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Article

Chemical Characterization of Two California-Grown Avocado Varieties (*Persea americana* Mill.) over the Harvest Season with an Emphasis on Sensory-Directed Flavor Analysis

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ABSTRACT: The research objective was to characterize avocado's aroma-active volatiles and use information about its overall composition, such as lipid profile, to discuss likely biosynthetic origins. To achieve this, two varieties, "Hass" and "3-29-5" (GEM), were evaluated during their commercial harvest period for dry weight, moisture content (freeze-drying), oil content (Soxhlet extraction), fatty acid composition, and aroma profile. Solvent-assisted flavor evaporation and aroma extract dilution analysis were performed on aroma extracts. Oleic acid (>50%) was the prominent fatty acid in the oil of both varieties. The majority of the aroma-active compounds in avocado are lipid-derived. The most notable compounds are 1-octen-3-one (mushroom) with a flavor dilution factor as high as 8192, hexanal (grassy), (*Z*)-4-decenal, an unknown, and (*E*,*E*)-2,4-nonadienal. Over the mid-to-late harvest season, a decline in hexanal and an increase in octanal were observed. In contrast to "Hass", the hexanal content was relatively stable in "3-29-5".

KEYWORDS: *avocado, Persea americana Mill., SAFE, GCO, AEDA, FAMEs, oleic acid, 1-octen-3-one, peroxidation, oxidation*

INTRODUCTION

U.S. consumers have a growing appetite for avocados. This is seen by consumption nearly quadrupling in the past 2 decades, from 1.00 kg/capita in 2000 to 3.64 kg/capita in 2018.[1] Over the past 40 years, the U.S. net production of avocado has ranged from 1.05 to 2.83 million kg annually.[2] The majority of U.S. avocados are grown in California, with about 92% of U.S. avocados grown in California during the 2018−2019 season.[2] Yet, the United States also relies on avocado imports, which reached 1.11 billion kg in 2019.[3]

The oil profile of avocado makes it unique as a fruit and is a driving factor for its popularity. For example, current research is exploring the health benefits of an avocado-a-day diet, probing its effect on lowering low-density lipoproteins (LDL) cholesterol and whether this effect is strictly linked to the monounsaturated fatty acid composition.[4] In a study evaluating the oil profile of "Hass" avocados from the United States, Mexico, Australia, and New Zealand, the oil content was found to be between 60 and 63% on a dry weight basis, composed predominantly of oleic acid (C18:1, 42−51% of the oil).[5] If the average moisture content of avocados is taken to be 78%,[6] this would correspond to approximately 13−14% oil on a wet weight basis. Roughly 75% of the oil is mono- or polyunsaturated.[5] In a study of 14 cultivars of avocados grown in Florida, the oil content ranged from 11.4% oil for "Simmons" to 25% for "PA-6206".[6] Eaks monitored the oil content of "Hass" and "Fuerte" avocados grown in southern California over a 13 month period for "Hass" and 8 months for "Fuerte" and showed that the oil content changes significantly over the season.[7] At peak concentration, the oil content was approximately 16 and 19% wet weight bases for "Hass" and "Fuerte", respectively.[7] Likewise, the fatty acid profile changed over the season, with the most significant changes observed with linoleic and oleic acids. Oleic acid concentrations in the oil peaked at nearly 60% in "Hass" and 70% in "Fuerte".

While oil content and composition are important for avocado mouthfeel and possibly taste,[8,9] the volatiles impacting avocado flavor are still largely unexplored. There is a growing body of work investigating the volatile compounds in avocados from cultivars such as "Hass",[10] "Fuerte",[11] "Barker", "Collinson", "Fortuna", "Geada",[12] and "Moro".[13] Several of the early volatile explorations have utilized simultaneous steam distillation/solvent extraction (Likens−Nickerson),[13−15] which provides insights into compounds that may be present in the avocado but can generate artifacts. Since avocado has a large percentage of its oil content coming from unsaturated fatty acids, the concentrations of lipid-derived volatiles are likely overestimated in heat-based extractions. For example, avocado pieces were boiled for a total of 8 h in one experimental protocol, and the researchers comment that the avocado extract obtained had a different odor quality than the fresh fruit.[14] In another study, researchers aimed to optimize avocado volatile extraction conditions in a modified Likens−Nickerson procedure by trying various extraction times and solvents, but the researchers had multiple cases where increasing the extraction time from 40 to 60 min decreased

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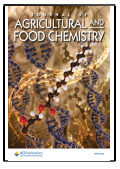

the total number of volatiles (peaks) obtained, i.e., from 17 to 12. However, with the same samples, increasing the extraction time from 60 to 70 min increased the number of volatiles.[12] This study failed to recognize that volatiles may be lost during the distillation process and that thermally generated compounds could form during long extractions; therefore, an extraction producing a greater number of volatiles does not necessarily mean that the extraction is better.

Obenland et al. used a number of precautions to minimize analytical artifacts, including the addition of sodium chloride to inactivate enzymes and a short, moderate heating time (30 min, 40 °C) using solid-phase microextraction (SPME).[10] Levels of 12 volatiles changed over the avocado harvest season, and this data, combined with the aroma threshold values, identified compounds that may be important to avocado flavor. By combining headspace analysis and sensory panels, Obenland et al. showed that several volatiles with "green" aroma attributes, namely, 1-penten-3-one, hexanal, (*E*)-2-hexenal, and 2,4-hexadienal, are associated with earlier season fruit and lower hedonics scores.[10] Mahendran et al. used purge and trap to collect avocado volatiles from an avocado puree for 1 h in a water bath at 45 °C and analyzed the extract by gas chromatography olfactometry (GCO) and gas chromatography-mass spectrometry (GC-MS).[11] While purge and trap is less prone to artifacts than Likens−Nickerson and better at trapping lower volatility compounds, no steps were taken to deactivate enzymes in the puree. Another pair of studies used microwave processing for ≤60 s to inactivate enzymes in avocado, with or without the presence of avocado leaves,[16,17] to develop a flavor-stable avocado puree. In the 2004 microwave processing study, the control was extracted by SPME after the avocado puree equilibrated at room temperature for 24 h,[16,17] which was no longer representative of fresh avocado. The more recent (2008) of the two studies included aroma extract dilution analysis (AEDA), which provides information about the relative importance of the aroma-active volatile compounds. The most important volatiles in microwaved (30 s) avocado at pH 5.5 were 1-penten-3-one, (*E*)-2-heptenal, octanol, and an unknown.[17] In the 2008 study, all avocados received a microwave treatment; therefore, it is unknown how the fresh avocado would have compared to the microwaved fruit. Solvent-assisted flavor evaporation (SAFE) can be applied to high-fat matrices (50% fat) to yield extracts free of nonvolatiles, thereby minimizing artifacts during analysis.[18] Further, SAFE can capture polar and mid-volatility (boiling points exceeding 300 °C) compounds. Applying AEDA to the SAFE extract identifies volatiles of significance from a flavor perspective. AEDA is crucial in identifying potent, low abundance volatiles that contribute to the overall flavor.

