compounds than those from water distillation and steam distillation (samples A and B) too. These differences are partially due to the fact that these components are dissolved or suspended in the water layer easier than in the oil layer of the distillate; when distillated in the L–N apparatus, these components could easily be extracted by the solvent.

ACKNOWLEDGMENT

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Effects of pH Adjustment and Heat Treatment on the Stability and the Formation of Volatile Compounds of Garlic

Tung-Hsi Yu,* Chung-May Wu, and Shyh-Yuan Chen[1]

Effects of pH adjustment after blending, 30-min standing, and heat treatment of garlic cloves on the stability and the formation of volatile compounds of garlic were studied with GC in this study. It was found that the amount of the two isomeric cyclic compounds 3-vinyl-4H-1,2-dithiin and 2-vinyl-4H-1,3-dithiin, which were artifacts from allicin, decreased with increasing pH values, whereas the amount of diallyl disulfide, propenethiol, propyl allyl disulfide, and diallyl sulfide increased with increasing pH values. Formation of diallyl trisulfide, methyl allyl trisulfide, 1,3-dithiane, 2,4-dimethylfuran, aniline, and *trans*-1-propenyl methyl disulfide were favored in neutral conditions, whereas formation of methyl allyl disulfide, methyl allyl sulfide, 1,2-epithiopropane, and methyl propyl disulfide were favored around pH 9.0.

Garlic (*Allium sativum* Linn.) has been known since ancient times as a flavoring agent and for its medicinal properties. Semmler (1892) established the importance of diallyl disulfide and diallyl trisulfide in the flavor of garlic distillate. It was also evident at an early stage that the odorous compounds of interest were not present in the plant as such but were formed (enzymically) when the cellular tissue was disrupted. Cavallito and Bailey (1944) described the isolation of the odoriferous antibacterial substance allicin (diallyl thiosulfinate) from extraction of

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garlic with ethanol at room temperature. Stoll and Seebeck (1948) reported that intact garlic cloves contain 0.24% by weight *S*-allylcysteine *S*-oxide (alliin), a colorless odorless solid, and the enzyme allinase, which converts alliin into allicin. In addition to alliin, three (possibly four) γ-L-glutamyl derivatives of *S*-alk(en)ylcysteine sulfoxides were identified in garlic (Virtanen, 1965). These compounds are not cleaved by allinase; they represent only "potentially available" flavor. Consequently, peptidases and transpeptidases, which "release" these secondary flavor precursors to first precursors, thiosulfinates, are important enhancers of the aroma of garlic and its products (Virtanen, 1965).

The crude cell-free garlic enzyme solution, of unspecified purity, utilized by Stoll and Seebeck (1947, 1948) showed a broad pH optimum (5–8) and a temperature optimum (37 °C) under the conditions used. With use of protamine and ammonium sulfate as precipitation agents followed

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by fractionation on G-200 Sephadex, Mazelis and Crews (1968) obtained a 6-fold purification of the enzyme solution and reconfirmed the observations of Seebeck and Stoll. The purified enzyme possessed an optimum pH of 6.5 with S-methyl-L-cysteine sulfoxide as substrate. We also reported that formation of allicin is favored around pH 6.5 (Yu and Wu, 1989a). Allicin is very unstable and even decomposes easily at room temperature (Brodinitz et al., 1971). Decomposition of allicin proceeds by several pathways (Block, 1985). In one of them, allicin self-decomposes to form two isomeric cyclic compounds, 2-vinyl-4H-1,3-dithiin and 3-vinyl-4H-1,2-dithiin. We also reported previously that allicin decomposed into 2-vinyl-4H-1,3-dithiin and 3-vinyl-4H-1,2-dithiin during gas chromatography (Yu and Wu, 1989b).

