which was injected. These observations suggest that the natural aroma of unprocessed rice is a blend of many compounds.

Our next objective is to prepare a synthetic rice aroma that equals the natural aroma in quality. Later the synthetic preparations should be evaluated in behavioral tests with *Rattus rattus mindanensis*. Our final objective is the preparation of a bait attractant that the rice farmer can use to protect his crops from rat damage.

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LITERATURE CITED

Bullard, R. W., M.S. Thesis, University of Denver, 1975.
Bullard, R. W., Shumake, S. A., *J. Wildl. Manage.*, in press (1977).
Collins, E., *J. Agric. Food Chem.* **19**, 533 (1971).
Fall, M. W., West, R. R., 4th National Conference of the Pest Control Council of the Philippines, Legaspi City, May 16–18, 1973.
Hougen, F. W., Quilliam, M. A., Curran, W. A., *J. Agric. Food Chem.* **19**, 182 (1971).
Koehler, P. E., Odell, G. V., *J. Agric. Food Chem.* **18**, 895 (1970).
Mulders, E. J., *Z. Lebensm. Unters-Forsch.* **151**, 310 (1973).
Schultz, T. H., Black, D. R., Bomben, J. L., Mon, T. R., Teranishi, R., *J. Food Sci.* **32**, 698 (1967).
Wang, P. S., Kato, H., Fujimaki, M., *Agric. Biol. Chem.* **33**, 1775 (1969).
Wang, P. S., Kato, H., Fujimaki, M., *Agric. Biol. Chem.* **34**, 561 (1970).
Withycombe, D. A., Ph.D. Dissertation, University of Wisconsin, 1974.

Received for review May 6, 1976. Accepted August 19, 1976. This research was conducted with funds provided to the U.S. Fish and Wildlife Service by the U.S. Agency for International Development under the project "Control of Vertebrate Pests: Rats, Bats, and Noxious Birds", PASA RA(ID) 1-67. Reference to trade names does not imply U.S. Government endorsement of commercial products. Presented at the 170th National Meeting of the American Chemical Society, Chicago, Ill., Aug 1975, Abstract AGFD-48.

Volatile Components of Papaya (*Carica papaya* L., Solo Variety)

Robert A. Flath* and Ralph R. Forrey

Volatile components of fresh papaya fruit were concentrated by several different methods. The concentrates were examined by combined gas chromatography–mass spectrometry (GC–MS), and a total of 106 compounds were identified. Linalool is the major component of these concentrates, followed by benzyl isothiocyanate. The relative proportions of the major components are shown to be dependent upon the treatment received by the fruit tissue before and during volatiles concentration.

Papaya (*Carica papaya* L.) has become a commercially important fresh fruit crop in the United States market. Most of the fruit is grown in Hawaii, and is air-freighted to the mainland for distribution, although there is some production in Florida as well. Attempts have been made to make use of sound but blemished or misshapen cull fruit in a processed form, but they have met with only partial success. One of the major difficulties is that the fruit material develops pronounced off-odors when the tissue is macerated and allowed to stand (Chan et al., 1973). This study of papaya flavor was therefore initiated with a twofold aim: to determine the identities of the components responsible for fresh ripe papaya flavor, and to establish a composition "baseline" for comparison with the makeup of processed papaya material. This paper deals with the composition of the volatiles from fresh papaya; organoleptic correlations are presently under study, and will be reported in a subsequent paper.

Relatively little information about the composition of papaya fruit appears in the literature. Most references deal with the presence of benzyl isothiocyanate and its glucosinolate precursor (Ettlinger and Hodgkins, 1956; Gmelin and Kjaer, 1970; Tang, 1970, 1971; Tang and Syed, 1972). Only one study on the general volatiles makeup of papaya has been reported (Katague, 1964; Katague and Kirch, 1965). In Katague's study the homologous series of normal primary alcohols from C_1 to C_6 and the primary isoalcohols from C_3 to C_5 were reportedly found, along with the corresponding acetate esters. 2-Heptanone was also listed as a component. Some of the present authors' preliminary results on papaya composition were reported in the paper by Chan et al. (1973).

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EXPERIMENTAL SECTION

Aroma Concentrate Preparation. Several different methods were used for the concentration of papaya volatile components. In none of the concentration runs was an attempt made to completely strip the volatile materials from the papaya sample, so the yields noted are not meant to represent the maximum possible. In all cases the starting fruit material was prepared in the following manner. Fresh papayas (Solo variety; three-quarters ripe, shipped by air-freight from Hawaii in shredded newsprint packing) were purchased in a retail market. Unblemished fruits were selected and held at room temperature until

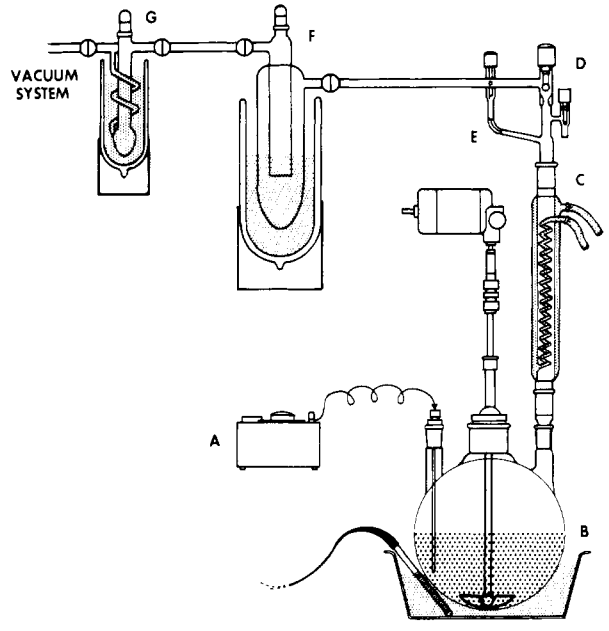

Figure 1. Apparatus used for vacuum stripping and trapping of papaya volatiles: (A) Null pyrometer with thermocouple; (B) flask (12 l.) containing fruit-water mixture, suspended in warm water bath; (C) double-walled reflux condensor held at 0 ± 2 °C; (D) main valve to traps and vacuum line; (E) capillary bypass to traps and vacuum line for slow initial pumpdown; (F and G) traps, cooled with liquid nitrogen.