With these considerations in mind, the research objective was to use, for the first time in avocados, SAFE followed by AEDA to provide a more complete flavor profile. Due to the dynamic composition of avocados during the commercial harvest season, moisture, oil content, and lipid profile of the fruit were also recorded chronologically over the harvest season, with three of the six sampling intervals including SAFE and AEDA analyses. The well-known commercial variety "Hass" was selected, as well as a newer commercial variety, "3-2-95", developed by the University of California (U.S. Plant Pat. No. 14 239 P3),[19] which is marketed internationally under the name of GEM. The primary goal of this research is to gain a better understanding of avocado flavor, specifically which volatiles make a significant aroma contribution to the flavor, how the flavor changes over the harvest season, and how the volatile odor profile is linked to oil and water contents.

METHODS

Avocados. Avocados were harvested from a commercial orchard near Saticoy, California, on an approximately monthly basis between February and July 2019 (Table 1). The "Hass" were harvested within its commercial season. The first harvest was early for "3-29-5", and all subsequent harvests were within its commercial season. The "Hass" trees were planted in 1978 and were grafted on seedling "Mexican" rootstock. The "3-29-5" trees were topworked into the same tree block in 2000 onto existing "Hass" trees so that "Hass" is an interstock on these trees. Topworking an existing tree is a common practice within the California avocado industry. The climatic conditions were typical of California growing conditions (Supporting Information). The fruit were harvested in the morning hours and transported in a climate-controlled vehicle at approximately 20 °C to Parlier, CA, which is approximately a 3.5 h drive. Upon arrival, the fruit were placed in cold storage (5 °C) for at least 5 days prior to ripening to help synchronize ripening. Subsets of the fruit were removed from cold storage for ripening at room temperature (20 °C). To promote ripening, the avocados were placed in an ethylene chamber with a continuous flow of humidified air (90−95% relative humidity) with ethylene gas at a concentration of 0.05 μL/mL. This concentration was achieved using a Matheson (Newark, CA) Dyna-Blender model 8280. The avocados' firmness was checked daily by hand using the method of White et al.[20] After ripening, the fruit was used immediately or placed back in cold storage (5 °C) until time of analysis, typically within 1 week. Arapia et al. have shown that preripened avocados stored at 5 °C for 1 or 2 weeks had no effect on hedonic score and only a slight (a few small areas) increase in body rot, the latter of which was mainly observed in early season fruit.[21]

Table 1. Harvest Dates of "Hass" and "3-29-5" Avocados

harvest no.	date
1	February 6, 2019
2	March 8, 2019
3	April 3, 2019
4	May 8, 2019
5	June 25, 2019
6	July 29, 2019

Chemicals. The standards, reagents, and solvents used are commercially available from Sigma-Aldrich (St. Louis, MO) or Fisher Scientific (Waltham, MA). For flavor standards, the purity was typically above 97%, and GC-flame ionization detection (GC-FID) was used to check the purity of any standard that was potentially oxidized or degraded.

Dry Weights. The fruit were held at 5 °C and allowed to warm to room temperature (20 °C) immediately before analysis. Dry weights were gravimetrically measured on four unripe avocados of each variety the day after harvest, except for harvests 2 and 3, which were measured 3 and 5 days after harvest, respectively. One plug of avocado was removed from each avocado using a 7 mm cork borer and placed on a preweighed watch glass and weighed. A sterilized acrylic plug was inserted into the avocado hole, and then the fruit was ripened in ethylene as described previously by Obenland et al.[10] The avocado plug was placed in an oven at 100 ± 10 °C and dried for 24 h or until a constant weight was obtained. Later in the season, the avocado plugs never reached a constant weight and the weight began to increase after several days in the oven. The oxidation of unsaturated fatty acids can lead to weight gains.[22] Therefore, the weight of the plug after approximately 30 h was used to calculate the dry weight percentage based on the equation

$$\text{dry weight (\%)} = \frac{\text{(avocado dry weight)}}{\text{(avocado wet weight)}} \times 100$$

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Moisture Content by Freeze-Drying. Three avocados of each variety were randomly selected for moisture content determination after ripening. The top and bottom third of the avocados were discarded and a wedge of the fruit was removed from the peel to the pit. If any rot, bruising, or other internal quality defects were observed, the whole fruit was discarded, and another avocado was selected. The avocado sample was cut into small slices, no more than 1 cm^2 by 3 mm thick. The avocado slices were placed on a weigh boat with a letter code, and the weight was recorded to the ten thousandth decimal place. The weigh boats were placed on a stainless steel tray and frozen with liquid nitrogen. The tray was then placed in a Labconco (Kansas City, MO) freeze dryer and dried for 48 h, achieving a vacuum of approximately 0.045 mbar. The samples were weighed immediately after removal from the freeze dryer and were then stored in a sealed glass jar at −80 °C until Soxhlet extraction.

Oil Content by Soxhlet. The freeze-dried avocado slices were brought to room temperature before removing them from the jar. The avocado samples were weighed again and coarsely ground in a mortar and pestle and then quantitatively transferred to a preweighed cellulose thimble. The mortar and pestle and transferring utensils were rinsed with petroleum ether, and the rinses were added to the thimble in the Soxhlet apparatus. Each thimble was covered with a preweighed amount of glass wool, which was used to cover the avocado sample contained in the thimble. The Soxhlet apparatus was assembled with a condenser at 3 °C and a total of 180 mL of petroleum ether. Two Soxhlets were run simultaneously, with the condensers connected in series. The heating controllers were set just high enough that a steady reflux would occur. After 18 h, the heating mantles were removed, and the flasks were allowed to cool. All petroleum ether remaining in the Soxhlet was poured into the round-bottom flask. The thimble was dried overnight in a fume hood before reweighing. The petroleum ether containing the oil was dried with sodium sulfate and filtered through a Whatman No. 1 qualitative filter. The solvent was removed by rotary evaporation (Buchi, New Castle, DE) until the oil was a constant weight. The oil was stored in the freezer at −17 °C until fatty acid methyl ester analysis.

Fatty Acid Methyl Esters (FAMEs). The avocado oil recovered by Soxhlet was methylated with sodium methoxide (reaction with carboxylic acids) and (trimethylsilyl)diazomethane (TMS-DM, reaction with free fatty acids) using the method of Salimon et al.,[23] with minor modifications. *n*-Hexane volume was increased to minimize emulsions during the addition of sodium methoxide, and methanol/toluene (2:1) was not evaporated before the addition of TMS-DM. The final dried hexane layer was diluted 1:50 for GC-FID analysis using an Agilent (Santa Clara, CA) 6890 GC equipped with a Select FAME column (Agilent). MIDI's Sherlock software (Newark, DE) and the MIDI calibration standard for edible oil methods aided in the identification and quantification of fatty acids present.

