

Table II. Evaluation of Characterized Blueberry Components

Flavor	Compound, ppm		
	Linal-ool	<i>cis</i> -3-Hexe-nol	<i>trans</i> -2-Hexe-nol
Lemon, woody	5		
Nice fresh green		5	
Green, sharp		10	
Spicy green apple			5
Green, olefinic			10
Blueberry	5	5	
Blueberry	5	10	
Blueberry-like, green apple	5		5
Green apple blueberry	5		10

it) possessed a blueberry character and only this fraction was studied further. In step III, an interesting phenomenon occurs. The desired flavor is not in any one fraction, but rather only in that combination of fractions which include both D and E. Step IV demonstrates that the component indicated by H is critical to blueberry flavor, but we do not know what other components are essential to the flavor. Step VI shows that the peak P is not critical to the flavor but that the two peaks encompassed in N are. In the final step, we observe that a blueberry character is developed by a combination of peaks containing fractions R plus T, but a sharper less desirable character results from a combination of S and T.

Standard identification techniques (GC/MS, GC/IR) were employed to characterize the components designated R, S, and T. These were found to be *cis*-3-hexenol, *trans*-2-hexenol, and linalool, respectively. The importance of linalool is not unexpected since Arctander (1969) indicates that linalool is used in imitation blueberry flavors.

Synthetic Blueberry Flavor. It cannot be expected that this approach will lead to a complete well-rounded flavor. On the other hand, the combination of the identified flavors in the correct proportion should produce a recognizable flavor. A number of mixtures were prepared

in the sucrose/acid base and were evaluated (Table II). These results reinforced the importance of *cis*-3-hexenol and linalool as major characterizing flavorings of blueberry and demonstrate that a blueberry character, though sharper and greener, results from a combination of linalool and *trans*-2-hexenol.

CONCLUSION

A procedure has been developed which enables the analyst to systematically determine the important flavor notes of a food. This technique facilitates the recognition of the critical components in a complicated chromatographic pattern. The sample is chromatographically fractionated into successively smaller portions which are evaluated both alone and in combination by modified profile evaluation panel techniques. The procedure is viable providing the aromatic concentrate possesses the desired character and that it can survive gas chromatography. It is useful even if the flavor pursued is not complete, providing that flavor profile panelists can recognize the desired note with a high degree of consistency. This ability to focus on only the important compounds has enhanced the usefulness of flavor analysis in the creation of synthetic flavors.

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Volatile Components of Unprocessed Rice (*Oryza sativa* L.)

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Laboratory tests showed that the volatile flavor components of unprocessed rice are attractive to Philippine ricefield rats (*Rattus rattus mindanensis*). These results suggested that a synthetic bait attractant could be made if the important volatiles in rice were identified. To identify the compounds, ground rice was tumbled at 50 °C in a modified rotary evaporator while helium swept the volatiles into a liquid nitrogen trap coated with dibutyl phthalate. The dibutyl phthalate solution containing the volatiles was analyzed by combination capillary-column gas chromatography and mass spectrometry. Altogether, 73 compounds were identified, including alcohols, aldehydes, alkyl aromatics, furans, ketones, terpenes, and naphthalenes. The structural types of another 31 compounds were indicated. Of the 73 compounds identified, only 9 have been previously reported in unprocessed rice and 54 have never been reported in any unprocessed cereal grain.

Rice is the world's largest cereal crop. It is produced in all of the continents and is the staple food of nearly half of the world's population. Only about 3%, however, moves in world trade and the rest is internally consumed by the producing countries. Over 90% of the world crop is

produced in East and Southeast Asia. Consequently, the developing countries in this area are highly dependent upon each rice crop to feed their people.

Because of dense rat populations, lack of effective control programs, and current farming practices, rice growers in the tropics are especially vulnerable to severe crop losses through rat damage. In the Philippines, wild ricefield rats, *Rattus rattus mindanensis*, damage rice in all growth stages—germinating seeds, growing seedlings, and

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panicle bearing plants. The rats also feed on harvested rice piled in the fields for drying, stored threshed rice, and hulled rice in retailers' bins. Damage surveys indicate that about 90% of the rice fields suffer rat damage and that total crop loss may occur in some areas (Fall and West, 1973). The man-rat conflict in Southeast Asia has led to research to develop effective means of curbing losses. Our research has focused on the development of rat baits and baiting procedures for rice agriculture. The need to assess the palatability of the common foods of *Rattus rattus mindanensis* led us to study the flavor components of rice.