fully ripe, as judged by their external color and degree of softness. After the stem and bud ends were removed, the fruits were quartered without cutting into the seed cavity. The intact greyish-black seeds were carefully removed to avoid rupturing the seed coats (Tang, 1970), and the skin was cut away from the light orange flesh. The sections were cut into thin slices and placed in the appropriate concentration apparatus pot.

A. *Vacuum Trapping Train.* Initial concentration attempts made use of the technique described by Forss et al. (1967). The apparatus shown in Figure 1 was employed. Fruit (4 kg) and distilled water (3.5 l.) were boiled under vacuum (6 mm), reaching a pot temperature of 8 °C. The reflux condensor caused condensation of most of the boiling water, while low concentration organic volatiles were collected in the traps. Ether (freshly distilled) extraction of the trap contents after 2 h, followed by distillation of the ether from the extract, yielded approximately 0.02 g (5 ppm) of residue.

B. *Codistillation–Extraction (Vacuum).* The second concentration method made use of the concurrent steam distillation–solvent extraction head shown in Figure 2. This is an extensively modified version of the device described by Likens and Nickerson (1964). Modifications incorporated were designed to provide more complete mixing of solvent and steam vapors and also to include more condensing surface area, particularly for use at reduced pressure. The sample pot containing papaya (4.64 kg) and distilled water (3 l.) was attached to the right leg of the apparatus. A small solvent flask containing purified isooctane (2,2,4-trimethylpentane, acid washed and distilled, 200 ml) was attached to the left leg, the system pressure was reduced to 10 cm, and both flasks were heated to boiling (sample pot temperature = 52 °C). The condensor was kept at approximately 0 °C by circulating a

