

Volatile Components of the Leaves of Various Avocado Cultivars

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The essential oils from the steam distillation of the leaves of avocados of three racial lineages were assayed by use of capillary column gas-liquid chromatography and mass spectroscopy. Estragole and two unidentified peaks were present only in the leaves of the five Mexican race avocados. The areas of the 54 major peaks in the 15 cultivars studied were subjected to principal component analysis, and the patterns of the three major factors were plotted. In all plots the members of the Mexican race were well separated into tight clusters but members of the Guatemalan and West Indian races formed loose, overlapping clusters. Previously unreported constituents of avocado leaves identified in this study were α -phellandrene, γ -terpinene, D-limonene, D-carvone, and *cis*-3-hexen-1-ol.

A comprehensive monograph on the taxonomy of the genus *Persea* in the western hemisphere by Kopp (1966) divided by the genus into the subgenera *Eriodaphne* and *Persea*. The subgenus *Persea* contains the commercial avocado *P. americana* Mill. Three botanically distinguished groups of avocados have long been recognized. On the basis of the respective centers of origin, as perceived at that time, these three groups of *P. americana* cultivars were assigned to three horticultural races: West Indian, Guatemalan, and Mexican (Popenoe, 1920). The main criteria used to assign a cultivar to a race were summarized by Bergh et al. (1973). The primary criteria of the Mexican race are anise-scented leaves and thin-skinned fruit. However, the characteristic scent was shown to result from estragole [1-methoxy-4-(2-propenyl)benzene] instead of anethole which is the major component of the oil of anise (Bergh et al., 1973). Principal components and cluster analysis, based on 67 characters, were applied to 38 cultivars representative of the pure races and hybrids (Rhodes et al., 1971). The results of the principal components analysis gave better agreement with the expected phenetic diversity of the germplasm than those of cluster analysis, but both analyses were needed to clarify indistinct groups.

Recently, in a study of the leaves of 44 cultivars of avocados, estragole was found to be present in all 14 cultivars assigned to the Mexican race at levels ranging from 0.018 to 1.21% by weight in the fresh leaves but was undetectable, i.e., <0.01%, in any of the 7 Guatemalan or 13 West Indian cultivars studied (King and Knight, 1987). Estragole levels in 16 Mexican hybrids ranged from not detectable to 0.79%, and estragole was not detectable in any hybrids containing no Mexican lineage. The importance of estragole as an insecticide has been shown in studies by Marcus and Lichtenstein (1979) in which the insecticidal properties toward house flies of 10 components of the oil of anise, including estragole, were determined. Also, estragole was recently found to have moderate insecticidal activity against the larvae of the Caribbean fruit fly, *Anastrepha suspensa* Loew (Benscoter and King, 1986), and also against house flies (*Musca domestica* L.) and fruit flies (*Drosophila melanogaster* M.) (Marcus and Lichtenstein, 1979). Estragole in leaves may afford protection from some species of insects and therefore confer a selective advantage to cultivars in which it is present.

Table I. Avocado Cultivars: Number, Race, and Location

no.	cultivar	race	location
1	Akbal	G	WA4-1-17-6
2	Kanan	G	W-4-1-7-6
3	Kayab	G	W-4-1-18-5
4	Nabal	G	W-4-1-9-5
5	Hass	G \times M ^a	Kendall Groves
6	Chapultepec Park	M	WA3-7-34
7	Duke	M	WA4-28-2
8	Las Campanas	M	WA2-19-32
9	Young	M	W-3-1-10-1
10	Younghans	M	WA2-13-37
11	Dade	WI	W-4-1-7-2
12	Fuchs	WI	WA3-7-42
13	Pollock	WI	W-3-1-6-3
14	Simmonds	WI	building 41
15	Wilson Popenoe	WI	W-4-1-5-16

^a The Hass cultivar is listed as pure Guatemalan by Bergh et al. (1973) and Rhodes et al. (1971) but as a hybrid by Malo et al. (1977). All cultivars are located at the USDA station except Hass, which is from a commercial orchard located in Dade County, FL.

The present work was undertaken to provide more information on the chemical constituents of avocado leaves and to find a chemometric method to differentiate avocados of the Guatemalan and West Indian races. The use of mass spectroscopy provided greatly increased sensitivity to detect and identify trace compounds.