Methods of fractionation were developed and several different rice fractions were tested to determine rat preferences. A rice volatiles formulation far outranked all others (Bullard and Shumake, 1977). This formulation was prepared by cryogenic trapping of volatiles from California Brown Pearl rice on granulated rice of the same variety. The consistently high acceptance of aroma-intensified rice formulations in all tests encouraged our development of a bait additive from rice volatiles. Since the extremely low yield would make commercial stripping of volatiles from rice too costly, our alternative was to develop a synthetic rice essence from the important components. Our earlier observation (see Results and Discussion) was that rice aroma is probably a blend of many compounds; therefore, a better understanding of the volatiles composition could help in the development of a synthetic additive for rodent baits.

EXPERIMENTAL SECTION

The major problem faced at the beginning of the experiment was to find an effective way of stripping volatile components from rice without causing any significant artificial changes due to the extraction process. Since the amounts of volatiles are very low we believed the process should be designed for maximum surface exposure of finely ground particles.

Collection of Volatiles. A modified rotary evaporator was used in the collection of volatiles from California Brown Pearl rice. A cold trap (Figure 1) replaced the Friedrich condenser. Unpolished rice was ground to 40-mesh size and placed in a modified 2-l. round-bottomed flask. Three 2-cm deep vertical "ribbed" depressions symmetrically located on the side of the flask caused tumbling of the material during rotation. This churning action in addition to a 50 °C internal temperature (measured by thermister) supplied by an infrared lamp provided appropriate conditions for the removal of volatiles. Helium gas (20 ml/min), which had been filtered through Molecular Sieve 5A, "swept" the volatiles from the rotating flask into the cold trap where they were collected.

After entering the cold trap (Figure 1), the effluent stream of helium containing the volatiles passed through an enlarged surface area consisting of 3.5 cm of 4 mm diameter glass beads (A). Then a 2 mm i.d. glass tube (B) directed the stream to the bottom of a 1 cm i.d. collection tube (C). The stream then passed upward through a 2-cm layer of silanized glass wool (D) before entering a 2 mm i.d. tube (E) which carried it back up through the trap interior and out through the exit port (F). During a typical collection of volatiles no odor could be detected at the exit port.

The interior of the trap, including glass wool, beads, and collection tube, was coated with 0.2 ml of dibutyl phthalate. This was accomplished by dissolving the dibutyl phthalate in about 3 ml of cold redistilled Freon 11. This solution was then added to the trap which had been cooled in a freezer. Hand rotation of the trap at room temperature

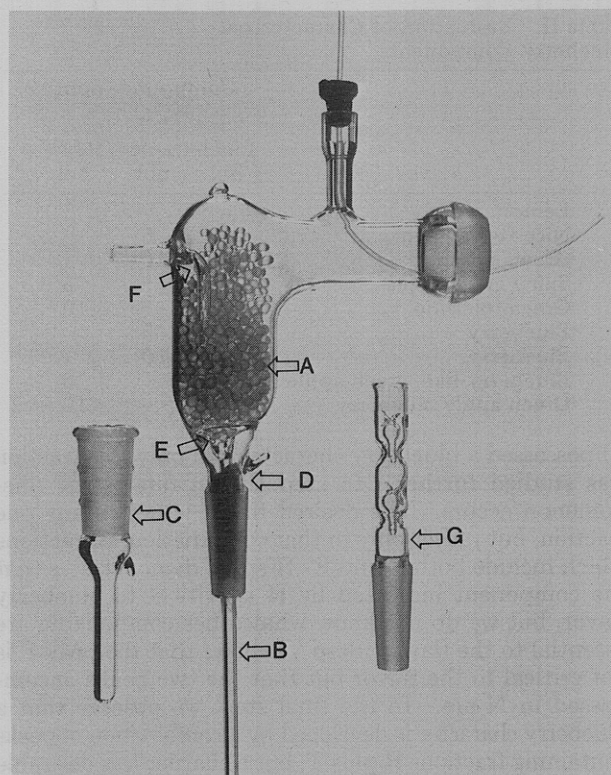


Figure 1. The cryogenic trap used in the collection of rice volatiles.

volatilized the Freon 11 and left the desired coating of dibutyl phthalate.