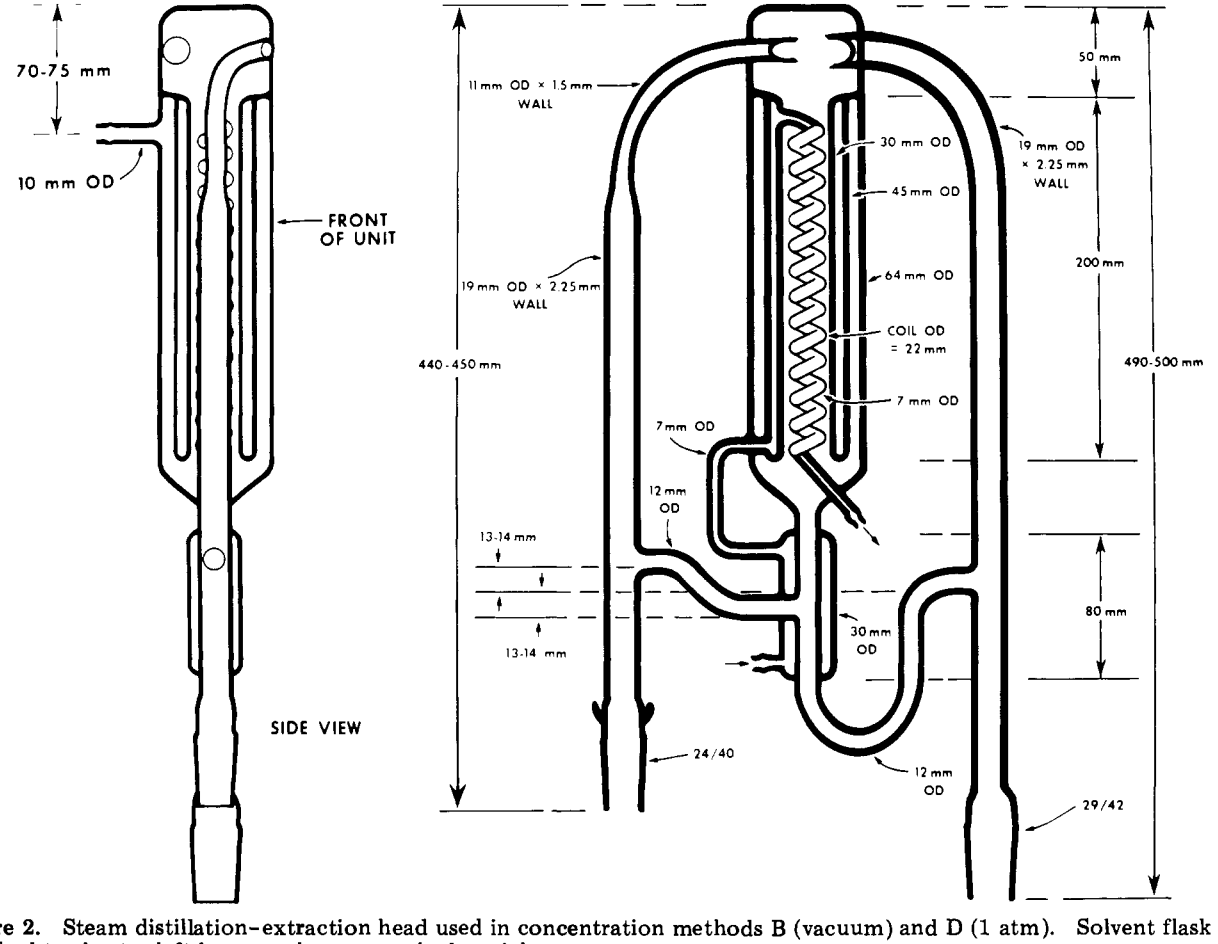

Figure 2. Steam distillation–extraction head used in concentration methods B (vacuum) and D (1 atm). Solvent flask attached to shorter left leg, sample pot attached to right.

chilled ethylene glycol–water solution through it. After 4.5 h the system was disassembled and solvent was distilled from the extract. Approximately 0.1 g of concentrate remained (22 ppm).

C. *Vacuum Distillation.* Several batches of fruit were stripped by distillation under vacuum (20 mm) as described in a previous paper (Forrey and Flath, 1974). The distillate was frozen in a cooled receiver during distillation, and was subsequently melted and extracted with freshly distilled ether. In a typical run, using 7.4 kg of fruit and 3 l. of water, 0.5 l. of distillate was collected, and approximately 0.03 g of concentrate was isolated after ether (5 × 100 ml) extraction, followed by concentration of the organic extract (4 ppm).

D. *Codistillation–Extraction (1 atm).* The steam distillation–extraction head (Figure 2) was also used at atmospheric pressure, with freshly distilled ether as a solvent. A mixture of papaya (6.4 kg) and distilled water (2.5 l.) was boiled for 3.75 h in air. Removal of solvent left 0.2 g of concentrate (31 ppm) having a pronounced cooked aroma, in contrast to the aromas of the concentrates prepared by any of the vacuum techniques previously described.

Concentration of Free Acids in Papaya Pulp. A quantity of papaya tissue (2.91 kg) was chopped and placed in a 12-l. flask. After 18 h at room temperature the material was strained, yielding 1.25 l. of cloudy juice, which was extracted with freshly distilled ether (4 × 200 ml). The combined ether extract was washed with 2 N sodium hydroxide solution (100 ml). This basic aqueous material was repeatedly extracted with freshly distilled ether and then was acidified with 6 N hydrochloric acid (35 ml). The acidic aqueous mixture was extracted with distilled ether (5 × 35 ml), and the ether extract was treated with diazomethane. Formic acid was added dropwise to react with excess diazomethane. The ether solution was dried with anhydrous sodium sulfate, and the bulk of the solvent was distilled, leaving 0.43 g of ether solution. This was shown to be approximately 82% solvent by GC, so the methyl ester yield was 77 mg.

Component Separation and Identification. Aroma concentrates and the esterified acid mixture were separated by gas–liquid chromatography (GLC), using 500 ft × 0.03 in. i.d. (152 m × 0.76 mm i.d.) stainless steel open tubular columns coated with methyl silicone oil (General Electric SF 96(50) containing 5% by weight of Igepal CO 880). The columns were temperature programmed in a standard manner for all runs. The oven was held at 50 °C for 10 min after injection, then programmed at 1 °C/min to 185 °C, with a final period of 50 min at 185 °C. A Hewlett/Packard 5831A gas chromatograph was used in most of this work to provide reproducible chromatograms and quantitative area percent data. To minimize column mass, newer 0.03 in. i.d. columns for the H/P 5831A were prepared in our laboratory from 0.058 in. o.d. (1.47 mm) tubing (Handy and Harman) instead of 0.063 in. o.d. (1.60 mm) material. A saving of approximately 20% in column mass is attained by this change (1.5 kg instead of 1.9 kg). In addition, the individual columns are coiled in the "honeycomb spool" format described by Verbeek and Maarse (1973) for better air-column contact.

Identifications are all based on unit resolution mass spectral data obtained with a GC–quadrupole mass spectrometer system. The gas chromatograph consists of a modified Beckman Thermotrac temperature programming oven with added injector. The mass spectrometer employs an Electronic Associates mass filter and Finnigan Corporation electronics. The GC separation is monitored via the integrator output of the Finnigan control unit. The separator interface is identical with that previously described (Forrey and Flath, 1974) except that a double layer of 0.001 in. thick methyl silicone membrane (General Electric) is used.

After tentative identification of a component by MS, the GC relative retention behavior of an authentic sample was always checked to be certain that it was eluted at the proper point in the chromatogram. Rather than combining the compound in question with a papaya concentrate sample for each verification, a normal hydrocarbon mixture was employed as a reference. A portion of the papaya volatiles concentrate being examined was co-injected with some of the hydrocarbon mixture. The resulting "calibrated" papaya chromatogram was then used for comparison with a chromatogram of the tentatively identified components mixed with a sample of the hydrocarbon mixture. Insufficient papaya extract was available for direct co-injection with all tentatively identified compounds.