Flavor Extraction. Six ripe avocados of each variety were randomly selected to make the flavor extract. The top and bottom third of the avocados were discarded, and the remaining fruit was minced into pieces approximately 1 cm^2 by 3−4 mm thick. If any rot, bruising, or other internal quality defects were observed, the whole fruit was discarded, and another avocado was selected. After mixing the avocado pieces, >200 g was placed into an aluminum ice cube tray with a levered cube divider. The avocado pieces were frozen with liquid nitrogen, and then the lever was pulled on the tray to release them. Small batches of avocado were ground in a Waring (Torrington, CT) blender with pulses followed by blending on the high-power setting. The resulting coarse powder was weighed into two Pyrex bottles, 100 g per bottle. The blender jar, Pyrex bottles, and other utensils were prefrozen. The internal standards 2-methyl-3-heptanone and 2-ethylbutyric acid were divided equally to each Pyrex bottle with a syringe to give a final concentration of 1 μg/g in the blended avocado. Diethyl ether (200 mL per Pyrex bottle) was added, and the bottles were shaken on a New Brunswick Innova (Eppendorf, Hauppauge, NY) 2100 platform shaker at 260 rpm for 30 min. The resulting green extracts were decanted and pooled into a third Pyrex bottle. The remaining avocado sludge was centrifuged at 110 rpm for 10 min to recover additional ether. The recovered avocado extract was then slowly fed into the SAFE apparatus (Ace Glass Inc., Vineland, NJ) with the receiving flask and distillation head recirculating water held at 35 °C. The SAFE apparatus reached a vacuum on the order of 10^{-6} bar. After all of the sample had been introduced, an additional 20 mL of ether was used to rinse the dispensing flask of the SAFE apparatus and the distillation then proceeded for 2 h. The recovered extract was dried with sodium sulfate, concentrated with a Vigreux column (approx. 20 cm) to remove the bulk of the solvent, and then concentrated under a stream of nitrogen gas until 1 mL remained. For AEDA, 1:2 serial dilutions of the extract were made using diethyl ether.

GCO and GC-MS. For GCO, an Agilent 6890N equipped with an FID and ODO II (SGE Analytical Science, now Trajan, Pflugerville, TX) was used for data acquisition. The helium carrier gas was a 2.0 mL/min constant flow through a HP-5 (Agilent) column, 30 m × 0.32 mm × 0.50 μm, or a VF-WAXms (Varian) column, 30 m × 0.25 mm × 0.25 μm. Injections (2 μL) were into a splitless inlet at 230 °C. The oven program was isothermal at 32 °C for 3.00 min, ramped 6.00 °C/min to 74 °C, ramped 8.00 °C/min to 260 °C and isothermal at 260 °C for 5.00 min, for a total run time of 38.25 min. The FID was set at 280 °C. The sniff port was supplemented with humidified air at approximately 10 mL/min. Three odor assessors (two male, one female, span of ages) were used for GCO, with at least two individuals sniffing each dilution series. Two odor assessors had prior experience with GCO. The odor assessors practiced on the avocado extracts until their odor evaluations were consistent from run to run, and sensitivity was improved. Initial results were compiled, and assessors prompted to re-evaluate areas of the eluent stream if two others detected an odor.

For GC-MS, an Agilent 7890A coupled to a 5975C inert XL MSD was used. Injections (2 μL) were into a splitless inlet at 280 °C. Separation was achieved using a HP-5 (Agilent) column, 30 m × 250 μm × 0.25 μm, or a VF-WAXms (Varian) column, 30 m × 0.25 mm × 0.25 μm. The oven program for the HP-5 column was as follows: isothermal at 32 °C for 5 min, ramped 6 °C/min to 280 °C, isothermal at 280 °C for 10 min for a total run time of 56.33 min. The oven program for the wax column was the same except that the final temperature was isothermal at 260 °C for 5 min, for a total run time of 48.00 min. A solvent delay of 4.10 min (HP-5 column) or 3.70 min (wax column) was used, and the sample was scanned from 40.0 to 200.0 amu at 7.96 scans/s. Transfer line, source, and quadrupole temperatures were 280, 230, and 150 °C, respectively.

GC × GC. To identify additional aroma-active unknowns, a LECO (St. Joseph, MI) Pegasus BT 4D time-of-flight MS, equipped with hot/cold modulators, was used for GC × GC. Injections (1 μL) were into a splitless inlet set at 230 °C. Helium carrier gas was set to a constant flow of 1.0 mL/min. Separation was achieved using a DB-5ms (Agilent) column, 30 m × 250 μm × 0.25 μm, in series with an Rxi-17sil MS (Restek) column, 2 m × 250 μm × 0.25 μm. The oven program for the GC × GC was as follows: isothermal at 32 °C for 5 min, ramped 6 °C/min to 280 °C, isothermal at 280 °C for 10 min, for a total run time of 56.33 min. Two modulator programs were used to achieve GC × GC separation. The modulation time was set to 2.0 s with either 0.4 s hot and 0.6 s cold or 0.6 s hot and 0.4 s cold. The secondary oven and modulator were set at 5 °C higher and 15 °C higher than the oven, respectively, and the transfer line was set at 300 °C. A solvent delay of 3.33 min was used, and mass spectral data was collected for 40.0−400.0 m/z at a rate of 200 spectra/s.

Identification. For positive identification, avocado volatiles must meet the following criteria: odor description agreement with an authentic standard, retention index match with the standard on two columns of different polarity, and mass spectral match with the standard. Retention indices were calculated with a series of standard alkanes, according to the method of van Den Dool and Kratz.[24] The table of compound identities (Table 4) lists which of these criteria were met for each compound.

Semiquantitation. The internal standard (IS, 2-methyl-3-heptanone) normalized integral of each analyte peak and known concentration of the IS were used to determine concentration. The normalization or response factor for each analyte was determined by

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J. Agric. Food Chem. 2020, 68, 15301−15310

Table 2. Average Dry Weight, Moisture, and Oil Content of "Hass" and "3-29-5" Avocados over the Harvest Season[a]

	harvest number					
	1	2	3	4	5	6
"Hass", % dry weight	23.50 ± 2.63 b[b]	25.77 ± 1.71 ab	26.48 ± 1.90 ab	26.04 ± 2.88 ab	26.82 ± 2.05 ab	29.23 ± 2.15 a
"3-29-5", % dry weight	20.01 ± 0.84 d	23.03 ± 1.41cd	24.96 ± 1.64 bc	26.07 ± 2.39 bc	28.60 ± 0.79 ab	31.72 ± 2.12 a
"Hass", % moisture	77.17 ± 2.21 ab	78.48 ± 3.12 a	73.08 ± 3.56 ab[c]	72.60 ± 3.62 b[d]	71.90 ± 1.25 ab	72.09 ± 1.90 ab[d]
"3-29-5", % moisture	81.65 ± 0.38 a	78.51 ± 2.04 ab	77.36 ± 1.19 ab[c]	71.77 ± 1.08 c[d]	70.13 ± 1.67 c[d]	69.36 ± 1.20 c[d]
"Hass", % oil	13.33 ± 0.76 ab	9.79 ± 0.60 b	15.61 ± 3.52 ab	17.47 ± 4.20 a	15.83 ± 1.64 ab	15.49 ± 3.10 ab
"3-29-5", % oil	9.30 ± 0.65 c	14.34 ± 0.79 ab[e]	12.97 ± 1.33 bc	17.76 ± 1.04 a[d]	16.51 ± 2.27 ab	18.22 ± 0.50 a

[a]$n = 3$ unless otherwise noted. Harvest dates were Feb 6, Mar 8, Apr 3, May 8, Jun 25, and Jul 6. [b]Different letters in each row denote significance at the $\alpha = 0.05$ level using Tukey's test. [c]$n = 5$. [d]$n = 4$. [e]$n = 2$.

