, and three alcohols, significantly changed in concentration during ripening (Table 5). The volatiles 1-penten-3-ol and 1-penten-3-one increased, while 1-pentanol, hexanal, furfural, 1-hexanol, methyl hexanoate and 2-nonenal decreased in amount.

4. Discussion

Preliminary work (Study 1) undertaken in this study to obtain a list of sensory attributes important to avocado flavor identified a combination of both textural and flavor attributes that act together to determine the quality of the eating experience (Fig. 1). The development of this list was necessary as reports in the literature concerning research that had dealt with sensory evaluation of avocados (e.g. Harding, 1954; Hatton et al., 1957b) had provided little guidance on the relative impact of different sensory attributes on likeability. A report from New Zealand had given us a list of attributes that were thought to be potentially of importance to determining flavor in New Zealand-grown 'Hass' avocados (Yahia and Woolf, 2011), but it was important for us to further refine this list to ensure its applicability for fruit grown in different geographical locations with various maturity levels. This was achieved by performing sensory evaluations throughout the year using 'Hass' avocados from Mexico, Chile, USA and Peru, important avocado-producing countries in the world. Those attributes found to be most closely linked to likeability were then used to develop the following line scales that panelists used to evaluate avocado samples in the remainder of the study: (1) watery to creamy; (2) bland to rich; and (3) grassy to not grassy.

As has been noted by previous researchers (Gamble et al., 2010; Harding, 1954; Lee et al., 1983) and was evidenced in this work by the ever-increasing hedonic ratings during the progression of the season (Fig. 2A), avocado eating quality increases as the fruit matures. In California the minimum maturity standards are a combination of dry weight and release dates which are based on fruit size (weight). In the maturity portion of this study we utilized fruit that ranged from 158 to 215 g. The release date, regardless of dry weight content, for this fruit size range was January 2 for the larger fruit and January 16 for the smaller fruit. Therefore, the fruit from harvest dates 1 and 2, while legally harvestable, were below the established minimum dry weight standard for 'Hass'. The minimum dry weight standard of 20.6% was not surpassed until the third harvest in this study. Although the hedonic score at this point had increased from a low of 5.3 (between dislike slightly and like slightly) to 6.3 (just above like slightly), the flavor of the fruit increased significantly above that point to a hedonic score of 7.0 (like moderately) as the season progressed. The close correspondence between changes in both the hedonic score and the measured sensory attributes (Fig. 2 and Table 4) indicates that the increase in hedonic score during avocado maturation is associated with the fruit becoming creamier and less watery in texture with a flavor that is richer with less grassiness (higher grassiness rating).

Our results were similar to other researchers (Liu et al., 1999; Meyer and Terry, 2008) in that D-mannoheptulose and perseitol were the most abundant carbohydrates in the unripe fruit. As has been previously observed (Liu et al., 1999), ripening led to a rapid

Table 5
Concentrations of aroma volatiles from homogenized 'Hass' avocado mesocarp tissue as influenced by fruit firmness in Study 3. Avocados were selected during ripening based on firmness to represent each of the seven firmness groups. Firmness values are means for the group. Volatiles were classified depending on whether concentrations were decreasing, increasing or had no clear pattern of change (mixed response). Only volatiles that were significantly different among the firmness groups are shown.

Aroma volatile	Descriptors[a]	AT[b] ($\mu g L^{-1}$)	Concentration ($\mu g L^{-1}$) Firmness (N)						
			238	196	155	122	65	14	4
Decreasing									
Methyl acetate	Ether, sweet		261.4a	254.4ab	222.5ab	218.5ab	189.9b	59.4c	48.4c
1-Pentanol	Green, grassy	4000[f]	38.9a	42.0a	46.0a	12.0b	3.0b	2.1b	0.0b
Hexanal	Fresh, green	4.5–5[c]	10551.3a	5895.4b	5112.1bc	3116.9cd	1820.1cd	693.0d	1554.3d
Furfural	Sweet, woody	23000[f]	12.6a	9.1ab	6.7bc	4.3bc	3.0c	2.2c	5.1bc
1-Hexanol	Grassy, sweet	2500[g]	36.0a	16.1bc	16.8b	4.3c	0.0d	0.0d	0.0d
Methyl hexanoate	Fruity	84[g]	3.6bc	3.2bc	3.4cd	4.2b	6.0a	1.5cd	0.3d
2-Nonenal	Fatty, tallowy	0.08[f]	110.1a	103.6a	103.6a	43.2b	15.1bc	0.0c	0.0c
Increasing									
1-Penten-3-ol	Green, vegetable	400[d]	9.2d	7.6d	6.5d	17.7cd	29.0bc	39.1ab	53.4a
1-Penten-3-one	Earthy, green	1.25[d]	1.6cd	1.3d	1.2d	1.9cd	2.8bc	3.8b	5.2a
Mixed response									
(E)-2-Hexenal	Green, fruity	17[c]	661.2b	358.2bc	352.5bc	301.1c	298.2c	470.6bc	1087.0a
2,4-Hexadienal	Green	60[e]	36.0a	16.1b	16.6b	15.6b	13.0b	15.2b	36.3a
Benzaldehyde	Almond, cherry	350[f]	6.0a	1.8b	1.7b	2.5b	2.2b	2.3b	3.8ab