RESULTS AND DISCUSSION

The neutral components identified in this study are listed in Table I. This table also provides two numbers for each compound entry. The first is the retention time of the component in the chromatogram in Figure 3, and the second number indicates the area percent of the component in a chromatogram of the mixture. The area percent values are only valid in detail for the sample chromatographed (vacuum distillate 5; method C in the Experimental Section); the relative amounts of the individual components vary considerably among the different concentrates (uncorrected area percent values are used in this discussion as a direct measure of component concentration). Linalool is always the major component, and benzyl isothiocyanate is usually the second most abundant. However, the amounts of the linalool oxides A and B as well as that of phenylacetonitrile are quite variable (Table II). This appears to be correlated with the treatment received by the papaya before and during the concentration step. The numbers shown in Table II are area percent values obtained from gas chromatograms of each sample, using the Hewlett/Packard 5831A instrument. Therefore, they do not indicate absolute amounts. For example, only 4.2% of the total chromatogram area is attributable to benzyl isothiocyanate in sample 1, while 8.3% of the area in sample 3 represents the same compound. This does not mean that twice as much benzyl isothiocyanate was isolated in 3 as in 1, but only that benzyl isothiocyanate comprises a greater proportion of 3 than of 1.

Some of the variation evident in Table II is probably due to fresh sample variability. Fruit was obtained at different times of the year, very likely from different growers and somewhat different areas. However, much of the variation can be correlated with differences in the concentration scheme conditions. In general, when the fruit pulp was held for longer periods of time, either before or during stripping, the concentration of benzyl isothiocyanate increased, relative to that of linalool, as did the concentrations of linalool oxides A and B. For example, the fruit material for concentrate 7 was held overnight before distilling, in contrast to the concentrate 6 papaya pulp, which was subjected to vacuum distillation immediately after preparation of the fruit. Higher temperatures and/or the presence of air brought about an accumulation of phenylacetonitrile; the fruit–water mixture used to prepare 8 was heated to boiling at atmospheric pressure in air, yielding a considerable increase in the percent concen-

Table I. Volatile Papaya Components

Alcohols	
Methanol	14.07[a] (tr)[b]
Ethanol	15.57 (tr)
2-Propanol	16.43 (0.06)
1-Propanol	19.54 (tr)
2-Butanol	21.41 (tr)
2-Methyl-1-propanol	23.95 (0.06)
2-Methyl-2-butanol	24.50 (tr)
1-Butanol	27.52 (0.47)
1-Penten-3-ol	29.34 (0.10)
2-Pentanol	30.17
3-Pentanol	30.17 (0.05)
3-Methyl-1-butanol	35.48 (0.09)
2-Methyl-1-butanol	36.04 (tr)
1-Pentanol	39.94 (tr)
3-Hexanol	42.51 (0.08)
2-Hexanol	43.16 (0.05)
cis-3-Hexen-1-ol	54.20 (tr)
1-Hexanol	54.78 (0.22)
trans-2-Hexen-1-ol	55.71 (tr)
1-Heptanol	70.34 (tr)
trans-2,6-Dimethyl-3,6-epoxy-7-octen-2-ol (A)	85.22 (4.86)
1-Octanol	85.97 (0.12)
cis-2,6-Dimethyl-3,6-epoxy-7-octen-2-ol (B)	87.56 (8.24)
Linalool	90.59 (67.69)
Benzyl alcohol	90.93 (0.33)
2-Phenylethanol	98.90 (tr)
cis-2,6-Dimethyl-2,6-epoxy-7-octen-3-ol (C)	100.71 (0.14)
trans-2,6-Dimethyl-2,6-epoxy-7-octen-3-ol (D)	101.60 (0.12)
α-Terpineol	103.25 (tr)
Geraniol	112.66 (0.17)
Aldehydes	
Acetaldehyde	13.89 (tr)
2-Methyl-2-pentenal	45.73 (tr)
Benzaldehyde	68.46 (tr)
Esters	
Ethyl acetate	21.17 (tr)
Methyl propionate	22.24 (tr)
Methyl 2-methylpropionate	27.02 (tr)
Methyl butyrate	31.20 (0.06)
Ethyl 2-methylpropionate	35.81 (0.08)
Methyl crotonate	36.26 (tr)
2-Methyl-1-propyl acetate	37.13 (tr)
Ethyl butyrate	41.23 (tr)
1-Propyl propionate	42.14 (0.05)
Methyl pentanoate	44.77 (tr)
Ethyl crotonate	47.62 (tr)
Ethyl 2-methylbutyrate	48.93 (tr)
Ethyl 3-methylbutyrate	49.33 (tr)
1-Propyl butyrate	49.75 (tr)
3-Methyl-1-butyl acetate	52.72 (tr)
Methyl hexanoate	60.20 (tr)
1-Butyl butyrate	71.41 (tr)
2-Methyl-1-propyl 2-methylbutyrate	73.42 (tr)
3-Methyl-1-butyl 2-methylpropionate	74.99 (tr)
Methyl heptanoate	75.93 (tr)
γ-Hexalactone	82.26 (0.24)
Methyl benzoate	87.90 (tr)
Esters (continued)	
γ-Heptalactone	96.53 (tr)
Ethyl benzoate	99.17 (tr)
γ-Octalactone	111.15 (0.14)
δ-Octalactone	115.10 (0.05)
Methyl decanoate	119.41 (tr)
2-Methyl-1-propyl benzoate	120.52 (tr)
Benzyl butyrate	122.39 (tr)
γ-Nonalactone	125.57 (tr)
1-Butyl benzoate	125.90 (tr)
γ-Decalactone	138.44 (tr)
Ethers	
1-Ethoxy-1-methoxyethane	25.49 (tr)
1,1-Diethoxyethane	32.64 (tr)
1,1-Diethoxy-2-methylpropane	51.35 (tr)
Halides	
Methylene chloride	17.18 (tr)
Chloroform	22.24 (tr)
1,2-Dibromoethane	43.02 (tr)
Heteroatom (N, S) Compounds	
Methyl thiocyanate	33.57 (tr)
Phenylacetonitrile	98.54 (0.37)
Benzothiazole	110.07 (tr)
Benzyl isothiocyanate	127.16 (13;11)
Hydrocarbons	
Hexane	20.86 (tr)
Heptane	29.82 (tr)
Methylcyclohexane	32.35 (tr)
Toluene	37.67 (tr)
Ethylbenzene	51.23 (0.06)
p-Xylene	52.72 (tr)
o-Xylene	56.25 (0.05)
2-Propylbenzene	61.21 (tr)
2-Propylcyclohexane	61.67 (tr)
1-Propylcyclohexane	63.26 (tr)
1-Propylbenzene	65.87 (tr)
m-Ethyltoluene	67.11 (tr)
p-Ethyltoluene	67.39 (tr)
1,3,5-Trimethylbenzene	68.60 (tr)
o-Ethyltoluene	69.88 (tr)
β-Pinene	70.50 (tr)
1,2,4-Trimethylbenzene	72.35 (tr)
Decane	74.55 (tr)
1,2,3-Trimethylbenzene	76.73 (tr)
p-Cymene	77.17 (tr)
Limonene	78.30 (tr)
1-Butylcyclohexane	79.38 (tr)
α-Ocimene	80.60 (tr)
1-Butylbenzene	82.00 (tr)
Tetradecane	131.32 (tr)
Ketones	
2-Butanone	20.31 (tr)
2-Pentanone	26.50 (tr)
4-Heptanone	51.85 (tr)
2-Heptanone	54.90 (tr)
2-Octanone	70.34 (tr)
β-Ionone	140.59 (tr)