Table 3. Average Fatty Acid Profile of "Hass" and "3-29-5" Avocados over the Harvest Season[a]

		% composition of the oil[b] harvest number					
fatty acid	variety	1	2	3	4	5	6
16:0 palmitic	"Hass"	18.1 ± 2.4 a[c]	15.3 ± 1.4 b	15.7 ± 0.6 ab	16.0 ± 1.8 ab	15.1 ± 0.6 b	15.1 ± 0.7 b
	"3-29-5"	15.4	15.0 ± 1.5 a	12.6 ± 0.6 b	14.7 ± 1.4 a	12.2 ± 0.2 b	14.9 ± 1.5 a
16:1n-9	"Hass"	0.1 ± 0.0 a	0.1 ± 0.0 a	0.1 ± 0.0 a	0.1 ± 0.0 a	0.1 ± 0.0 a	0.1 ± 0.0 a
	"3-29-5"	0.1	0.1 ± 0.0 a	0.1 ± 0.0 a	0.1 ± 0.0 a	0.1 ± 0.0 a	0.1 ± 0.0 a
16:1n-7 palmitoleic	"Hass"	7.9 ± 0.1 a	6.6 ± 0.5 b	6.3 ± 0.7 bc	6.6 ± 0.7 b	5.6 ± 0.2 c	6.2 ± 0.6 bc
	"3-29-5"	3.6	3.2 ± 0.3 c	2.8 ± 0.4 c	4.0 ± 0.4 ab	3.5 ± 0.5 bc	4.5 ± 0.5 a
16:1n-5	"Hass"	n.d.[d]	n.d.	n.d.	n.d.	n.d.	trace[e]
	"3-29-5"	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
17:0 iso	"Hass"	0.1 ± 0.0	0.1 ± 0.0 a	0.1 ± 0.0 a	0.1 ± 0.0 a	0.1 ± 0.0 a	0.1 ± 0.0 a
	"3-29-5"	0.1	0.1 ± 0.0 a	0.1 ± 0.0 a	0.1 ± 0.0 a	0.1 ± 0.0 a	0.1 ± 0.0 a
18:0 stearic	"Hass"	0.5 ± 0.1 a	0.4 ± 0.1 a	0.4 ± 0.0 a	0.4 ± 0.1 a	0.4 ± 0.0 a	0.4 ± 0.0 a
	"3-29-5"	0.5	0.6 ± 0.0 a	0.5 ± 0.0 ab	0.5 ± 0.0 ab	0.4 ± 0.0 b	0.5 ± 0.0 ab
18:1n-9 oleic	"Hass"	51.1 ± 0.6 c	54.7 ± 1.1 ab	56.4 ± 1.3 a	53.7 ± 2.6 bc	53.4 ± 1.1 bc	52.1 ± 0.6 bc
	"3-29-5"	58.2	61.3 ± 2.5 a	64.2 ± 0.8 a	61.9 ± 1.2 a	62.9 ± 0.7 a	57.4 ± 2.9 b
18:1n-7 c-vaccenic	"Hass"	6.5 ± 1.4 b	7.1 ± 0.6 ab	6.9 ± 0.6 ab	7.3 ± 0.4 ab	7.8 ± 0.4 a	8.1 ± 0.2 a
	"3-29-5"	4.6	4.4 ± 0.4 d	4.7 ± 0.1 cd	5.2 ± 0.1 bc	5.6 ± 0.6 ab	5.9 ± 0.2 a
unknown	"Hass"	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	"3-29-5"	0.2	0.1 ± 0.1	n.d.	n.d.	n.d.	n.d.
18:2n-6 linoleic LA	"Hass"	14.1 ± 1.0 c	14.2 ± 1.0 c	13.1 ± 1.5 c	14.4 ± 0.7 bc	16.1 ± 0.8 ab	16.4 ± 0.4 a
	"3-29-5"	15.5	13.7 ± 1.0 bc	13.6 ± 0.3 bc	12.5 ± 1.1 c	14.0 ± 0.6 b	15.7 ± 1.0 a
18:3n-3 ALA	"Hass"	0.8 ± 0.1 ab	0.8 ± 0.0 ab	0.7 ± 0.2 b	0.8 ± 0.1 ab	0.9 ± 0.0 a	0.9 ± 0.0 a
	"3-29-5"	0.7	0.6 ± 0.1 a	0.8 ± 0.1 a	0.6 ± 0.1 a	0.6 ± 0.1 a	0.6 ± 0.1 a
20:0 arachidic	"Hass"	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	"3-29-5"	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
unknown	"Hass"	0.6 ± 0.1 a	0.4 ± 0.1 ab	0.2 c	0.3 ± 0.1 bc	0.3 ± 0.1 abc	0.2 ± 0.1 bc
	"3-29-5"	0.8	0.7 ± 0.4 a	0.5 ± 0.4 a	0.3 ± 0.1 a	0.4 ± 0.1 a	0.3 ± 0.1 a
20:1n-9 gondoic	"Hass"	0.2 ± 0.0 a	0.2 ± 0.0 a	0.2 ± 0.0 a	0.2 ± 0.0 a	0.2 ± 0.0 a	0.2 ± 0.0 a
	"3-29-5"	0.2	0.2 ± 0.0 a	trace	0.2 ± 0.0 a	0.2 ± 0.0 a	0.2 ± 0.1 a
22:2n-6 docosadienoic	"Hass"	trace	trace	n.d.	n.d.	n.d.	n.d.
	"3-29-5"	n.d.	trace	n.d.	n.d.	n.d.	n.d.
24:0 lignoceric	"Hass"	n.d.	n.d.	n.d.	n.d.	n.d.	trace
	"3-29-5"	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

[a]Harvest dates were Feb 6, Mar 8, Apr 3, May 8, Jun 25, and Jul 6. [b]For H1 "Hass", $n = 4$; for H2–H4 "Hass", $n = 5$; for H5–H6 "Hass", $n = 6$; for H1 "3-29-5", $n = 1$; for H2–H5 "3-29-5", $n = 6$; for H6 "3-29-5", $n = 5$. [c]Different letters within a row indicate statistical difference at $\alpha = 0.05$ using Tukey's test. [d]n.d. indicates that the area was below the integration level. [e]Trace indicates that for at least one replication, the area was below the integration level.

linear regression analysis of a 5- or 6-point plot of mass ratios (analyte to IS) against area ratios. Quantitation was on the Agilent GC-MS equipped with the HP-5 column, and ion extraction was unique for each analyte: hexanal (t_R = 10.56 min, m/z 56); 2-methyl-3-heptanone (t_R = 15.06 min, m/z 128); 1-octen-3-one (t_R = 16.29 min, m/z 70); octanal (t_R = 17.02 min, m/z 84); (*E*)-2-octenal (t_R = 18.62 min, m/z 70); nonanal (t_R = 19.88 min, m/z 98); (*E*)-2-nonenal (t_R = 21.35 min, m/z 67); and decanal (t_R = 22.52 min, m/z 112). All response factor curves had a coefficient of correlation of ≥0.985. In the case of hexanal, the avocado extracts were diluted to be in the linear range of the detector. The analyte and internal standard recovery were assumed to be 100%. This assumption was made because it was of main interest to compare the relative amounts of flavor compounds, rather than to determine the absolute concentrations. As a result of this assumption, the data are semiquantitative.