MATERIALS AND METHODS

Gas Chromatography. Gas-liquid chromatography (GLC) data were obtained on a Hewlett-Packard Model 5880A instrument equipped with a level 4 terminal for instrument control and peak analysis, a flame ionization detector, and a glass capillary column (Supelco, Inc., Bellefonte, PA; SPB-5, 1.0 μ m, 0.75 mm i.d., 30-m length). For assays the following conditions were used: nitrogen carrier (15 mL/min) and makeup gas (45 mL/min); injection port, 250 $^{\circ}$ C; oven programmed from 50 to 250 $^{\circ}$ C at 8 $^{\circ}$ C/min; detector, 300 $^{\circ}$ C.

Mass Spectra. A Finnigan MAT Model 700 ion trap detector (ITD) in conjunction with a Hewlett-Packard Model 5710A gas chromatograph equipped with a 30-m capillary column (0.32 mm i.d., 1- μ m DB5, from J&W Scientific, Inc.) was used for separation and mass spectral identification. The oven temperature was programmed as follows: 50 $^{\circ}$ C initial, 0 time hold, 8 $^{\circ}$ C/min program rate, 250 $^{\circ}$ C final temperature for 2 min. Helium carrier gas was used at 1 mL/min column flow with a 50:1 split ratio and a 5- μ L injection.

Steam Distillation Procedure. The sample preparation procedure was identical to that previously reported, except for sample size, by King and Knight (1987). Mature leaves were

Table II. Retention Times and Areas of GLC Peaks from Avocado Leaf Essential Oils

time, min	peak areas for each cultivar														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
4.09	428	484	402	628	718	380	404	410	640	459	473	354	559	420	380
4.15	0	0	136	0	0	0	0	0	0	0	0	0	0	0	0
4.20	0	74	0	106	83	60	0	0	0	0	0	46	0	0	0
4.78	14	18	13	20	18	13	9	13	27	11	13	10	15	16	13
4.89	571	496	455	738	545	435	275	406	746	364	461	334	512	471	441
5.08	28	0	0	28	30	8	0	0	19	7	23	0	0	17	16
5.20	35	0	34	37	35	34	0	33	32	33	33	0	0	32	43
5.60	0	0	0	0	0	0	0	0	4	0	10	0	0	5	9
6.16	33	0	0	0	0	0	5	10	0	0	6	0	20	0	0
6.31	431	221	73	469	22	95	244	314	102	70	1431	177	906	448	206
6.59	6	0	0	0	0	0	5	0	0	0	45	0	20	5	0
7.00	1936	0	162	0	23	249	457	712	201	211	0	0	1193	0	161
7.10	486	383	125	846	41	150	380	448	136	87	2504	304	1376	763	321
7.23	83	21	0	46	0	0	26	43	8	0	318	18	165	40	20
7.49	0	0	0	57	0	0	2	0	0	0	610	4	37	28	29
7.76	0	0	0	0	0	0	0	0	0	0	53	0	0	0	0
8.05	477	93	60	266	16	71	133	182	50	57	860	59	539	126	76
8.26	0	0	0	15	0	0	0	0	0	0	437	9	244	33	39
8.52	5	0	0	0	0	0	0	0	0	0	23	0	5	0	0
8.69	59	0	0	0	0	0	0	0	0	0	19	0	48	0	11
9.05	0	0	0	0	0	0	0	0	0	0	123	36	147	72	0
9.23	12	0	0	0	0	0	0	0	0	0	1258	8	3401	20	540
9.69	0	0	0	0	0	0	0	0	0	0	124	0	0	0	0
10.02	0	0	0	0	0	0	0	0	0	0	97	0	0	0	0
10.51	0	0	0	0	0	0	0	0	0	0	28	0	0	0	0
10.73	25	0	0	0	0	0	2	0	0	0	13	0	10	0	7
11.05	0	24	0	0	0	0	0	0	0	0	0	0	0	0	0
11.28	0	0	0	0	0	0	0	0	0	0	74965	24886	69795	30107	39106
11.91	0	0	0	0	0	0	0	0	0	0	35	30	0	0	0
12.05	0	0	0	0	0	0	0	0	0	0	77	0	32	0	75
12.31	0	0	0	0	0	0	0	0	0	0	261	58	85	50	0
12.56	0	0	0	0	0	0	0	0	0	0	145	18	188	53	0
13.36	0	0	0	0	0	0	0	0	0	0	24	0	0	0	0
13.49	0	0	0	0	0	0	0	0	0	0	0	198	0	0	0
13.61	0	0	0	5	0	0	0	41	19	0	0	0	0	0	0
13.68	3	0	0	0	0	5	5	0	0	0	15	0	28	0	0
14.08	48	0	0	34	0	0	2	20	10	0	150	24	142	73	32
14.31	14	10	0	27	0	6	15	49	23	20	0	0	0	0	0
14.37	0	0	0	0	0	0	0	0	0	0	432	123	1033	208	205
14.82	240	346	28	438	26	39	168	205	85	70	277	101	919	190	167
15.34	36	37	0	61	0	0	17	28	8	0	34	8	108	28	14
15.46	0	0	0	0	0	0	0	0	0	0	84	0	48	0	0
15.74	116	47	10	143	11	16	38	72	36	30	207	16	206	51	21
15.95	25	14	0	32	0	18	5	7	0	17	95	0	37	67	7
16.23	96	72	1	219	0	14	65	127	30	14	281	14	232	586	75
16.76	337	197	22	0	0	0	0	0	0	0	554	17	722	90	54
16.92	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0
17.13	78	20	0	65	0	0	4	17	0	0	99	0	68	42	0
17.26	36	45	65	80	71	37	39	45	44	50	152	60	187	88	97
17.62	0	0	0	0	0	0	0	0	0	0	21	0	20	0	0
18.01	0	0	0	0	0	0	0	0	0	0	35	0	0	22	11
18.20	0	0	0	40	0	0	0	0	0	0	8	17	554	0	0
18.79	50	59	9	186	0	0	0	0	0	0	0	0	0	0	0
18.87	0	0	0	0	0	0	0	0	0	0	177	15	218	321	142
total	5708	2661	1610	4586	1639	1629	2308	3182	2221	1501	87090	26946	83818	34470	42317