Several methods have been reported for the quantitation of thiosulfinates (Barnard and Cole, 1959; Carson and Wong, 1959; Watanabe and Komada, 1966; Nakata et al., 1970). It might appear that the best method for measuring the flavor and aroma is gas–liquid chromatography (GLC) under carefully controlled conditions (Bernhard, 1968), except that the method is imperfect in that it measures secondary compounds of the enzymatic action and the relative contribution of these compounds to overall flavor and aroma is not known. In this study, GC was used to determine the effects of pH adjustment after blending, 30-min standing, and heat treatment of garlic cloves on the stability and the formation of volatile compounds of garlic.

EXPERIMENTAL SECTION

Sample Preparation. Garlic cloves were purchased at a local market. A total of 300 g of fresh peeled garlic cloves was blended with 750 mL of distilled water for 5 min in a Waring blender; the homogenate was then stood for 30 min. After standing, the pH value of the homogenate was adjusted by adding 1.0 M sodium hydroxide or 1.0 M hydrochloric acid solution. The mixtures ranged from pH 2.0 to 10.0 with 1.0 unit/interval. Volatiles of each mixture were extracted for 2 h by using the modified Likens–Nickerson apparatus (Romer and Renner, 1974), in which steam is the heating source; diethyl ether (40 mL, 99.5%; E. Merck) was used as the extracting solvent. Distillation was carried out immediately after pH adjustment. Dipropyl disulfide stock solution (Wako; 2 mL, 0.5093 g in 100 mL of diethyl ether) was then added to the extracts as an internal standard. The extracts were dried with anhydrous Na_2SO_4 and concentrated to minimal volume by using a spinning band distillation apparatus (Kontes).

Gas Chromatography. Gas chromatography was conducted on a Shimazu GC-9A equipped with a flame ionization detector (FID). A 50 m × 0.22 mm fused silica column (Chrompack International, B.V.) coated with CP-Wax 52 CB was used. The oven temperature was programmed from 50 to 200 °C at 2 °C/min. The injector and detector temperatures were 250 °C. The carrier gas was nitrogen at a flow rate of 0.75 mL/min. The data were recorded on a Shimadzu C-R3A integrator. Values reported were from the average of two analyses. The linear retention indices of the volatile components were calculated with n-paraffins (C_8–C_{25}; Alltech Associates) as references (Majlet et al., 1974).

Gas Chromatography–Mass Spectrometry. GC–MS was conducted with a Hewlett-Packard 5985B system. The gas chromatograph was installed with a fused silica capillary column (bonded CP-Wax 52 CB; 50 m × 0.32 mm). Operational parameters were as follows: carrier gas, helium; ionization voltage, 70 eV; ion source temperature, 200 °C.

Chemical Syntheses. 2-Vinyl-4H-1,3-dithiin and 3-vinyl-4H-1,2-dithiin were synthesized by the method of Bock et al. (1982).