Statistical Analysis. One-way analysis of variance (ANOVA) (SPSS, Chicago, IL) was used to determine harvest differences within each variety for dry weight, percent moisture, percent oil, and fatty acids. Arcsine transformation was performed on percentages prior to

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J. Agric. Food Chem. 2020, 68, 15301−15310

analysis, with the original mean values presented. Tukey's test (α = 0.05) was used to provide mean separations.

RESULTS AND DISCUSSION

Harvest dates are shown in Table 1, and all other tables and figures denoting harvest number correspond to these dates. The dry weights, moisture contents, and oil contents of the avocados are shown in Table 2. The "Hass" avocados are well over the California Code of Regulation (CCR) minimum maturity standard of 20.8% dry weight,[25] even at harvest 1, when the dry weight was 23.5%. The minimum maturity standard for "3-29-5" is 22.8%.[26] "3-29-5" does not meet this standard at harvest 1, when the dry weight is 20.01%; however, the fruit exceed the dry weight minimum requirement by harvest 2. For "Hass", the dry weight and oil content are stable across the harvest season, with the dry weight only showing a statistically significant increase when comparing harvests 1 and 6. The oil content is statistically the same at harvests 1 and 6 but statistically increases between harvests 2 and 4. In contrast to the limited increases observed in "Hass", the dry weight of "3-29-5" continues to increase through harvest 6. The data shows the overall trend that as dry weight increases over the harvest season, the oil content also increases and the moisture content declines. The positive relationship between dry weight and oil content has been previously noted and is the basis for the current means of determining maturity and eating quality in avocados.[27] Although the use of dry weight to provide an easily measured estimate of fruit maturity has been useful, there is a recognition in the avocado industry that this measure oversimplifies avocado-eating quality and that other factors besides oil content are involved.

The fatty acid profile of the avocados is shown in Table 3. Across all harvests, oleic acid was the dominant fatty acid in both varieties. "3-29-5" consistently contained a higher level of oleic acid than "Hass", with oleic acid comprising 64.2% of the oil at harvest 3 (April) in "3-29-5" versus 56.4% of the oil in "Hass". The second most abundant fatty acids were palmitic and linoleic acids, each comprising 12.5−18% of the oil. Palmitoleic and vaccenic acids were found in the range of 3−8%. Approximately nine other fatty acids, including α-linoleic acid, were present at levels of 1% or less of the total oil content. As will be discussed later, the unsaturated fatty acids are the origin of many of avocado's flavor compounds. For avocados from Riverside, CA, Eaks found that the oleic acid content of "Hass" avocados was highest in February to April, at 55−60%,[7] which agrees closely with our results. Oleic acid is consistently the most abundant fatty acid in avocado, followed by palmitic and linoleic acids (either may take second place in abundance) and then palmitoleic acid.[6,7,28−31] Although most researchers examining the fatty acid content of avocado focus on the predominant fatty acids, Aliakbarzadeh et al. found 15 fatty acids in avocado and 17 fatty acids in the seed.[28] The most notable difference in the current work, where 14 fatty acids were found, is that α-linoleic acid was detected in the avocado pulp, which Aliakbarzadeh et al. only found in the seed.[28] Notably, there is considerable geographic variation among "Hass" avocados. The work of Ferreyra et al. examined 50 preharvest variables on the fatty acid profile of "Hass" avocados grown in 12 localities in Chile.[32] Among these regions, oleic acid was found to vary from 66.6 to 75.4%,[32] at least 10% above the oleic acid content of California "Hass". Altitude of the avocado grove, the average annual maximum temperature, and January absolute maximum temperature were among the climate factors affecting the oleic acid content of Chilean avocados.[32] Lower altitudes and cooler temperatures correlated to higher oleic acid content and lower palmitic acid content. The Saticoy experimental site average climatic conditions (Supporting Information) were between the "high middle" and "high" climatic zones reported by Ferreyra et al.[32] Results from this study were comparable for palmitic acid, but oleic acid was lower in California.

Figure 1a,b shows that oleic and linoleic acid contents exhibit an approximately inverse relationship over time. While oleic acid content in the oil exhibited an inverted U pattern over the harvest season, with a maximum oleic acid content at harvest 3, the linoleic acid was at its minimum at this point. Table 3 shows that palmitic and palmitoleic acids both declined slightly in "Hass" over the harvest season. In contrast, palmitoleic acid increased slightly in "3-29-5" over the harvest season. Vaccenic acid increased slightly over time in both "Hass" and "3-29-5". The saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) exhibited little change over the season, as shown in Figure 1c. In contrast to Figure 1a−c, Figure 1d represents the fatty acid content as the percentage in the avocado by wet weight, rather than the percent composition of the oil. Palmitoleic and vaccenic acids were also present at slightly higher quantities in "Hass" than in "3-29-5".

It has been established that higher oil contents generally correspond to better sensory ratings, although the predictive power of oil content on sensory ratings does not hold for late season fruit.[27,33] The low levels of free fatty acids in oil are also the primary source of oil's flavor. For example, the oil detection thresholds for oleic acid, canola oil, and canola oil with 3.8 mM oleic acid in a fat-free milk-based emulsion, thickened with gum arabic, were determined to be 0.19, 13.35, and 7.51% fat in the sample, respectively.[34] With such a low threshold for oleic acid, it is clear that even low levels of fatty acids impact the overall flavor of avocado. The FAME methodology utilized in this work liberated the fatty acids of triglycerides for analysis, as well as analyzing the initial free fatty acids. Thus, the percent of free oleic acid in the avocados was not determined.

The flavor profile of "Hass" and "3-29-5" avocados by AEDA is given in Table 4, along with the chromatographic and spectral evidence to support identification. Approximately 30 compounds were detected that have a flavor dilution (FD) factor of 2 or higher. The compounds of greatest significance are 1-octen-3-one (mushroom, earthy), hexanal (green, grassy), unknown (exhaust), and (*E,E*)-2,4-nonadieanal (floral/vinegar chip). Two cucumber aroma compounds, nonanal and (*E*)-2-nonenal, are also important. From this data, it is also observed that there is a large subset of flavor compounds that have weak aromas, with FD factors between 4 and 16. The aldehydes octanal, nonanal, and decanal are all present and have oily or green-fatty (cucumber-like) aromas.