Volatiles were usually collected from 3 kg of ground rice. Six 500-g batches were each stripped for 4 h. At the end of the collection period the contents of the cold trap were rinsed into the collection tube with three 1-ml volumes of Freon 11. The upper portion of the trap was held in a plastic dry ice container and the lower portion, i.e., the collection tube, protruded through the bottom of the container. This permitted the removal of the lower collection tube. A micro-Snyder column (G of Figure 1) was attached to the collection tube and Freon 11 was distilled off by hand warming. Most of the trap contents, except those trapped in ice crystals (from moisture in the ground rice), were thereby removed from the system. The dibutyl phthalate solution of volatiles had a characteristic rice aroma.

The ice, however, had a strong earthy odor. It was thawed, transferred to a small separatory funnel, and extracted with Freon 11. About 50 μ l of a dibutyl phthalate solution remained after evaporating Freon 11 from the lower layer. This provided a concentrated source of the less volatile components. These two fractions of rice essence were subsequently analyzed separately and in combination.

Component Separation and Identification. All gas chromatographic separations were carried out with 0.03 in. i.d. \times 500 ft and 1000 ft stainless steel open-tubular columns prepared and coated in our laboratory. The columns were coated with OV-101 or OV-225 (Ohio Valley), both containing 5% Igepal CO-880 (General Aniline and Film), or with Carbowax 20M (Union Carbide).

Analyses were conducted on an Aerograph 200 gas chromatograph interfaced by a Watson-Bieman separator to a Nuclide 1290G mass spectrometer. The operating parameters of the mass spectrometer were: 70 eV ionizing potential; 200 °C source temperature; 200 °C separator temperature; 1.2×10^{-5} Torr source pressure and 5000 V

Table I. Aroma Constituents from Unprocessed Rice

1. Acetaldehyde ^{a,b}	75. 1,2,3-Trimethylbenzene
3. Acetone ^{a,b}	76. <i>p</i> -Cymene
4. Ethanol ^a	78. <i>trans</i> - β -Methylstyrene
7. Isobutanol ^{a,b}	79. Saturated aldehyde ^c
8. 1-Propanol ^a	83. <i>p</i> -Diethylbenzene
9. 2-Butanone ^b	84. 2-Octenal
10. Butanol ^{a,b}	85. <i>m</i> -Diethylbenzene
11. Branched ketone (mol wt 86) ^c	86. 1,3-Dimethyl-5-ethylbenzene
13. Aldehyde (mol wt 84) ^c	87. <i>o</i> -Diethylbenzene
15. Isopentanal ^{a,b}	88. <i>p</i> -Methylbenzaldehyde
16. 2-Methylbutanal	90. 1,3-Dimethyl-4-ethylbenzene
17. Benzene	91. 1,4-Dimethyl-2-ethylbenzene
18. 3-Penten-2-one	92. 2-Nonanone
19. 2-Pentanone	94. Nonal
20. Pentanal ^{a,b}	95. 2,4-Octadienal
21. 2,5-Dimethylfuran	96. 1,2-Dimethyl-3-ethylbenzene
22. Branched ketone (mol wt 100) ^c	97. Branched ketone (mol wt 156) ^c
26. 2-Methylpentanal	98. 1,2,4,5-Tetramethylbenzene
27. Ketone (mol wt 100) ^c	99. 1,2,3,5-Tetramethylbenzene
28. Toluene	105. Alkylbenzene (mol wt 148) ^c
29. 2-Hexanone	108. 2-Nonenal
30. Hexanal ^{a,b}	109. 1,2,3,4-Tetramethylbenzene
31. Branched ketone (mol wt 114) ^c	110. Alkylbenzene (mol wt 148) ^c
34. Alkylfuran (mol wt 110) ^c	111. Alkylbenzene (mol wt 148) ^c
37. 2-Hexenal	112. Alkylbenzene (mol wt 148) ^c
40. Ethylbenzene	113. Alkylbenzene (mol wt 148) ^c
41. <i>p</i> -Xylene	114. Alkylbenzene (mol wt 148) ^c
42. <i>m</i> -Xylene	115. Branched ketone (mol wt 170) ^c
43. Branched ketone (mol wt 128) ^c	116. Alkylbenzene (mol wt 148) ^c
44. 2-Heptanone	117. Branched ketone ^c
45. Heptanal	119. 2-Decanone
46. 2-Butylfuran	120. Naphthalene
47. 2,4-Hexadienal	122. Decanal
48. <i>o</i> -Xylene	123. Alkylbenzene (mol wt 148) ^c
49. Saturated aldehyde ^c	125. Branched ketone (mol wt 170) ^c
50. Branched ketone (mol wt 128) ^c	126. 2,4-Nonadienal
53. 2-Acetylfuran	127. Alkylfuran (mol wt 152) ^c
58. α -Pinene	129. Branched ketone ^c
59. 2-Heptenal	134. 2-Decenal
60. <i>n</i> -Propylbenzene	139. Branched ketone ^c
61. 1-Ethyl-4-methylbenzene	140. Branched ketone ^c
62. Benzaldehyde	141. Branched ketone ^c
63. 1,3,5-Trimethylbenzene	142. 2-Undecanone
64. Branched ketone (mol wt 142) ^c	144. 2-Methylnaphthalene
65. 6-Methyl-5-hepten-2-one	147. Branched ketone (mol wt 184) ^c
66. 2-Octanone	148. 2,4-Decadienal
68. 2- <i>n</i> -Pentylfuran	149. 1-Methylnaphthalene
69. Octanal	158. Phenylacetaldehyde
70. 1,2,4-Trimethylbenzene	164. 2-Dodecanone
72. Branched ketone (mol wt 142) ^c	165. 2-Ethyl-naphthalene
73. 2,4-Heptadienal	166. Branched ketone (mol wt 198) ^c
74. Δ^3 -Carene	174. 2-Dodecenal