^a Cultivars: 1, Akbal; 2, Hass; 3, Kanan; 4, Kayab; 5, Nabal; 6, Dade; 7, Fuchs; 8, Pollock; 9, Simmonds; 10, Wilson Popenoe; 11, Chapultepec Park; 12, Duke; 13, Las Campanas; 14, Young; 15, Youngmans.

collected for this study from February 16 to 24, 1988. Leaves were assayed on the day of collection as follows: A 5- or 10-g sample of leaf, 20 mL of hexane, and 100 mL of water were blended in a sealed 500-mL Eberbach blending container for 1 min. The mixture was transferred to a 1000-mL round-bottom flask. The container was rinsed with 150 mL of water, which was then added to the flask. The mixture was distilled, using ice water to cool the Liebig condenser and an ice bath to cool the 100-mL round-bottom collecting flask, until about 70 mL of water-hexane condensate was collected. The condensate was transferred to a separatory funnel, 5 g of NaCl was added, and the mixture was shaken. The hexane layer was separated, and about 10 mL was dried over 1 g of anhydrous sodium sulfate in a 12-mL culture tube. The hexane layer was then assayed using the ITD as described. Samples were also assayed by GLC-FID for quantification and to obtain retention indices. Further studies to

identify unknown components were performed using the original distillants, stored at -20 °C, and additional leaf samples which were prepared as needed.

Reagents. The hexane used as solvent was of UV grade from Burdick and Jackson, Inc. Chemicals used as qualitative standards were purchased at the highest available purity (95-99+%) from the following sources: Aldrich Chemical Co., Milwaukee, WI; Sigma Chemical Co., St. Louis, MO; Fluka Chemical Corp., Ronkonkoma, NY; or Pfaltz and Bauer, Inc., Waterbury, CT. The steam distillation procedure can concentrate volatile impurities in water up to 5-fold in the procedure used. Therefore, water used for steam distillation or as a component of the mobile phase in liquid chromatography was purified by redistilling distilled water through a packed column. The first 10% distillate was discarded and the following 80% collected for