Each concentration value is the mean of 10 fruit that were individually measured, with the exception of the initial (238 N) and final (4 N) ripening stages where 9 and 6 fruit were measured, respectively. Fruit were harvested on harvest date 4. Volatile concentration values followed by a different letter are statistically significant at $P \leq 0.05$ using the Bonferroni correction to adjust for the number of comparisons made.

[a] Flavor descriptors from the University of Florida Citrus Flavor and Color Database (Rouseff, 2010), Cornell University Flavornet (http://www.flavornet.org/flavornet.html), and the Good Scents Company (http://www.thegoodscentscompany.com/index.html).
[b] Aroma threshold.
[c] Rouseff (2010), in water.
[d] Buttery et al. (1971), in water.
[e] Teranishi et al. (1974), in water.
[f] Buttery et al. (1988), in water.
[g] Takeoka et al. (1990), in water.

decline in these and other soluble carbohydrates, leading to an almost total disappearance of carbohydrates by the time the fruit were fully ripe. The direct influence of carbohydrates on the flavor of ripe fruit, therefore, is not likely to be great given the small concentrations present. This is supported by our findings in the initial portion of the study to identify key flavor attributes that found sweetness to be of low importance (data not shown). However, while carbohydrates may not directly impact flavor in ripe fruit, they do play an important role in flavor formation during ripening by providing substrate to support respiratory changes and to supply precursors for aroma volatiles (Defilippi et al., 2009).

The composition of aroma volatiles that we observed in avocados was heavily represented by aldehydes, a result that is consistent with prior work (Pereira, 2010; Pino et al., 2004; Sinyinda and Gramshaw, 1998). This is not surprising given that aldehydes are derived from lipid degradation (Schwab et al., 2008) and that avocados are exceedingly high in lipids (Lewis, 1978). One of the clearest associations of aroma volatiles with flavor was found with the changes that occur in hexanal, 2-hexenal and 2,4-hexadienal, three aldehydes that have a green or grassy aroma (Table 3). These compounds are some of the most abundant volatiles present in avocados and are most associated with low maturity fruit (Fig. 3). Although the use of odor thresholds that were determined in water must be used with caution due to the potential of interaction of the aroma volatiles with the fruit matrix (Plotto et al., 2008, 2004), the amounts present in the fruit relative to the published thresholds (Table 3) indicate that these aldehydes likely have some influence on flavor. As the fruit matured, the concentrations of all three of these compounds declined greatly in conjunction with the decrease in the grassiness sensory rating (Fig. 2), indicating a likely link between concentration and the lessening of the perception of grassiness in mature fruit. Although present in less abundance, 1-penten-3-one also has a green aspect to its aroma and through its decline in amount with maturation may also add to the loss in grassiness. Another aldehyde with a grassy aroma that was found, 2,4-heptadienal, is less likely to have an impact on flavor due to its relatively low concentration in the fruit and low odor threshold.

