Chromatographic Analysis of the Volatile Components of Papaya Fruit

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The volatile components of two varieties of papaya fruits (*Carica papaya* Linn.) were analyzed by gas and thin-layer chromatography. At least 18 components were found in one variety (greenhouse), whereas only 13 were identified in the fruit obtained on the open market. The changes of the relative concentration of the former also were determined at various stages of ripeness.

THE CHEMICAL composition of the volatile components of various fruits has been the subject of a number of investigations (1). Quantitative analysis of some of the volatile substances as related to the various stages of ripening in banana pulp has been reported by Hultin and Proctor (2).

This report concerns a similar study using papaya fruits.

EXPERIMENTAL

Samples.—The fruits used in this investigation were obtained from a female papaya tree grown under typical greenhouse conditions. The dates of cross-pollination and harvesting of the fruits were used as the basis for establishing three stages of ripening. The samples were characterized as follows: half-ripe, 16–18 weeks; ripe, 20–22 weeks; over-ripe, 24–26 weeks.

For purposes of comparison and as a check of the reproducibility of the procedures used, commercial fruits purchased on the open market were used also.

Preparation of the Extracts.—Weighed portions of homogenates of the fruit were mixed with an equal volume of water and then steam distilled. The resulting distillate was saturated with salt and subsequently extracted with ether. This extract after drying over anhydrous sodium sulfate was concentrated in a rotary flash evaporator and under reduced pressure. The resulting concentrate was either analyzed immediately or stored in an airtight container in a refrigerator until used.

Gas Chromatography.—A Beckman GC-2 chromatograph with a thermal conductivity detector connected to a Sargent SR recorder equipped with an integrator was used. The column consisted of 0.25-in. copper tubing and was 6 ft. long. Carbowax 400 (10%) and DEGS (20%) on Chromosorb B were used as the liquid phases. The following parameters of operation were found to be ideal: temperature, 100°C.; flow rate of helium, 30 p.s.i.; filament current, 250 μamp. Five-microliter samples were used.

For purposes of identification, synthetic blends were compared not only with the unknown but also with pure standards. The peaks were confirmed by the enrichment method (3) and the dual column technique. The relative percentages agreed in both methods. Those peaks not verified by either of the above procedures were collected and analyzed by thin-layer chromatography and in some cases checked by infrared spectrography.

Thin-Layer Chromatography.—The apparatus is essentially that used by Rollins (4) using either 1 × 3 in. microscope slides or in some cases 2 × 8 in. glass plates. Silica Gel G (Merck) was used as adsorbant. The mobile phase consisted of a 1:1 mixture of petroleum ether and benzene. Saturated iodine solution or iodine vapor was used to detect the spots (5).

Infrared Spectrography.—Infrared spectra were determined in a Beckman IR-4 spectrophotometer.

RESULTS AND DISCUSSION

Since the supply of papaya fruit as grown under typical greenhouse conditions was rather limited, some fruits were purchased on the open market and used not only to check the usual methods of extraction of aroma concentrates but also to ascertain the reproducibility of the procedures used in the isolation of the volatile components.

The per cent composition, as shown in Tables I–III, represents relative percentages of the various peaks based on the integration and is the average of three determinations. To eliminate any discrepancies due to solvent peaks, correction was made in all the computations of these percentages.

While the comparison of retention times of unknown peaks with certain standards was used for purposes of identification, confirmation of this identification was made by co-chromatography of the sample and standards. In addition, a method known as the two phase concept of analysis was used (6, 7). Identities of certain components (methyl alcohol, ethyl acetate, butyl alcohol and its acetate, isoamyl and hexyl acetate) were verified by thin-layer chromatography after separation of the mixture by gas chromatography. This is the same method first described as a means to separate a mixture according to increasing molecular weight or dispersion forces (8). The R_f values of the various standards were compared with those of compounds present in certain fractions both singly and simultaneously (Table IV).