The biochemical and/or chemical origin of these odorants will be considered. Lipid peroxidation occurs in avocados, which is an enzyme catalysis process.[35] The plant enzyme lipoxygenase (LOX) is responsible for producing hydroperoxides from linoleic and linolenic acid,[36] and avocado LOX's activity and optimal activity conditions have been characterized.[37] Oleic acid, the most abundant fatty acid in avocado, does not react with LOX because it lacks the *(Z,Z)*-1,4-pentadiene structural motif necessary for reaction with LOX.[35] Garciá-Rojas et al. explored the gene expression of avocado LOX at various storage conditions and firmness values

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J. Agric. Food Chem. 2020, 68, 15301−15310

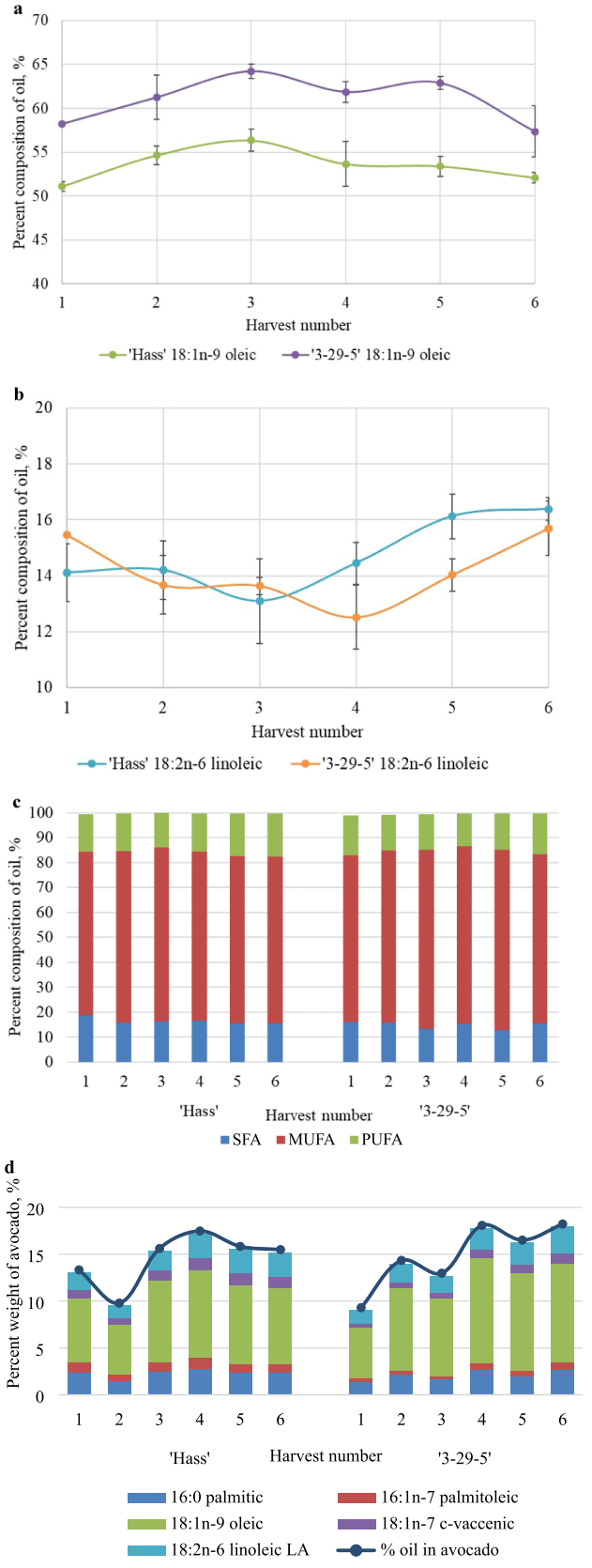

Figure 1. Changes in the distributions of key fatty acids, oleic acid (a) and linoleic acid (b), degree of saturation (c), and percentage of fatty acid and oil composition (d) in "Hass" and "3-29-5" over the harvest season (SFA: saturated fatty acid, MUFA: monounsaturated fatty acid, PUFA: polyunsaturated fatty acid). Harvest dates were Feb 6, Mar 8, Apr 3, May 8, Jun 25, and Jul 6.

of avocado.[38] After cold storage and application of 100 μL/L of ethylene for 24 h, the LOX gene expression continued to increase with decreasing firmness.[38] The authors' discussion indicates that LOX is actively producing hydroperoxides, and subsequently flavor molecules, while the fruit is ripening.[38] Hydroperoxide lyase, cis−trans isomerases, and alcohol dehydrogenases also play a role in forming aldehydes and alcohols after the action of LOX is complete.[37,39] Numerous flavor compounds that originate from linoleic acid were found to be odor-active in our study: hexanal, octanal, 1-octen-3-one, (*E*)-2-octenal, (*E*)-2-nonenal, and (*E*,*E*)-2,4-nonadienal. One product from linolenic acid was also identified: (*E*)-2-hexenal.

The other mode of forming hydroperoxides in foods is lipid oxidation, in which oxygen, particularly singlet oxygen, interacts with unsaturated fatty acids. These hydroperoxides are one of the chain propagating steps in lipid oxidation, also known as autoxidation.[35] The hydroperoxides formed by lipid oxidation eventually lead to numerous flavor compounds after the hydroperoxides undergo β-scission to form smaller molecules. β-Scission occurs at the allylic position, leading to two direct products. In the case of both linoleic and linolenic acids, there are two locations for β-scission to occur in the molecule. The β-scission products may continue to react, which can lead to a huge variety of aroma-active products. The aldehydes octanal, nonanal, and decanal are formed by lipid oxidation of oleic acid,[35] which was observed as the primary fatty acid in the avocados by FAMEs. Principal component analysis of various oils with their fatty acid profiles and volatile compositions demonstrated a high correlation between oleic acid and C8−C10 saturated aldehydes and ketones.[40] Given that LOX has no activity toward oleic acid, due to its lack of the necessary chemical motif, lipid oxidation is likely the cause of some of the octanal formation and the main source of C9 and C10 aldehyde formation. It is unclear if this lipid oxidation occurs in the orchard or during storage. In support of this idea, Meethaworn and Siriphanich found that octanal content increased 11-fold during the storage of young, trimmed coconut between weeks 2 and 4 of storage.[41] During this time, LOX activity was low and declining, and lipase activity was only slightly increasing. The researchers concluded that the enzymes were not solely responsible for octanal formation and that lipid oxidation was likely playing a role.[41]

Determining the role and/or importance of lipid oxidation within the intact fruit is a complex challenge. In the intact fruit, the enzyme cannot be denatured to monitor lipid oxidation, and extracting the avocado oil fundamentally changes the matrix, due to light and chlorophyll exposure, because chlorophyll is a pro-oxidant.[31,35] LOX can act extremely fast after tissue disruption, especially when fruit tissue is homogenized,[42] which is likely the reason LOX receives the most attention when discussing lipid breakdown products in plant tissues. The best way to explore lipid oxidation products (as opposed to peroxidation products) in avocado would be to cut the whole fruit while it is submerged in calcium chloride solution, as Steinhaus et al. did with guava,[42] and immediately extract the tissue afterward.