Table III. Retention Times of Volatile Compounds from Avocado Leaves

peak	time, min	RI ^a	compound	ID ^b
1	4.09	791	hexanal	RI
2	4.15	796		
3	4.20	800	octane	RI
4	4.78	839		
5	4.89	844	<i>cis</i> -3-hexen-1-ol	RI + MS
6	5.08	858	1-pentanol	RI
7	5.20	865		
8	5.60	893		
9	6.16	924		
10	6.31	932	α -pinene	RI + MS*
11	6.59	948	camphene	RI
12	7.00	972	sabinene	RI*
13	7.10	977	β -pinene	RI + MS*
14	7.23	987	β -myrcene	RI*
15	7.49	1002	α -phellandrene	RI + MS
16	7.76	1016	α -terpinene	RI + MS
17	8.05	1029	cineol + <i>D</i> -limonene	RI + MS
18	8.26	1044	β -ocimene	RI*
19	8.52	1053		
20	8.69	1064		
21	9.05	1088	γ -terpinene	RI + MS
22	9.23	1096		
23	9.69	1122		
24	10.02	1140		
25	10.51	1166	1-octanol	RI
26	10.73	1177		
27	11.05	1195		
28	11.28	1208	estragole	RI + MS*
29	11.91	1249	<i>D</i> -carvone	RI
30	12.05	1258		
31	12.31	1276		
32	12.56	1292		
33	13.36	1345		
34	13.49	1354	α -cubebene	RI
35	13.61	1361	neryl acetate	RI
36	13.68	1368		
37	14.08	1392		
38	14.31	1407	methyleugenol	RI
39	14.37	1412	1-decyl acetate	RI
40	14.82	1437	β -caryophyllene	RI + MS*
41	15.31	1468	α -humulene	RI + MS*
42	15.46	1481		
43	15.74	1498		
44	15.95	1512		
45	16.23	1530		
46	16.76	1568		
47	16.92	1579	1-dodecanol	RI
48	17.13	1595		
49	17.26	1602	hexadecane	RI
50	17.62	1628		
51	18.01	1657		
52	18.20	1669		
53	18.79	1711		
54	18.87	1717		

^a The retention indices are from a 30-m Supelco 1.0- μ m film SPB-5 0.75-mm column programmed from 50 to 250 °C at 8 °C/min.^b The retention index (RI) and mass spectral (MS) comparisons were based on authentic standards. Samples marked with an asterisk were previously reported by Bergh et al. (1973).

use. The redistilled water gave no interfering peaks in the analyses involved.

RESULTS AND DISCUSSION

The cultivars tested, the racial type, and the location of each cultivar are shown in Table I. The assigned racial lineages, which are in agreement with those of Malo et al. (1977), were based on plant records of this station and the opinions of one of the authors (R.J.K.). The cultivar Hass, listed here as a Guatemalan-Mexican hybrid, is assigned to the Guatemalan race by Bergh et al. (1973) and Rhodes et al. (1971).

The retention times and areas for the major peaks determined by GLC for volatiles found in the leaves are

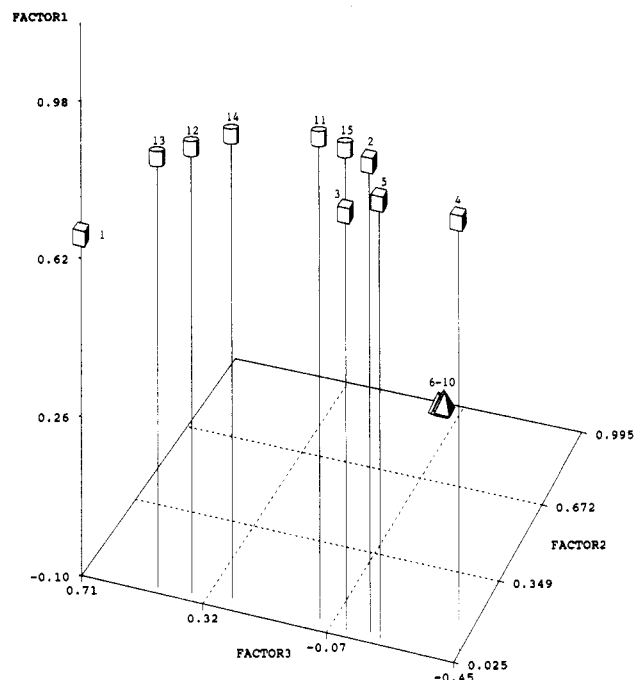


Figure 1. Plot of factor patterns for factors 1-3 obtained from principal component analysis of 54 peak areas for each of 15 avocado cultivars. The symbols used are as follows: cubes for the Guatemalan race and Hass; cylinders for the West Indian race; and pyramids for the Mexican race. The numbers corresponding to each cultivar are listed in Table I.