Thirteen components were identified in the ether extracts of the steam distillates obtained from the commercial fruit (Table I). The values as tabulated and listed in Tables I and II are the averages of two determinations and are corrected for solvent peak discrepancies.

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TABLE I.—ANALYSIS OF PAPAYA FRUIT EXTRACT (COMMERCIAL) ON DEGS[a]

Peak No.	Retention Time, min.	Standard Compd. (Retention Time)		Comp., %
1	1.4	Ether (solvent)	(1.3)	...
2	2.1	Methyl alcohol	(2.0)	
		Methyl acetate	(2.3)	9.5
3	2.6	Ethyl alcohol	(2.6)	21.4
4	4.0	Ethyl acetate	(4.0)	
		Butyl alcohol	(4.3)	19.1
5	4.9	Butyl acetate	(4.7)	
		Isobutyl acetate	(5.0)	5.8
		Isobutyl alcohol	(5.3)	
6	5.7	Amyl acetate	(5.5)	30.3
7	7.0	Isoamyl acetate	(7.2)	3.0
8	8.3	Hexyl acetate	(7.9)	10.7
		Isoamyl alcohol	(8.3)	
9	11.4	Hexyl alcohol	(11.4)	0.2
10	16.8	High-boiling components		Trace

[a] Twenty per cent w/w on Chromosorb P at 100°C., 250 μamp., 30 p.s.i., 6-ft. column.

TABLE II.—ANALYSIS OF PAPAYA FRUIT EXTRACT (GREENHOUSE) ON CARBOWAX[a] (RIPE FRUIT)

Peak No.	Retention Time, min.	Standard Compd. (Retention Time)		Comp., %
1	1.4	Ether	(1.3)	...
2	1.8	Methyl acetate	(1.8)	10.5
3	2.1	Methyl alcohol	(2.1)	11.6
		Ethyl acetate	(2.1)	
4	2.3	Isopropyl alcohol	(2.3)	
		Ethyl alcohol	(2.4)	18.9
5	2.7	Isobutyl acetate	(2.8)	2.3
6	3.0	Propyl alcohol	(3.2)	6.8
7	3.5	Isobutyl alcohol	(3.5)	
		Propyl acetate	(3.7)	2.9
8	4.5	Butyl acetate	(4.4)	
		Butyl alcohol	(4.6)	7.5
9	5.0	Isoamyl acetate	(4.8)	
		Amyl acetate	(5.0)	18.9
10	6.5	Isoamyl alcohol	(6.4)	3.6
11	7.1	2-Heptanone	(7.0)	
		Amyl alcohol	(7.2)	0.2
12	9.6	Hexyl acetate	(9.8)	4.3
13	10.8	Hexyl alcohol	(10.5)	12.4
14	13.0	High-boiling components		0.1

[a] Ten per cent w/w on Chromosorb P at 100°C., 250 μamp., 30 p.s.i., 6-ft. column.

TABLE III.—ANALYSIS OF PAPAYA FRUIT EXTRACT (GREENHOUSE) ON DEGS[a] (RIPE FRUIT)

Peak No.	Retention Time, min.	Standard Compd. (Retention Time)		Comp., %
1	1.4	Ether	(1.3)	...
2	2.3	Methyl alcohol	(2.0)	31.6
		Methyl acetate	(2.3)	
		Ethyl alcohol	(2.6)	
3	3.3	Propyl alcohol	(3.5)	
		Isopropyl alcohol	(3.6)	9.1
4	4.0	Ethyl acetate	(4.0)	7.1
5	4.3	Propyl acetate	(4.2)	
		Butyl alcohol	(4.3)	8.3
6	4.9	Butyl acetate	(4.7)	
		Isobutyl acetate	(5.0)	4.5
		Amyl alcohol	(5.1)	
		Isobutyl alcohol	(5.3)	
7	5.7	Amyl acetate	(5.5)	5.2
8	7.2	Isoamyl acetate	(7.2)	13.7
9	7.9	Hexyl acetate	(7.9)	
		Isoamyl alcohol	(8.3)	7.9
10	9.8	2-Heptanone	(9.8)	0.1
11	11.5	Hexyl alcohol	(11.4)	12.3
12	16.8	High-boiling components		0.2

[a] Twenty per cent DEGS on Chromosorb P at 100°C., 250 μamp., 30 p.s.i., 6-ft. column.