As already mentioned, 1-octen-3-one is one of, if not the most important, avocado odorants. This aroma compound has an FD factor of at least 512 and up to 8192 in H4 "3-29-5". Identifying 1-octen-3-one was challenging because the mass spectral data is buried under other peaks. Notably, 1-octen-3-ol, which also has a mushroom aroma, elutes at an RI of 978 on the HP-5 column, nearly identical to 1-octen-3-one's RI of 982.

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J. Agric. Food Chem. 2020, 68, 15301−15310

Table 4. Aroma Extraction Dilution Analysis of "Hass" and "3-29-5" Avocados at Three Points of the Harvest Season (Apr 3, May 8, and Jul 6)

					FD[a] factor					
					"Hass"			"3-29-5"		
RI[b] (HP-5)	RI (Wax)	identity	descriptor	identity based on[c]	H3	H4	H6	H3	H4	H6
<700	978	unknown	butterscotch		n.d.[d]	n.d.	16	n.d.	n.d.	32
<700	1447	acetic acid	vinegar	odor, RI HP-5, RI wax, MS	16	n.d.	64	n.d.	n.d.	2
799	1069	hexanal	grassy	odor, RI HP-5, RI wax, MS	4096	256	256	512	512	256
822	1099	unknown	acid/vinegar, skunky		n.d.	4	4	4	4	4
842	1675	3-methylbutyric acid	sweaty socks	odor, RI HP-5, RI wax, LECO	4	4	8	16	4	16
856	1219	(*E*)-2-hexenal	fruity/sour candy	odor, RI HP-5, RI wax, MS	32	4	2	n.d.	n.d.	n.d.
904	1462	methional	potato	odor, RI HP-5, RI wax	n.d.	n.d.	256	n.d.	32	16
924		unknown	off/fatty		4	conc[e]	8	n.d.	n.d.	n.d.
960		(*E*)-2-heptenal	cheese biscuits, crackers	odor, RI HP-5, LECO	4	4	n.d.	4	4	4
982	1298	1-octen-3-one	mushroom	odor, RI HP-5, RI wax, MS SIM/LECO	512	256	512	2048	8192	1024
998		2-pentylfuran	rancid	RI HP-5, LECO	8	4	4	n.d.	n.d.	n.d.
1007	1285	octanal	grain/fatty	odor, RI HP-5, RI wax, MS	n.d.	n.d.	64	16	8	128
1053		ethyl 2-furoate	curry/yeast/grain/fatty	odor, RI HP-5, LECO	n.d.	4	16	16	16	4
1062	1432	(*E*)-2-octenal	flour/playdough/fatty	odor, RI HP-5, RI wax, MS	8	8	4	64	64	128
1095	1396	nonanal	cucumber/fatty	odor, RI HP-5, RI wax, MS	32	32	16	128	128	256
1134		unknown	citrus/sweet		n.d.	n.d.	2	n.d.	n.d.	64
1155	1547	(*E*)-2-nonenal	spicy/cucumber/rancid	odor, RI HP-5, RI wax, MS SIM/LECO	256	128	256	256	64	512
1169		unknown	exhaust/sneakers		256	64	64	1024	64	32
1201	1495	decanal	oily/oxidized	odor, RI HP-5, RI wax, MS	32	conc	32	16	2	8
1224		(*E,E*)-2,4-nonadienal	clean, floral/vinegar chip/dough	odor, RI HP-5	128	16	64	512	128	256
1259		*carvenone*[f]	mint	LECO	4	2	2	16	4	16
1344		*α-cubebene*	fresh/floral/minty/fennel seed	LECO	32	16	8	128	16	n.d.
1362		unknown	playdough/corn chip/clean, floral		n.d.	n.d.	4	n.d.	8	32
1444		*sesquisabinene*	spicy/metallic/sulfur	LECO	n.d.	n.d.	n.d.	32	n.d.	n.d.
1477		farnesene isomer	paper	odor, RI HP-5, MS	n.d.	n.d.	8	n.d.	n.d.	64
1490		unknown	sunflower seeds/metallic		4	n.d.	16	n.d.	64	32

[a]FD: flavor dilution. [b]RI: retention index. [c]All means of identification are listed for each compound. Odor: the odor of the analyte in the avocado extract matched the commercial standard; RI HP-5/wax: the retention index of the analyte in the avocado extract matched the retention index of a commercial standard on the specified column; MS: the mass spectra of the analyte matched the NIST library spectra and an authentic standard; MS SIM: select ion monitoring was used to find 2−3 ions of the analyte in the correct relative abundances; LECO: GC × GC was used to separate the analyte and match its identity to the NIST library and an authentic standard. [d]n.d.: not detected. [e]conc: concentrated extract. [f]Compounds in italics were identified by mass spectrometry alone, and a commercial standard was unavailable. These are tentative identifications only.

Yet, 1-octen-3-ol could be eliminated as the mushroom odorant by data from the wax column, where 1-octen-3-ol was observed to elute at 1447. Mushroom aroma was smelled at 1303 but not at 1447. The compound 2-octanone was also observed by GC-MS on the wax column at 1283, an RI close enough to make it a potential candidate; however, it has a harsh, sour aroma. Other researchers have also had difficulty identifying this compound, and Guzmán-Gerónimo et al. erroneously identified this mushroom aroma as (*E*)-2-heptenal.[17] By our odor evaluation, (*E*)-2-heptenal is described as clean, fatty, and spice and elutes earlier at 963. Only 1-octen-3-one is able to match the detected odor property and the observed RI on both polar and nonpolar columns.

Hexanal was found at moderately high levels at harvest 3, with an FD factor of 512 and 4096 in "3-29-5" and "Hass", respectively, and diminished to an FD of 256, for both varieties, by harvest 6. Hexanal, a grassy, green aroma, is expected to be more abundant in early season fruit, as found by Obenland et al.[10] If the flavor portion of the research had begun earlier in the season, hexanal would have been expected to have had even higher FD values for both varieties. Some increases in the lipid oxidation product octanal are observed from harvest 3 to harvest 6, as shown by an increase in FD from 16 to 128 in "3-29-5" and n.d. to 64 in "Hass". The compounds (*E*)-2-octenal and nonanal also show a 1-fold increase from harvest 3 to harvest 6, but that increase is too small for high certainty in its significance.