^a Volatile compound previously identified from unprocessed rice (see Bullard (1975) for literature references). ^b Volatile compound previously identified from processed rice (see Bullard (1975) for literature references). ^c Mass spectrum indicates given structural type.

accelerating voltage. After sample injection the temperature was programmed from 50 to 185 °C at 1 °C/min and then held isothermally until the emergence of dibutyl phthalate. The column was then vented through a rotary valve to prevent dibutyl phthalate from going into the mass spectrometer.

Two criteria were required for identification of the rice components. First, mass spectra of the tentatively identified rice components must match that of the pure compounds (either purchased or obtained from other laboratories) run on the same system under the same conditions. Secondly, their gas chromatographic properties must be the same. We used a peak enhancement or enrichment technique (Schultz et al., 1967) to overcome the errors which often accompany the use of relative retention times. A 0.5- μ l sample of the rice essence was injected in admixture with five of the known compounds (0.05–0.1 μ g each) that have widely different retention times. Enhancement of the appropriate peak by a known compound

having the same mass spectrum constituted positive identification.

RESULTS AND DISCUSSION

A gas chromatogram (total ion current monitor) of the combined essence fraction of California Brown Pearl rice is shown in Figure 2. The chromatogram indicates the presence of over 150 compounds of which 73 were identified (Table I). In addition, 31 compounds had mass spectra that could be interpreted to the extent of indicating the structural types of the compounds.

Only 10 of the identified compounds have been previously reported for rice, processed and unprocessed combined, and only nine if only unprocessed rice is considered. Compounds previously identified for rice are listed in Table I. Fifty-four of the compounds have not been reported previously for any of the uncooked cereal grains.

One of our greatest concerns was that the isolation process may alter some of the volatile components. The