RESULTS AND DISCUSSION

Figure 1 shows the capillary gas chromatograms of volatile components of garlic formed at (A) pH 2, (B) pH 6, and (C) pH 10. The comparison of the volatile constituent contents of different pH is shown in Table I.
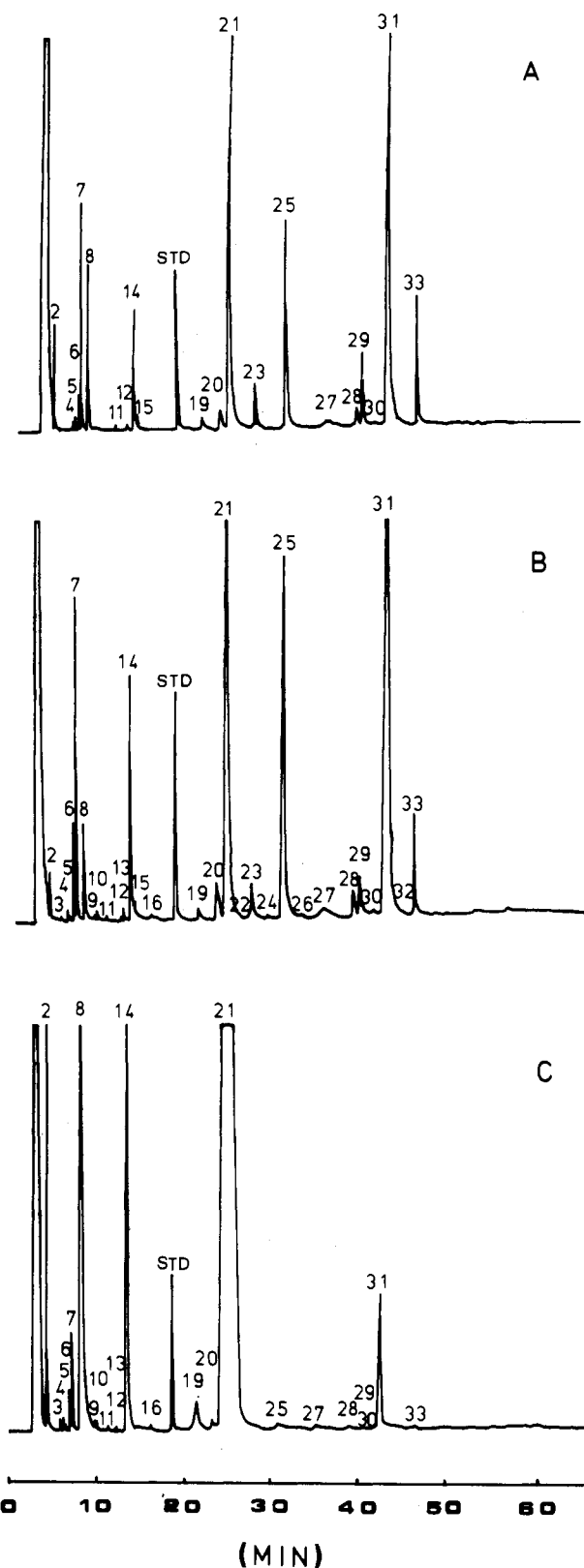

Figure 1. Capillary gas chromatograms of volatile components of garlic formed at (A) pH 2.0, (B) pH 6.0, and (C) pH 10.0.

Identification of the compounds was reported previously (Yu et al., 1989). The total amount of flavor compounds increased with increasing pH values from pH 6.0 to pH 9.0, whereas little decreased after pH 9.0. It seems that the 30-min standing time is not enough for complete enzymatic reaction or that formation of volatile compounds of garlic involved another kind of enzyme system besides allinase. In addition to alliin, three (possibly four) γ-
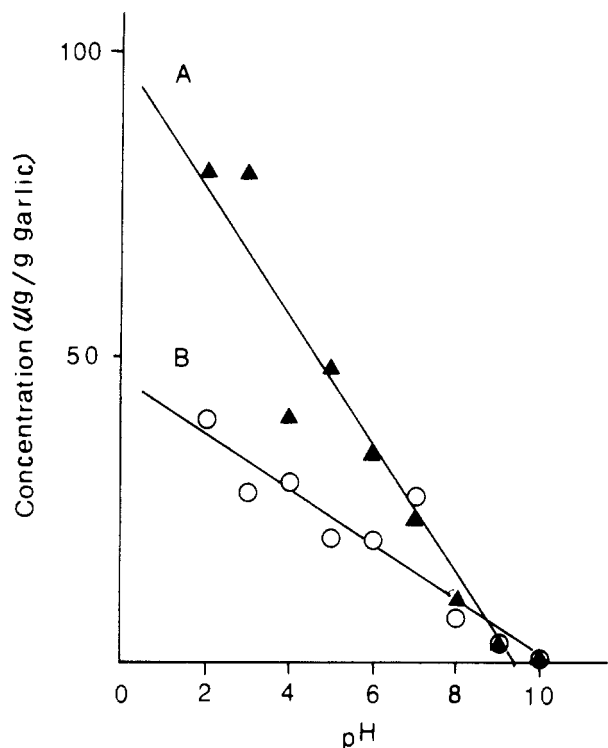
Figure 2. Changes of the amount of (A) 2-vinyl-4*H*-1,3-dithiin [$y = 99.320 - 10.583x$, $R = 0.96$] and (B) 3-vinyl-4*H*-1,2-dithiin [$y = 46.741 - 4.518x$, $R = 0.92$] at different pHs with heat treatment.
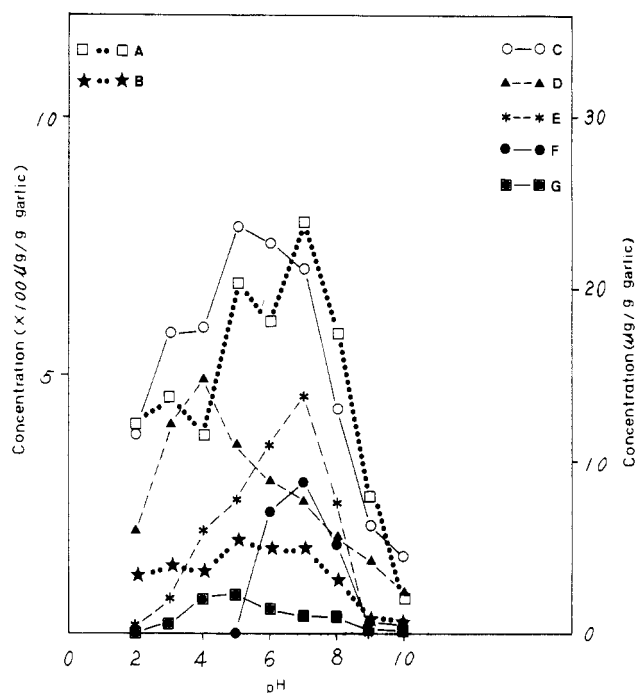