[a] Retention time in minutes in Figure 3. [b] Area percent of component peak in Figure 3. "tr" signifies an area percent of less than 0.05.

Table II. Major Composition Differences in Papaya Concentrates, Area Percent

	Concentrate no.							
	1 (A)[a]	2 (A)	3 (A)	4 (B)	5 (C)	6 (C)	7 (C)	8 (D)
Linalool oxide A	0.8	1.3	0.3	5.8	4.9	4.3	8.0	11.2
Linalool oxide B	0.9	1.6	0.4	10.4	8.2	4.3	8.4	13.8
Linalool	87.3	88.4	82.0	71.8	67.7	73.1	47.3	43.1
Linalool oxide C	tr[b]	tr	tr	0.1	0.1	tr	0.1	0.8
Linalool oxide D	tr	tr	tr	0.1	0.1	tr	0.1	0.4
Phenylacetonitrile	tr	tr	tr	0.3	0.4	0.1	1.3	15.3
Benzyl isothiocyanate	4.2	3.5	8.3	10.3	13.1	11.3	30.7	3.4

[a] Letter denotes concentration method used; see text. [b] Area percent less than 0.05.

tration of this compound, as compared with the results obtained under vacuum (concentrate 4). These results are consistent with those noted by Challenger (1959) in his references to other mustard oils.