TABLE IV.—R_f VALUES OF STANDARD COMPOUNDS (THIN-LAYER CHROMATOGRAPHY)[a]

Normal Series		Acetates	Propionates	Butyrates
Alcohols and Esters				
Methyl	0.02	0.36	0.52	0.60
Ethyl	0.18	0.40	0.60	0.66
Propyl	0.20	0.56	0.68	0.78
Butyl	0.30	0.68	0.76	...
Amyl	0.36	0.78	...	...
Hexyl	0.40	0.82	...	...
Iso or Branched				
Isopropyl	0.10	0.30	0.52	...
Isobutyl	0.20	0.50	0.64	0.74
Isoamyl	0.36	0.80	...	0.92
sec-Butyl	0.24	...	...	...
tert-Butyl	0.16	...	...	...

Aldehydes		Ketones	
Aldehydes and Ketones			
Acetaldehyde	0.06	Acetone	0.94
Propanal	0.16	2-Butanone	0.82
Butanal	0.26	2-Pentanone	0.46
2-Hexen-1-al	0.40	3-Pentanone	0.40
		2-Heptanone	0.22
Aliphatic Acids			
Acetic	0.46	Butyric	0.54
Propionic	0.50	Valeric	0.58

[a] Adsorbant, Silica Gel G (Merck); solvent, benzene–petroleum ether (b.p. 30–60°C.), 1:1 mixture.

At least 18 components were identified in extracts prepared from the fruit grown in the greenhouse. Ethyl, propyl, butyl, and hexyl alcohols, and methyl, ethyl, amyl, and isoamyl acetates were found to be present in a relatively high concentration (greater than 5%). On the other hand, methyl, isopropyl, and isoamyl alcohols, and isobutyl and hexyl acetates were found in amounts less than 5%. Amyl and isobutyl alcohols and heptanone-2 were found to be present in quantities less than 1%.

The retention times of some of the peaks as obtained with Carbowax 400 were too close for verification using the enrichment techniques. Using a column of slightly different polarity (DEGS), the presence of most of the components could be confirmed (Table III).

The presence of isobutyl acetate, propyl alcohol, isoamyl alcohol and its acetate, amyl acetate, hexyl alcohol and acetate, and methyl ethyl ketone was verified by this method. The other components were confirmed by thin-layer chromatography. In addition, both amyl and isoamyl acetates were further identified by the use of infrared spectrography.

Comparing the results of analysis of ripe commercial fruit with those of the greenhouse fruit, the following differences were noted: propyl and isopropyl alcohols were not detected in the commercial fruit, but were found in appreciable amounts (as high as 9.1% in the ripe samples) in the greenhouse fruit. Similar results were observed with the acetates. Propyl acetate was present in the greenhouse fruit; none could be detected in the commercial fruit. Two minor components, amyl alcohol and heptanone-2, while present in the greenhouse fruit, were not detected in the commercial samples.

Aside from the above qualitative differences, certain quantitative differences were noted also. While the concentration of methyl and ethyl alcohol and butyl and isobutyl acetates and of the high boiling components were about the same in the samples, differences in the amounts of the other components could be observed. (Tables I and III.)

It has been reported that volatile components of fruits, particularly bananas (2), change at the different stages of ripeness. One of the purposes of this investigation was to determine the extent of these changes, if any, in the papaya fruit grown under typical greenhouse conditions. Fruits at the various stages of ripeness were analyzed immediately after harvesting. Figure 1 represents a typical chromatogram of half-ripe and over-ripe papaya fruit extracts.