Figure 4. Volatile compounds of garlic formed at different pHs with heat treatment: A, diallyl trisulfide; B, methyl allyl trisulfide; C, $C_6H_{10}S_2$ (peak 20 in Table I); D, 1,3-dithiane; E, 2,4-dimethylfuran; F, aniline; G, *trans*-1-propenyl methyl disulfide.
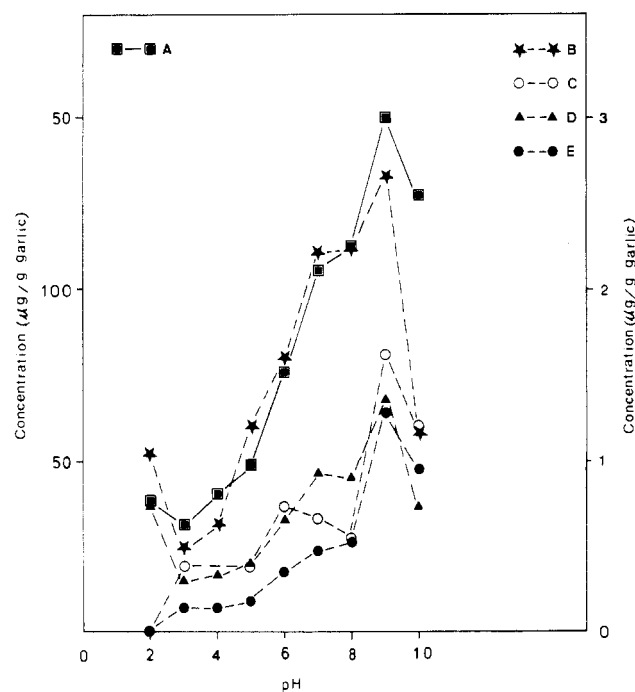

Figure 3. Volatile compounds of garlic formed at different pHs with heat treatment: A, diallyl disulfide; B, propenethiol; C, propyl allyl disulfide; D, diallyl sulfide.