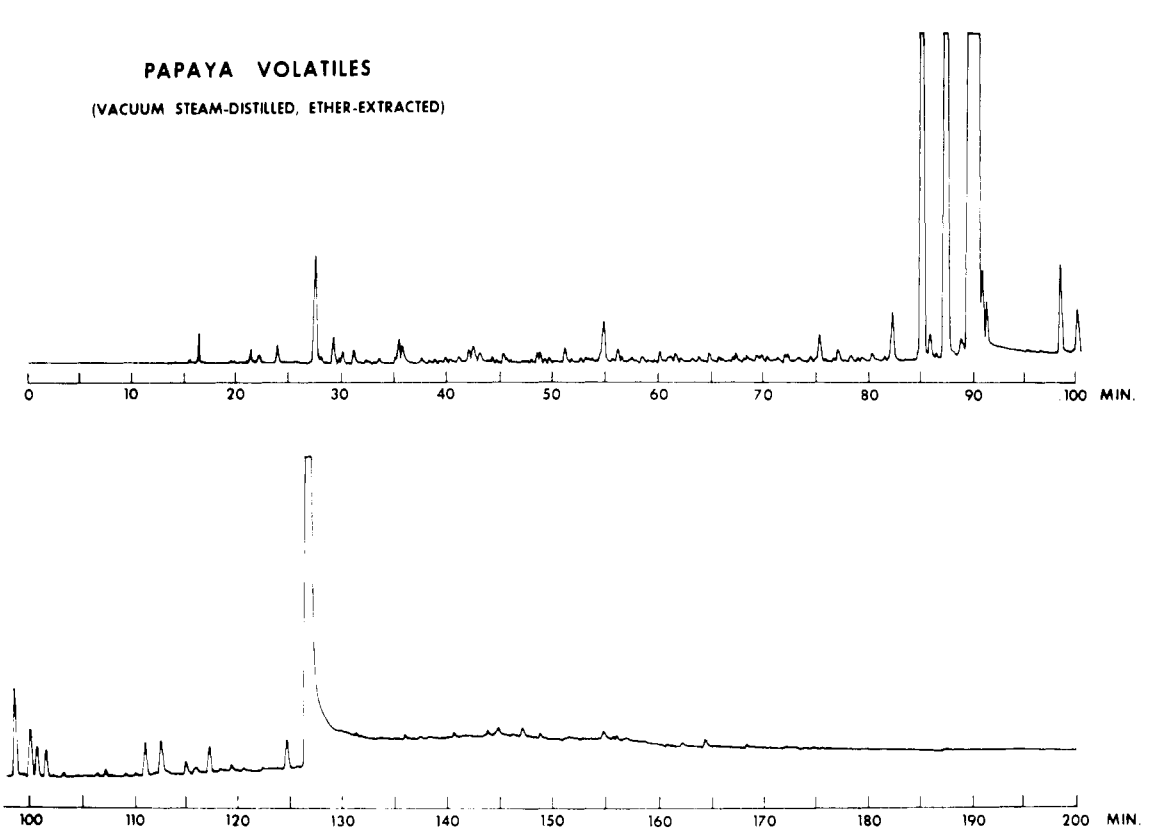

Figure 3. Temperature-programmed open-tubular column GC analysis (FID) of papaya volatiles concentrate 5 prepared by method C; 500 ft × 0.03 in. i.d. (152 m × 0.76 mm i.d.); stainless steel open-tubular column coated with methyl silicone oil SF 96(50) containing 5% Igepal CO 880; column held at 50 °C for 10 min, then programmed at 1.0 °C/min to 185 °C, and held at 185 °C for 50 min.

Table III. Methyl Esters of Extracted Free Acids

Methyl ester	Area %
Propionate	0.1
2-Methylpropionate	0.1
n-Butyrate	73.8
Crotonate	tr[a]
3-Methylbutyrate	tr
n-Pentanoate	0.6
n-Hexanoate	19.0
2-Hexenoate	0.1
n-Heptanoate	0.2
Benzoate	tr
n-Octanoate	5.8
Phenyl acetate	tr
n-Nonanoate	tr
Salicylate	tr
n-Decanoate	0.2
n-Dodecanoate	0.1

[a] Less than 0.1%.

Table III lists the identified acids extracted from papaya juice. The table also indicates the relative amounts of the acids extracted, as determined from a gas chromatographic separation of the corresponding methyl esters (no response factors were employed). *n*-Butyric acid is by far the major volatile acid extracted, followed by *n*-hexanoic and *n*-octanoic. Methyl acetate was detected at low concentration in preliminary runs, but could not be found in the concentrate examined for Table III. Because of the volatilities of the lower methyl esters, some loss of these compounds may have occurred during solvent removal, although considerable care was taken to minimize such occurrence. The tabulated results in Table III are consistent with preliminary findings reported earlier (Chan et al., 1973).

Most of the components detected by GC (Figure 3) have also been identified; the major exceptions are the peaks at 75.36, 91.38, and 117.31 min. The material eluted at 75.36 min is an intractable mixture of overlapping components and that at 91.38 min is masked by the linalool tail. The material at 117.31 min appears to be a single component with a molecular weight of 169. The compound is not removed from the concentrate by an acid wash. No satisfactory structure has been deduced as yet, and attempts to isolate sufficient material for examination by other methods have been unsuccessful. An appreciable number of low concentration, long retention time components are unidentified, largely due to the usual problems of higher MS background, greater possibility of structural diversity (with implied lesser likelihood of having suitable reference spectra available), and incomplete gas chromatographic separation. Two components, eluting at 100.03 and 124.71 min, appear to be methylsiloxane compounds (Wurst et al., 1973), but have not been fully characterized. They are thought to be artifacts, introduced with the silicone oil lubricant used in a glass stirrer bearing on the sample pot.

Thirty alcohols are included in Table I. Linalool is by far the major component, followed by the two tetrahydrofuranyl linalool oxides. The total alcohol peak area, excluding linalool, is approximately 2%. These minor alcohols include all straight-chain isomers from C_1 to C_6. 3-Methyl-1-butanol, 2-methyl-1-butanol, *cis*-3-hexen-1-ol, *trans*-2-hexen-1-ol, and 1-hexanol, the alcohols commonly obtained from macerated fruit material, are all present, as are the four isomeric linalool oxides (Stevens et al., 1966).

A relatively small number of carbonyl components has been found. Of the nine compounds (total area percent = 0.1), β-ionone is probably the most potent odorant (threshold in water = 0.007 ppb; Buttery et al., 1970). Assuming a typical volatiles concentration in the fresh fruit

of 10 ppm, which is certainly a conservative estimate, a calculated β-ionone concentration of 2 ppb is indicated (area percent in Figure 1 is actually 0.02; called tr in Table I). One of the ketones, 2-heptanone, was previously reported by Katague and Kirch (1965).

As is usually the case in fruit volatiles composition studies, a rather diverse group of 32 esters (represented by 0.87% of the chromatogram area) was identified. A series of lactones makes up a large proportion of the total ester concentration. γ-Hexalactone and γ-octalactone are present in significant concentrations, as is δ-octalactone. γ-Heptalactone, γ-decalactone, and γ-nonalactone are also present, at quite low levels.

Only minimal amounts of the three acetals were detected. It seems unlikely that these are artifacts produced in the volatiles concentrate during storage, for little if any of the corresponding alcohol or aldehyde precursors were found in the concentrates. If not present in the whole fruit, they may have formed in the macerated tissue before or during stripping.

Because of their relatively simple, yet characteristic spectra, lower molecular weight halogenated compounds are relatively easy to identify at very low levels. Only three such compounds were found: methylene chloride, chloroform, and 1,2-dibromoethane. The origin of the methylene chloride is unknown, but chloroform is used for cleaning syringes in our laboratory, and traces might easily be retained in the 1-μl syringes used for GC sample handling. Papayas for export from Hawaii are fumigated for 2 h with 1,2-dibromoethane after an initial hot water wash (Chan, 1972), so it is likely that the dibromo compound was introduced at this point.