In most fruits, the development of aroma or odor parallels the process of ripening. This could be observed also in the case of papayas. While the half-ripe fruit has hardly any odor, it could be observed that as ripening progressed to the yellow color, a typical aroma emanated from the fruit. This development of aroma is in line with the changes in concentration of the various constituents and is similar to the finding recently reported by McCarthy *et al.* (9) on their work on the volatile components of bananas.

As shown in Table V, there seems to be a decrease in the concentration of methyl alcohol and methyl acetate, ethyl acetate, and hexyl alcohol as the fruit ripens, while there is a marked increase in the concentration of ethanol from half-ripe to the over-ripe

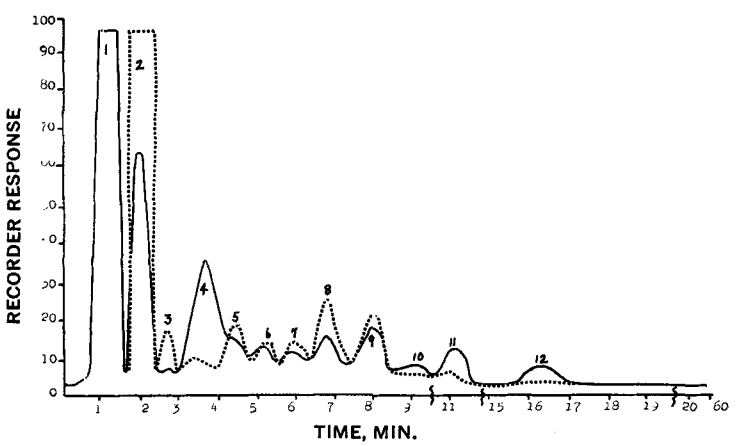

Fig. 1.—Typical chromatograms of half-ripe and over-ripe papaya fruit extracts on DEGS. Key: ———, half-ripe; ....., over-ripe.

TABLE V.—ANALYSIS OF PAPAYA FRUIT AT VARIOUS STAGES OF RIPENESS[a]

Components	Half-Ripe	Total	Ripe	Total	Over-Ripe	Total
Methyl alcohol	6.5		4.5		3.8	
Methyl acetate	12.3		10.5		8.7	
Ethyl alcohol	1.6	20.4	16.6	31.6	26.4	38.9
Propyl alcohol	1.6		6.8		6.6	
Isopropyl alcohol	0.7	2.3	2.3	9.1	2.2	8.8
Ethyl acetate	17.7		7.1		6.5	
Propyl acetate	2.0		2.0		2.2	
Butyl alcohol	5.8	7.8	6.3	8.3	6.5	8.7
Butyl acetate	1.2		1.2		1.1	
Isobutyl acetate	2.1		2.3		2.3	
Amyl alcohol	0.1		0.1		0.1	
Isobutyl alcohol	0.9	4.3	0.9	4.5	0.9	4.4
Amyl acetate	3.7		5.2		5.3	
Isoamyl acetate	6.9		13.7		13.8	
Hexyl acetate	4.0		4.3		4.2	
Isoamyl alcohol	2.4	6.4	3.6	7.9	3.4	7.6
Methyl amyl ketone	1.2		0.1		0.1	
Hexyl alcohol	13.2		12.3		5.6	
High-boiling components	16.1		0.2		Trace	

[a] Individual percentages for multiple components' peaks were based from results in both the DEGS and Carbowax columns.

stage. Similar increases in the concentration of isoamyl acetate during the process of ripening were noted.

The decrease in the methanol content as the fruit ripens is not characteristic of papaya alone. Hultin (2) reported similar results with his work during the ripening of bananas. Biale and Young (10), reporting on their work on tropical and semitropical fruits, suggest that the main reactions involved in the ripening of fruits were directly or indirectly related to the oxidation (respiratory) and formentation (glycolic) processes collectively referred to as biological oxidation.

SUMMARY

The volatile components in two varieties of papaya fruits were determined. At least 18 components were found in the fruit grown in the greenhouse, while 13 compounds were found in the fruit obtained on the open market. Differences in concentration at the various stages of ripening in the one (greenhouse) variety could be established also.

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