Figure 5. Volatile compounds of garlic formed at different pHs with heat treatment: A, methyl allyl disulfide; B, methyl allyl sulfide; C, $C_6H_{10}S$ (peak 10 in Table I); D, 1,2-epithiopropane; E, methyl propyl disulfide.

glutamyl derivatives of *S*-alk(en)ylcysteine sulfoxides were identified in garlic (Virtanen, 1965). These compounds are cleaved by peptidases and transpeptidases other than allinase. Schwimmer and Austin (1971) found that γ-glutamyl transpeptidase (GGT) acts optimallyl at pH 9.0. The reason why the total amount of volatiles of garlic reached the highest level around pH 9.0 was postulated that the formation of these compounds at pH around 9.0 was due to high activity of GGT.

Figure 2 shows the effect of pH and heat treatment on the formation of 2-vinyl-4*H*-1,3-dithiin and 3-vinyl-4*H*-1,2-dithiin, which were confirmed to be the major artifacts

from allicin during the gas chromatography by Block (1985) and Yu and Wu (1989b). The amount of these two compounds decreased with increasing pH values. It seems that allicin was more stable in acidic conditions than in other pHs.

Figure 3 shows the effect of pH and heat treatment on the formation of diallyl disulfide, propenethiol, propyl allyl disulfide, and diallyl sulfide. Among these compounds, diallyl disulfide was the most abundant compound formed. The amount of these compounds increased with increasing

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Table I. Effects of pH and Heat Treatment on the Stability and the Formation of Volatile Compounds of Garlic

peak no.[a]	compound	$I_K{}^c$ (CP-Wax 52 CB)	yield[d] $\times 10^{-6}$ g/g garlic bulb at pH								
			2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0
2	propenethiol		14.41	6.94	6.90	3.66	5.58	9.02	13.90	45.27	92.67
3	1,2-epithiopropane	924	-[e]	0.14	0.14	0.18	0.35	0.48	0.53	1.28	0.95
4	methyl allyl sulfide	956	1.05	0.50	0.64	1.20	1.59	2.20	2.22	2.65	1.16
5	dimethyl disulfide	1077	1.94	1.20	0.93	1.04	1.42	1.83	1.85	1.75	1.00
6	2,4-dimethylfuran	1093	5.87	12.10	14.80	10.93	8.81	7.67	5.63	4.19	2.53
7	2-propen-1-ol	1125	37.89	22.17	22.12	32.47	41.63	48.45	56.50	23.36	17.81
8	diallyl sulfide	1148	45.18	29.82	41.65	17.58	41.45	43.38	68.20	225.71	478.48
9	tetrahydro-2,5-dimethylthiophene	1197	-	0.49	0.65	0.46	0.86	0.71	0.75	0.63	0.58
10	$C_6H_{10}S$ [m/e 45 (100), 42 (86), 43 (86), 23 (86), 55 (64), 73 (48), 71 (28), 64 (28)][b]	1233	-	0.38	0.67	0.38	0.73	0.66	0.54	1.62	1.20
11	methyl propyl disulfide	1252	0.75	0.30	0.34	0.40	0.65	0.93	0.89	1.36	0.74
12	3-methyl-2-cyclopentene-1-thione	1261	1.02	1.31	0.41	0.27	0.41	1.34	0.32	0.11	0.18
13	*trans*-1-propenyl methyl disulfide	1270	-	0.53	2.03	2.15	1.39	1.07	1.06	-	0.30
14	methyl allyl disulfide	1282	38.10	30.85	40.39	48.83	76.14	105.03	112.74	149.91	127.59
15	1,3-dithiane	1296	0.44	1.97	5.96	7.84	10.99	13.81	7.70	-	-
16	aniline	1328	-	-	-	-	7.11	8.83	5.24	0.64	0.60
19	propyl allyl disulfide	1432	5.37	4.11	6.08	5.04	8.09	10.12	12.58	20.47	26.37
20	$C_6H_{10}S_2$ [m/e 73 (100), 146 (99), 81 (79), 41 (57), 45 (54), 105 (45), 39 (35), 71 (30)]	1471	11.67	17.48	17.75	23.67	22.67	21.12	13.02	6.27	4.45
21	diallyl disulfide	1490	287.14	325.92	377.21	472.19	720.26	1002.06	1264.41	2223.27	2324.33
22	1,2-dimercaptocyclopentane	1519	-	-	-	-	-	-	0.42	-	-
23	unknown [m/e 103 (100), 104 (64), 45 (39), 119 (16), 39 (15), 69 (11), 105 (11), 74 (8)]	1532	22.17	18.34	12.95	10.86	8.91	6.21	3.38	0.44	-
24	4-methyl-5-vinylthiazole	1560	-	0.40	1.97	0.32	1.57	0.96	0.77	-	-
25	methyl allyl trisulfide	1593	110.84	133.27	120.39	182.02	167.39	169.60	100.94	32.89	2.01
26	2-methylbenzaldehyde	1644	-	-	10.49	0.32	0.31	-	-	-	-
27	3,5-diethyl-1,2,4-trithiolane	1682	36.43	24.50	21.17	23.72	42.95	25.83	13.78	2.23	1.22
28	isobutyl isothiocyanate	1753	14.22	7.51	4.14	16.50	14.60	11.70	15.33	10.38	2.30
29	3-vinyl-4H-1,2-dithiin	1761	39.95	27.92	29.89	20.51	20.34	27.27	7.53	2.77	0.54
30	unknown [m/e 146 (100), 74 (73), 73 (64), 117 (62), 72 (55), 71 (32), 138 (30), 45 (23)]	1772	3.17	3.96	6.32	3.31	6.58	3.81	2.55	2.43	1.05
31	diallyl trisulfide	1806	404.57	457.93	384.88	685.52	598.05	832.63	581.89	268.62	66.07
32	unknown [m/e 138 (10), 111 (92), 109 (64), 110 (62), 95 (60), 123 (50), 77 (48), 151 (36)]	1851	-	-	0.39	-	-	-	-	-	-
33	2-vinyl-4H-1,3-dithiin	1872	80.39	80.48	40.52	48.41	34.56	23.57	10.39	3.17	0.90
	total		1162.57	1210.52	1171.78	1218.11	1845.39	2380.29	2305.06	3303.22	3155.03