Several heteroatom (S,N) compounds are included in Table I. The presence and concentration variability of benzyl isothiocyanate have been noted above. Tang (1971) has previously reported the presence of benzyl isothiocyanate in macerated fruit pulp. Challenger (1959) has reviewed much of the earlier work dealing with the common co-occurrence of isothiocyanates and nitriles in natural mustard oils. Methyl thiocyanate is present at rather low concentration in the concentrate sample that was used for Figure 1 and Table I. However, the apparent concentrations of many of the more volatile constituents are probably artificially low, for these were more likely to be partially removed during solvent stripping. Methyl thiocyanate and the corresponding isothiocyanate isomer have somewhat similar mass spectra, but the isothiocyanate was definitely ruled out on the basis of the abundances of m/e 45, 46, and 47 fragments relative to the 73 base peak, as determined from mass spectra of authentic samples of each compound.

Identification of benzothiazole is based primarily upon the presence and relative abundances of the fragment ion peaks at m/e 108 and 135 in the GC–MS data, as well as the retention behavior of a sample of the compound. This material has been found in a variety of plant materials in recent years, including potatoes (Buttery et al., 1970; Meigh et al., 1973), peanuts (Walradt et al., 1971), filberts (Kinlin et al., 1972), and giant cordgrass (Mody et al., 1974) (see Watanabe and Sato, 1972, for other references). Some of these plant sources have undergone a roasting or boiling treatment before or during the concentration of aroma materials, but this is not always the case.

A rather diverse group of hydrocarbons, both aliphatic and aromatic, has been identified (total area % = 0.73). The majority of these are aromatic, but the total concentration of these aromatics in the concentrate examined in detail is very low. Johnson et al. (1969) have discussed some possible sources of aromatic hydrocarbons in foods and related materials, but production via the routes mentioned requires rather vigorous heating of precursors in most cases. Contamination is always a possibility; the papaya fruit may have been exposed to various kinds of petroleum solvents during growth (Chan, 1972), and the harvested fruit was packed in shredded newspaper for shipping to the mainland. When fruit was prepared for stripping, the skin and a layer of underlying tissue approximately 0.125–0.25 in. thick was cut away, so any concentration of externally applied material in the internal tissue would be a function of the contaminant's mobility in the skin and underlying tissue. Very few terpene hydrocarbons were identified. There is some MS evidence for the presence of additional terpenes, but no others could be identified with any degree of confidence. The stereochemistry of the central double bond in α-ocimene is unknown, but the MS and GC relative retention behaviors of the papaya component are identical with that of the α-ocimene isolated from opopanax oil (Flath et al., 1966).

As is evident from Figure 1 and Table I, most compounds reported are present in very low concentrations. Only four components have concentrations greater than 0.5% in the sample mixture. This means that relatively few of the constituents provided strong mass spectra. In addition, many compounds in the papaya concentrates were found to be only partially resolved by the gas chromatographic system, so spectra obtained were composites of two or more individual spectra, the relative amounts of each varying as consecutive mass spectra were recorded. These factors introduce a degree of uncertainty into identifications arrived at by the GC–MS process alone. For this reason, the authors took particular pains in checking the relative retention behavior of tentatively identified components. While no numerical degree of confidence can be assigned to each identification, the authors are confident that the components listed in Table I are indeed present, and are eluted where indicated in Figure 3.

LITERATURE CITED

Buttery, R. G., Seifert, R. M., Guadagni, D. G., Ling, L. C., *J. Agric. Food Chem.* **19**, 524 (1971).
Buttery, R. G., Seifert, R. M., Ling, L. C., *J. Agric. Food Chem.*, **18**, 538 (1970).
Challenger, F., "Aspects of the Organic Chemistry of Sulfur", Butterworths, London, 1959, pp 120–132.
Chan, H. T., Jr., private communication, 1972.
Chan, H. T., Jr., Flath, R. A., Forrey, R. R., Cavaletto, C. G., Nakayama, T. O. M., Brekke, J. E., *J. Agric. Food Chem.* **21**, 566 (1973).
Ettlinger, M. G., Hodgkins, J. E., *J. Org. Chem.* **21**, 204 (1956).
Flath, R. A., Lundin, R. E., Teranishi, R., *Tetrahedron Lett.* **3**, 295 (1966).
Forrey, R. R., Flath, R. A., *J. Agric. Food Chem.* **22**, 496 (1974).
Forss, D. A., Jacobsen, V. M., Ramshaw, E. H., *J. Agric. Food Chem.* **15**, 1104 (1967).
Gmelin, R., Kjaer, A., *Phytochemistry* **9**, 591 (1970).
Johnson, A. E., Nursten, H. E., Self, R., *Chem. Ind. (London)*, 10 (1969).
Katague, D. B., Ph.D. Thesis, University of Illinois, 1964.
Katague, D. B., Kirch, E. R., *J. Pharm. Sci.* **54**, 891 (1965).
Kinlin, T. E., Muralidhara, R., Pittet, A. O., Sanderson, A., Waldradt, J. P., *J. Agric. Food Chem.* **20**, 1021 (1972).
Likens, S. T., Nickerson, G. B., *Am. Soc. Brew. Chem. Proc.*, 5 (1964).
Meigh, D. F., Filmer, A. A. E., Self, R., *Phytochemistry* **12**, 987 (1973).
Mody, N. V., Bhattacharyya, J., Miles, D. H., Hedin, P. A., *Phytochemistry* **13**, 1175 (1974).
Stevens, K. L., Bomben, J., Lee, A., McFadden, W. H., *J. Agric. Food Chem.* **14**, 249 (1966).