Acetaldehyde is most often thought of as an anaerobic metabolite but it is also formed in fruit as a result of the normal ripening and maturation process (Dixon and Hewett, 2000). In citrus, for example, acetaldehyde increases in concentration gradually during on-tree maturation, the amount of increase differing by citrus type (Davis, 1970; Shi et al., 2007). In this study we found acetaldehyde to be present at each maturity stage with the concentration being the greatest in fruit harvested at the later harvest dates (Table 2). Pesis et al. (1998) reported that application of acetaldehyde to avocados reduced tissue browning. Interestingly, we have previously found that avocados of early maturity, such as those from harvest 1, brown much more readily than later maturity fruit, suggesting the idea that the natural acetaldehyde content of the fruit may have some role in helping to inhibit browning. Also, many aldehydes including acetaldehyde, benzaldehyde, hexanal, 2-hexenal show a fungicidal effect on fruits and could serve as natural antifungal compounds (Pesis, 2005; Song et al., 2007). In terms of flavor, acetaldehyde has a pungent, solvent-like aroma (Rouseff, 2010) and could, given the high concentration relative to the odor threshold, have a direct or indirect role. Acetaldehyde is also readily metabolized to ethanol by the action of alcohol dehydrogenase, which may then be utilized in the production of other volatiles such as esters (Rudell et al., 2002). Moreover, acetaldehyde can be metabolized to acetyl CoA which can lead to the synthesis of other compounds, including mevalonate, which can be utilized to produce monoterpenes (Eskin, 1979; Pesis, 2005). We were not, however, able to detect any ethanol in the headspace of our avocado samples. Ethanol has been reported to be present in avocados in some of the limited number of studies on avocado volatiles available (López et al., 2004; Pino et al., 2004) and was found to increase in concentration when exogenous acetaldehyde was applied (Pereira, 2010). It is possible that ethanol was present but could not be detected using our analytical techniques even though we have been successful doing this with other fruit extracts (Obenland et al., 2011).

Methyl acetate, pentanal, β-myrcene and nonanal all significantly increased in concentration during fruit maturation and could also potentially influence flavor (Table 3). Of particular interest was pentanal, which has an aroma described as being fruity and nutty. Nuttiness is an attribute of avocados that is associated with good avocado flavor (Hatton et al., 1964) and was found in the initial portion of this study to be most prevalent in fruit scored high on the hedonic scale (Fig. 1). Nonanal was present in amounts far in excess of the aroma threshold at each of the harvest dates but was much more abundant at the later harvest dates (Table 3). It has a fatty, citrus-like aroma that may act to modify avocado flavor during maturation. Enhanced levels of methyl acetate or β-myrcene, on the other hand, would be associated with greater sweetness or mustiness, respectively.

In contrast to other typical climacteric fruit for which there is an enhanced accumulation of some key aroma volatiles that contribute to flavor development (Defilippi et al., 2009), avocado volatiles for the most part either declined or remained unchanged in amount during ripening. Out of the 12 compounds identified during the ripening study that significantly changes only 1-penten-3-ol and 1-penten-3-one increased in amount with ripening, with the former compound being present at levels well below its aroma threshold. These results were similar to those of Pereira (2010) who reported that mature-green and mid-ripe stages contained greater numbers and concentrations of volatiles than did the ripe stage. Most notable in our data was the large decline in hexanal concentration, especially given its probable contribution to the grassy flavor that is sometimes noted in avocados. This decline in hexanal was also noted by El-Mageed (2007) in examining the changes in volatiles that occurred in whole avocados during ripening. A sizeable decrease in hexanal and in other associated aldehydes also was found to occur during peach and kiwifruit ripening and was linked to declines in lipoxygenase and hydroperoxide lyase gene expression (Zhang et al., 2010, 2009). A similar situation may also exist in avocados, although decreases in linoleic and linoleic acids that have been reported during avocado ripening (Villa-Rodríguez et al., 2011) may also play some role.

In summary, avocados that were most liked by our panelists were described as having a creamy, smooth, buttery texture with richness, nuttiness and a minimum of grassy flavor. This study demonstrated that avocado flesh contains aroma volatiles that likely participate in determining this flavor. The volatiles most clearly associated with flavor were aldehydes with a grassy aroma, although other volatiles are likely involved as well. These aldehydes declined in abundance with fruit maturation at the same time as a lessening in the sensory perception of grassiness in the fruit. Although not a point of study in this work, changes in lipid content that occur during maturation in avocados may act to modify the effect of these volatile compounds on flavor given the known effects of lipids on volatile release and perception (Bayarri et al., 2006). Ripening also led to an overall decline in the amount of these grassy components, largely due to a loss in hexanal. Aroma volatiles offer an additional means to help predict and explain avocado flavor in a more complete manner than what can be currently accomplished by the use of oil content and dry matter alone.

Acknowledgements

This work was funded in part by grants-in-aid from the California Avocado Commission and the Pinkerton Growers' of California. The authors wish to thank Paul Neipp for his technical assistance in data collection; Edna Pesis for her critical review of the manuscript; Roger Essick for suggesting this line of research; Nathan White for donating fruit used in the second study; and Victor Tokar and Reuben Hofshi for their helpful suggestions.

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