[a] Number refers to Figure 1. [b] Number in parentheses indicates relative percentage. [c] Calculated Kovats' retention indices. [d] Average of two experiments using dipropyl disulfide as internal standard. [e] Nondetected.

pH values. It seems that these compounds came from the decomposition of allicin directly under the heat treatment.

Figure 4 shows the effect of pH and heat treatment afterward on the formation of diallyl trisulfide, methyl allyl trisulfide, $C_6H_{10}S_2$ (peak 20 in Table I), 1,3-dithiane, 2,4-dimethylfuran, aniline, and *trans*-1-propenyl methyl disulfide. Formation of these compounds was favored in the neutral or weak acidic pH range. Among these compounds, diallyl trisulfide and methyl allyl trisulfide were the two most abundant compounds formed. The reasons in the optimum formation pH for these compounds was postulated that they were more stable at this pH range than at other pHs under heat treatment or that allicin was decomposed easier into these compounds at pH ≈ 6.0 than in other pHs under heat treatment.

Figure 5 shows the effect of pH and heat treatment on the formation of methyl allyl disulfide, methyl allyl sulfide, $C_6H_{10}S$ (peak 10 in Table I), 1,2-epithiopropane, and methyl propyl disulfide. Formation of these compounds was favored around pH 9.0. Among these compounds, methyl allyl disulfide was the most abundant compound formed. The reason why the amounts of these compounds reached the highest level around pH 9.0 was postulated

that allicin decomposed into these compounds easier at pH ≈ 9.0 than at other pHs or that formation of these compounds was due to the high activity of GGT.