Tang, C. S., *J. Chem. Educ.* **47**, 692 (1970).
Tang, C. S., *Phytochemistry* **10**, 117 (1971).
Tang, C. S., Syed, M. M., *Phytochemistry* **11**, 2531 (1972).
Verbeek, A. M., Maarse, H., *J. Chromatogr.* **84**, 389 (1973).
Walradt, J. P., Pittet, A. O., Kinlin, T. E., Muralidhara, R., Sanderson, A., *J. Agric. Food Chem.* **19**, 972 (1971).
Watanabe, K., Sato, Y., *J. Agric. Food Chem.* **20**, 174 (1972).
Wurst, M., Lömker, F., Kelker, H., *Chromatographia* **6**, 359 (1973).

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A Study of the Volatiles Isolated from a D-Glucose–Hydrogen Sulfide–Ammonia Model System

Takayuki Shibamoto and Gerald F. Russell*

The compounds produced by heating a D-glucose–hydrogen sulfide–ammonia–water model system were extracted with methylene chloride using a liquid–liquid continuous extractor. Fifty-one compounds were identified by a combination of gas chromatographic and mass spectrometric techniques. The main constituents of the reaction mixtures were thiophenes, furans, pyrazines, and thiazoles. With small amounts of ammonia in the starting mixture, the reaction products consisted mainly of thiophenes and furans and possessed a somewhat sulfury aroma which was also akin to that of rare cooked meat. When the amount of ammonia was increased, however, the reaction mixture contained a large proportion of pyrazines, a smaller proportion of thiazoles, thiophenes, and furans and the mixture possessed a nutty or burnt aroma.

The authors have recently reported on the volatile chemicals generated in a heated D-glucose–hydrogen sulfide–ammonia model system (Shibamoto and Russell, 1976) and identified a number of compounds which have been associated with cooked meat aromas (Persson and von Sydow, 1973; Nonaka et al., 1967). Among the chemicals identified were thiols, sulfides, thiophenes, thiazoles, and furans. Most of the compounds identified in this system have also been found in various food products (Maga and Sizer, 1973; Maga, 1975; Shankaranarayana et al., 1974).

Boelens et al. (1974) studied the reactions between fatty aldehydes, hydrogen sulfide, thiols, and ammonia and subsequently identified sulfides, thiols, and nitrogen- and sulfur-containing heterocyclic compounds in their reaction products. They showed that the reaction of ethanal, hydrogen sulfide, and ammonia produced 2,4,6-trimethyldihydro-1,3,5-dithazine, which has been found in beef broth (Brinkman et al., 1972).

Heterocyclic compounds (thiophenes, furans, thiazoles, and pyrazines) are among the major volatiles which have been identified in cooked meat (Mussinan and Walradt, 1974). For example, Maga (1975) reported the relative percentages of pyrazines and furans found in cooked pork liver volatiles to be 40.96 and 20.90, respectively. Pyrazines are well-known compounds which occur in cooked foods, including meat (Maga and Sizer, 1973). Persson and von Sydow (1973) identified 26 thiophenes and 11 thiazoles in cooked meat.

The precursors and formation pathways of these heterocyclic compounds are not well understood. One reason for this is that the amounts of these compounds formed in foods are very low—usually lower than micrograms per kilogram levels in food (Schutte, 1974); therefore, detection of these compounds has been difficult. This has been especially true when stainless steel columns have been used for gas–liquid chromatographic (GLC) analysis because sulfur compounds at low levels do not pass through a stainless steel column in sufficient quantities to give a detector response (Withycombe and Walradt, 1975). Shibamoto and Russell (1976) suggested that carbonyls, hydrogen sulfide, and ammonia could be precursors of compounds which are associated with cooked meat aromas.

It is well known to sensory analysts that the relative proportions of volatiles in an aroma complex have a very strong influence on its sensory properties. This appears to be especially true with cooked meat aroma in general and the previously reported model system in particular. It was the purpose of the present study to vary reaction conditions and monitor the products to gain further insight into the role of the reactants in the D-glucose–H_2S–NH_3 model system.

EXPERIMENTAL SECTION

Materials. D-Glucose, ammonium hydroxide, and hydrogen sulfide were obtained commercially. Authentic reference compounds were obtained from reliable commercial sources or were donated by Ogawa & Co., Ltd., Tokyo, Japan.

Reaction of D-Glucose, Hydrogen Sulfide, and Ammonia. Hydrogen sulfide gas was bubbled through aqueous solutions (100 ml) each containing 0.1 mol of D-glucose for 10 min at 0 °C in a Kjeldahl flask. Ammonium hydroxide solutions of 0.10, 0.15, 0.20, and 0.50 mol of NH_3 for experiments 1–4, respectively, were then added to each of the above solutions. The necks of the flasks were flame-sealed and the flasks placed in an oven at 100 °C for 2 h. Volatiles were isolated from each reaction mixture with 200 ml of methylene chloride using a liquid–liquid continuous extractor for 6 h. The qualitative and quantitative analyses of volatiles were conducted following the gas chromatographic–mass spectrometric (GC–MS) methods described previously (Shibamoto and Russell, 1976).

RESULTS AND DISCUSSION

Boelens et al. (1974) reported nine saturated heterocyclic compounds formed from the reaction of fatty aldehydes,

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