The unknown detected at an RI of 1169 on the HP-5 column, described as "exhaust", was very important to one panelist, and the other two panelists occasionally detected it. This presented a challenge to represent its importance in the table, and best efforts to average its importance across the panelists were taken. Another compound of interest is acetaldehyde, which several others have identified as an avocado volatile.[10,11,13] Acetaldehyde is an anaerobic fruit metabolite, and application of acetaldehyde vapor to half-peeled avocado has been shown to reduce browning.[43] Obenland et al. showed that acetaldehyde increased over the harvest season.[10] Acetaldehyde was aroma-active in the analyzed samples and was also detected by GC-MS. However,

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J. Agric. Food Chem. 2020, 68, 15301−15310

Table 5. Concentration of Selected Aroma-Active Volatiles over the Harvest Season[a,b]

	concentration of compound in avocado, ng/g						
	H3 "Hass"	H4 "Hass"	H6 "Hass"	H3 "3-29-5"	H4 "3-29-5"	H6 "3-29-5"	odor threshold[c]
hexanal	7305.31	213.23	142.44	1163.06	773.05	1692.95	4.5[d]
octanal	8.88	10.83	6.07	9.85	14.50	26.49	8[e]
(*E*)-2-octenal	14.60	4.97	2.81	30.29	36.51	46.49	4[e]
nonanal	295.31	140.44	97.42	111.38	193.34	161.33	1[d]
decanal	4.96	3.27	1.98	2.81	3.34	4.62	5[e]
1-octen-3-one	2.83	1.85	0.95	2.91	3.88	1.53	0.005[f]
(*E*)-2-nonenal	5.89	2.09	1.80	2.86	1.30	3.31	0.15[e]

[a]Harvest dates were Apr 3, May 8, and Jul 6. [b]Concentrations are based on a single extraction of six avocados. [c]Orthonasal odor threshold in water, ng/g. [d]Guadagni et al.[44] [e]Rychlik et al.[45] [f]Buttery et al.[46]

the solvent, diethyl ether, contained acetaldehyde, so another technique, such as headspace, would need to be used to determine the odor importance of acetaldehyde to avocado. As a result, acetaldehyde has been excluded from Table 4.

Semiquantitation was performed on several of the most potent, positively identified compounds, as shown in Table 5. The majority of compounds were present on the order of 1−100 ng/g, although hexanal was more abundant, on the μg/g scale in some samples. Only small changes were observed in the concentrations of compounds quantified, such as in the decrease of (*E*)-2-octenal from 14.60 to 2.81 ng in "Hass" avocados from harvest 3 to 6. This agrees with the AEDA data, which also showed minor changes in the odor intensities of compounds over the harvest season. The only exception to this is the decline in the hexanal concentration in Hass from harvest 3 to 4 from 7305.31 to 213.23 ng. This sharp decline in hexanal was also observed by AEDA in "Hass": the FD factor decreased from 4096 to 256 from harvest 3 to 4. Interestingly, "3-29-5" did not show this decline in hexanal over time, exhibiting hexanal concentrations around 1000 ng (fluctuating within a factor of 2) at harvests 3−6. The odor activity values (OAVs) for the quantified compounds are given in Table 6.

Table 6. Odor Activity Values (OAVs) of Selected Aroma-Active Volatiles over the Harvest Season[a,b]

	H3 "Hass"	H4 "Hass"	H6 "Hass"	H3 "3-29-5"	H4 "3-29-5"	H6 "3-29-5"
hexanal	1623	47	32	258	172	376
octanal	1	1	1	1	2	3
(*E*)-2-octenal	4	1	1	8	9	12
nonanal	295	140	97	111	193	161
decanal	1	1	0	1	1	1
1-octen-3-one	566	370	189	582	775	307
(*E*)-2-nonenal	39	14	12	19	9	22

[a]Harvest dates were Apr 3, May 8, and Jul 6. [b]Odor activity values are based on a single extraction of six avocados. Odor activity value is the concentration of compound (ng/g) divided by its threshold (ng/g).

OAVs are determined by dividing the concentration of a compound by its odor threshold. The threshold used in this calculation was determined orthonasally (by smell directly, as opposed to smell via the oral cavity) in water. Since avocado contains approximately 20% oil and other matrix materials, the OAVs in Table 6 are expected to be high relative to avocado. This data indicates that hexanal, 1-octen-3-one, and nonanal are the most important odorants, with some contribution from (*E*)-2-nonenal. Notably, the potent odorant 1-octen-3-one was present at only 1−5 ng across all avocado samples evaluated, which emphasizes how important odor threshold is in determining a compound's importance to flavor. AEDA and OAV findings agree on the key odorants in avocado.

This research provides a comprehensive view of two varieties of avocado from an analytical perspective, which allows interactions between moisture content, oil content, oil profile, and aroma-active volatiles to be seen more clearly. As expected, moisture content and oil content showed an inverse relationship. Additionally, oleic acid and linoleic acid contents are related in an inverse manner. When oleic acid peaked mid-season (harvest 3), linoleic acid was at its lowest abundance. Although lipid oxidation products were known to be prevalent volatiles in avocados, sensory-directed research utilizing tools such as AEDA had been lacking. Further, too many avocado volatile studies have relied on extraction methods with high heat input or long extraction times without minimizing enzymatic activity. By using liquid nitrogen during extraction and SAFE for preparing the samples for analysis, artifact formation was minimized. AEDA indicates that 1-octen-3-one, hexanal, (*Z*)-4-decenal, (*E,E*)-2-4-nonadienal, and (*E*)-2-nonenal are key aroma volatiles. Similarly, 1-octen-3-one, hexanal, and nonanal are key odorants on the basis of OAVs. The current literature has recognized hexanal and nonanal as important avocado volatiles, but 1-octen-3-one has been overlooked, likely due to its low concentration. This earthy, mushroom-like compound is one of the two most important avocado volatiles (with hexanal). Thus, further investigations of 1-octen-3-one concentration and its sensory impact on consumers would be valuable. Complementary sensory studies will be published separately.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.0c05917.

Climate conditions of the growing site of the avocados in this study (PDF)

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Notes

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Chemical Characterization of Two California-Grown Avocado Varieties (*Persea americana* Mill.) Over the Harvest Season with an Emphasis on Sensory-Directed Flavor Analysis

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Table S1. Climate characteristics of experimental site near Saticoy, California.[a]

Altitude[b] (m.a.s.l.)	ETO (mm yr^{-1})	Average Annual Temperature (°C)	Absolute Maximum Temperature[c] (°C)	Average Maximum Temperature (°C)	Average Minimum Temperature (°C)	Average Annual RH (%)	Absolute Minimum RH (%)[z]
47.2	1313 ± 0.04	16.09 ± 0.10	36.70 ± 0.25	22.89 ± 0.12	9.98 ± 0.11	70.2 ± 0.50	2.00 ± 0.51

[a] Weather data for 2 years (1/1/2018 – 12/31/2019) that bracket the time of fruit development and collection were downloaded from the California Irrigation Management Information System (C.I.M.I.S.; http://cimis.water.ca.gov; accessed August 30, 2020). Data was from Station 198 in Santa Paula which was 5.33 km from the research site and Station 152 in Camarillo which was 14.17 km from the research site. The research site is situated at the transition zone between a strong coastal influence (Station 152) and inland Valley (Station 198). Data was averaged for each site across years.

[b] Altitude is for Saticoy, CA which is situated 2 km from research site.

[c] The absolute maximum temperature and the absolute lowest relative humidity for both weather stations and for both years occurred in October. The high temperatures are associated with strong seasonal winds which bring low relative humidity.