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Volatile Components of Pineapple Guava

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Volatile components of pineapple guava (Feihoa sellowiana Berg) fruit were identified by gas chromatography–mass spectrometry. Major constituents were germacrene D, bicyclogermacrene, methyl benzoate, β-caryophyllene, (Z)-3-hexenyl benzoate, linalool, humulene, and 3-octanone. Of the 85 compounds identified, 47 were not previously reported in pineapple guava.

Intrigued by reports that guava species in Hawaii were major hosts for the Mediterranean fruit fly (Vargas et al., 1983a) and the oriental fruit fly (Vargas et al., 1983b), we decided to determine the composition of volatile compounds of pineapple guava. Presumably, gauva volatiles act as attractants for these fruit flies, so identification of the volatiles plus testing for attractancy could lead to recognition of compounds potentially useful in fruit fly lures.

Volatile constituents of the common guava (Psidium guajava, L.) have been extensively investigated. In a distillate from guava puree, 22 components—mostly alcohols and esters—were identified (Stevens et al., 1970). An additional 21 compounds were identified by GC–MS analysis of the headspace of volatiles obtained via high vacuum from partially freeze-dried guava puree (Torline and Ballschmieter, 1973). Comparison of the content of eight sesquiterpenes in the leaf essential oils from wild guava trees allowed classification into three main chemotypes (Smith and Siwatibau, 1975). Twelve terpene hydrocarbons from a dichloromethane extract of guava puree and the possible significance of these compounds as insect attractants were reported by Wilson and Shaw (1978). Shiota (1978) reviewed studies of guava fruit flavor and, in addition, analyzed volatile components of purees, peels, and leaves of guava by GC–MS, identifying 39 compounds. From an isopentane extract of a guava vacuum steam distillate, MacLeod and Gonzalez de Troconis (1982) also identified 39 compounds and evaluated their contribution to guava aroma. Volatiles from fresh guava fruit pulp obtained by vacuum distillation with subsequent solvent extraction when analyzed by GC, GC–MS, and GC/FTIR spectroscopy contained 154 identifiable compounds (Id-

stein and Schreier, 1985). Five of these were terpenes, but no sesquiterpenes were reported. In contrast, a study of the essential oil from guava fruit peels showed 7 terpenes and 10 sesquiterpenes as well as caryophyllene oxide among the 21 compounds definitely identified (Oliveros-Belardo et al., 1986). Relative amounts of aroma compounds (88) in two Egyptian guava cultivars were compared and the odors of many characterized (Askar et al., 1986).

Pineapple guava (Feihoa sellowiana Berg) fruit volatiles have also been of interest. Notable among the volatiles distilled from the fruit were methyl benzoate and ethyl benzoate, which provided much of the distinctive aroma (Hardy and Michael, 1970). Three other benzoate esters were among the 56 compounds found in a steam distillate of fruit of a New Zealand cultivar by Shiota et al. (1980). This group also compared the volatile compounds in steam distillates from guava, strawberry guava (Psidium cattleianum Sabine), and yellow guava (Psidium cattleianum Sabine var. lucidem Hort). Collected headspace volatiles from a New Zealand feihoa fruit contained 11 compounds, 2 of these not previously reported (Shaw et al., 1983). The GC analysis of essential oil of ripe feihoa fruit cultivated in Georgia (USSR) indicated more than 250 volatile components of which 47 were identified (Starodubtseva and Kharebava, 1986).

EXPERIMENTAL SECTION

Materials. Mature abscissed fruits from a F. sellowiana plant in Berkeley, CA, were collected and surface cleaned. Part of the collection was put into a plastic bag and stored at −30 °C. The remainder was allowed to stand overnight at room temperature before being extracted with acetone.

Acetone Extraction. Whole fruits (2000 g) were blended with distilled acetone in a Waring Blendor. The mixture was filtered, and the filter cake was rinsed with acetone before being reblended with fresh solvent. Combined extracts amounted to 7 L. Acetone was removed from the extract by distillation under reduced pressure, and the distilled acetone plus some volatile fruit com-

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