shown in Table II. The areas listed are the average of two replicate samples. Using the five largest peaks for each cultivar, the deviations of replicate peak areas from their mean were calculated. For these 70 duplicate peak areas, the mean deviation was 9.2% with a standard deviation of 7.5%. A total of 54 peaks are listed, but each cultivar had only a fraction of the peaks. Retention times, indices, and compound identifications are shown in Table III. Compounds were identified by comparing the retention index and mass spectrum of authentic samples in this laboratory. Tentative identification of other compounds was based on published retention indices and mass spectral compilations. Bergh et al. (1973) reported the major constituents of the essential oil from the leaves of *P. americana* cultivars as β -pinene, *p*-cymene, caryophyllene, farnesene, humulene, estragole, ϵ -cadinene, and anethole. Minor constituents reported were α -pinene, sabinene, β -myrcene, alloocimene, β -ocimene, 1,8-cineol, farnesol, and geranyl acetate. Additional compounds identified in this study were α -phyllandrene, γ -terpinene, *D*-limonene, *D*-carvone, and *cis*-3-hexen-1-ol. Peaks 1, 4, 5, 10, 13, 17, 40, 43, and 49 were found in all cultivars and peaks 28 (estragole), 39 (1-decyl acetate), and 54 (unidentified) only in cultivars assigned to the Mexican race. The occurrence of estragole in the leaves of only pure or hybrid Mexican cultivars was reported in a previous publication (King and Knight, 1978), but no compound was found to be unique to either the Guatemalan or West Indian races. In this study estragole, 1-decyl acetate, and an unidentified compound (peak 54) were present in all leaf samples from the Mexican race cultivars but not in any other samples. The total area of the peaks ranged from 26 946 to 87 090 for Mexican race samples and from 1610 to 5708 for all other samples. Most of the difference in peak area is due to the large amount of estragole in samples of the Mexican race cultivars, which in some cases exceeds 1% by weight in fresh leaves (King and Knight, 1987). In most cases the chromatograms of distillates from samples of the Mexican

race give a greater number of peaks than observed for other samples.

Because none of the previously discussed data provided any obvious chemical means of distinguishing between the Guatemalan and West Indian races of avocados, the data were analyzed using the FACTOR procedure of the SAS Institute to perform a principal component analysis (SAS Institute, 1985). The output from this procedure includes all of the eigenvalues and the pattern matrix for eigenvalues greater than 1. Three factors that had corresponding eigenvalues greater than 1 were retained, and the three two-dimensional graphs were examined. In all three plots the Mexican race avocados resulted in a well-separated tight cluster, whereas the other two races produced looser, overlapping clusters. In the three-dimensional plot of the three principal factors shown in Figure 1, the cluster for the five Mexican cultivars forms a well-separated, very tight group. The remaining cultivars do show clustering but not sufficiently to assign an unknown cultivar to a particular race. The application of the SAS CLUSTER procedure using the Ward method (SAS Institute, 1985) to the data to obtain pseudo *f* statistics (Sarle, 1983) indicated that three, four, or five clusters, in that order, best fitted the data. This result is in good agreement with the patterns visually observed in Figure 1 since either or both points 1 and 5 could be considered as outliers.

The presence of estragole, the total amount of volatile oils, and the results of principal components analysis of the GLC data from an assay of these oils all indicate that the Mexican race is distinct from the others. These data indicate no way to distinguish between the West Indian and Guatemalan races. The Guatemalan and West Indian races have often been placed in the same taxon and the Mexican race has been separated into a distinct botanical variety (Kopp, 1966). Bergh and Storey (1964) noted that the genetic behavior suggests that the three races not only belong to one species but that the Mexican race is no more distinct from the Guatemalan and West Indian races than they are from each other. The chemical data presented here indicate that the Mexican race is more different from the other two than they are from each other. Although taxonomic relationships have historically been determined on the basis of morphological and genetic data, this study demonstrates that chemical data can be a significant supplement.

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Registry No. Hexanal, 66-25-1; octane, 111-65-9; *cis*-3-hexen-1-ol, 928-96-1; 1-pentanol, 71-41-0; α -pinene, 80-56-8; camphene, 79-92-5; sabinene, 3387-41-5; β -pinene, 127-91-3; β -myrcene, 123-35-3; α -phellandrene, 99-83-2; α -terpinene, 99-86-5; cineol, 470-82-6; D-limonene, 5989-27-5; β -ocimene, 13877-91-3; γ -terpinene, 99-85-4; estragole, 140-67-0; D-carvone, 2244-16-8; α -cubebene, 17699-14-8; neryl acetate, 141-12-8; methyl eugenol, 93-15-2; 1-decyl acetate, 112-17-4; β -caryophyllene, 87-44-5; α -humulene, 6753-98-6; 1-dodecanol, 112-53-8; hexadecane, 544-76-3; 1-octanol, 111-87-5.