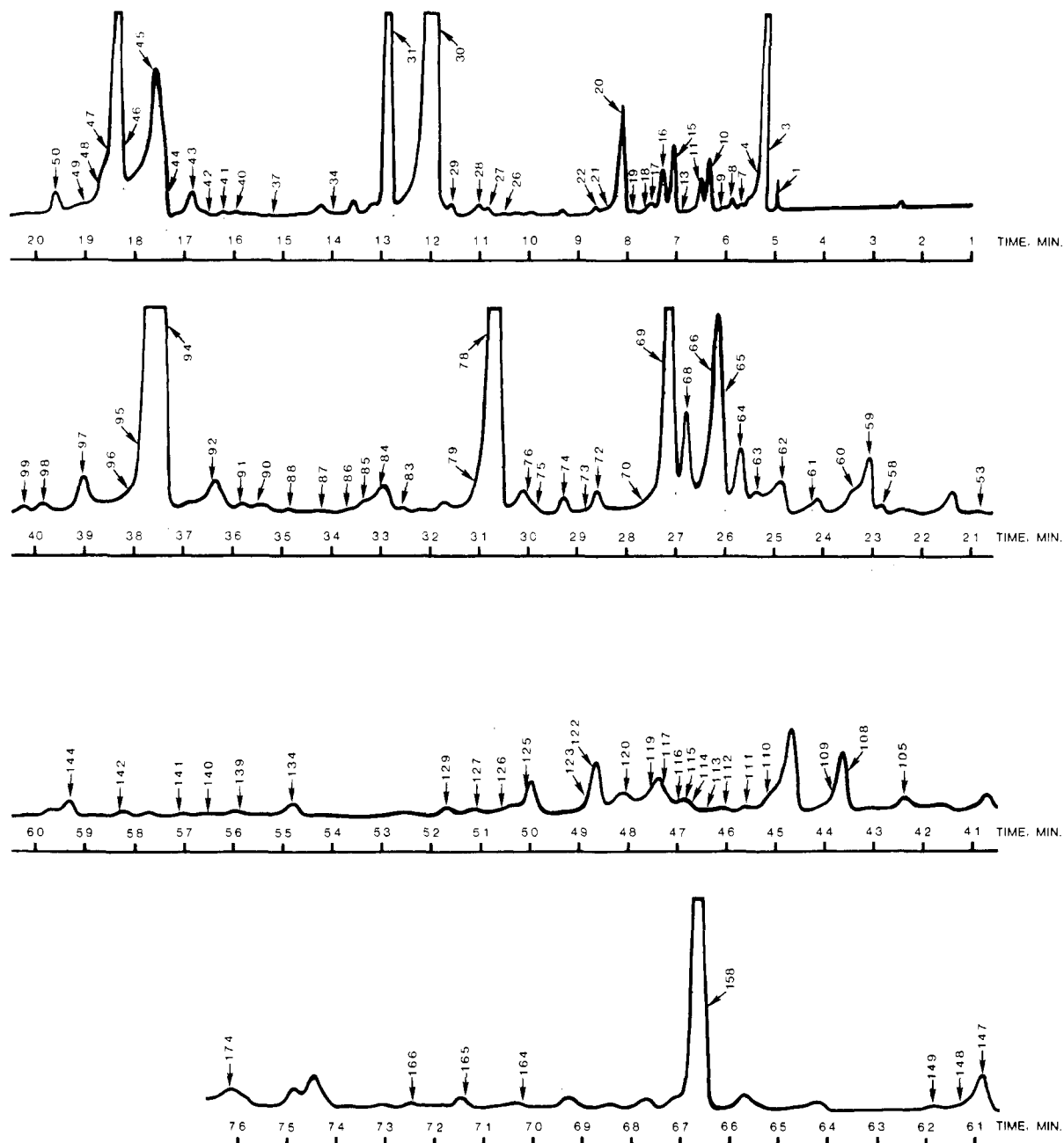


Figure 2. Gas chromatogram of rice essence.

objective was to avoid autoxidation, thermal decomposition, nonenzymatic browning, and other reactions that can cause flavor changes. Previous work indicates that even atmospheric steam distillation can cause formation of pyrazines (Koehler and Odell, 1970).

We tested the possibility of producing an atypical volatile composition by comparing gas chromatogram profiles of volatiles collected at ambient temperatures and at 50 °C. The period for collection at ambient temperatures was 7 h whereas that for the 50 °C temperature was the normal 4 h. The chromatograms for the two collections were similar and new peaks or shifts in peak height ratios were not evident. Furthermore, we did not have mass spectral evidence of any of the numerous pyrazines, thiophenes, pyrroles, and lactones found in roasted barley (Wang et al., 1969, 1970; Collins, 1971), processed wild rice (Withycombe, 1974), or white bread (Mulders, 1973).

Hougen et al. (1971) reported that the volatiles from several varieties of maize, wheat, rye, and triticale were qualitatively similar but quantitatively different. They

reported the tentative identification of 11 compounds in wheat and that the same general chromatogram profile was characteristic of other cereal grains. Rice was not included in their investigation, but if the same trend applies, most of the volatiles listed in Table I would be found in other cereal grains. A literature review to some extent supports that conjecture. Only 30 carbonyls, 10 alcohols, and 2 amines have been identified as volatiles from uncooked cereal grains, and 19 of these compounds were found in the volatiles from rice. One might expect the agreement to have been even better if the isolation and identification systems had been the same.

None of the individual compounds identified had the characteristic aroma of uncooked rice. Also, no character impact (characteristic aroma) compounds could be detected by sniffing the effluent of a 1:10 splitter. A character impact compound could have been retained in the stainless steel system, but this is considered unlikely since a capillary cold trap collection of the total volatiles from the 1:10 splitter smelled similar to the rice essence

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

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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 C₁ to C₆ and the primary isoalcohols from C₃ to C₅ 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).

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

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