

celery, lovage, foenugreek flavors; the 6-butyl-4-methyl- $\alpha$ -pyrone was judged to have the best quality. The presence of branching within the alkyl chain of these derivatives tends to produce weaker, less characteristic, less desirable organoleptic properties. By contrast, the 6-alkyl-4-methyl-tetrahydro- $\alpha$ -pyrones which we synthesized have unpleasant oily, musty, chemical flavors, totally different from either the corresponding 6-alkyl-4-methyl- $\alpha$ -pyrones or the 6-alkyltetrahydro- $\alpha$ -pyrones.

From our results and those reported by Nobuhara (1968, 1969a,b), a number of conclusions may be drawn concerning the flavor properties of the 6-alkyl- $\alpha$ -pyrones and related compounds so far evaluated. The compounds with the most desirable qualities are the C<sub>10</sub> lactones with one or two double bond(s) in conjugation with the carbonyl group and an unbranched alkyl substituent at position 6 on the ring. The alterations in the organoleptic quality created by homologating the 6-alkyl side chain are less significant than the marked changes brought about by the addition of alkyl substituents in the 4 or 5 positions of the ring or by the degree and position of any unsaturation in the lactone ring.

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## Volatile Constituents of Tamarind (*Tamarindus indica* L.)

Peter L. Lee,\* Greg Swords, and G. L. K. Hunter

The volatile constituents of tamarind have been investigated by the combined technique of gas chromatography-mass spectrometry with 61 major components identified and confirmed. Five additional compounds identified as artifacts appeared to originate from the vacuum steam distillation apparatus during isolation of the volatiles.

Several pyrazines and thiazoles, normally formed during roasting of a variety of foods, were found in tamarind. The results of this study suggest that the overall aroma of tamarind consists of citrus notes and warm spice-like flavors with some roast-character.

Tamarind (*Tamarindus indica* L.), a native fruit of Africa and cultivated in India since prehistoric times, grows well in other parts of tropical Asia, South America, and southern Florida. The fruit, ranging from 3 to 7 in. long and about 1 in. in breadth, is a slightly curved, plump, brownish pod, having a brittle shell. The brown edible pulp is high in sugar (ca. 20%) and minerals, particularly calcium and phosphorus (Mowry et al., 1967). It is rich in thiamine but contains virtually no vitamin A or ascorbic acid (Miller and Bazole, 1945). Because of its pleasant acid taste and rich aroma, tamarind has been used in many commercial products such as meat sauce and more recently in beverage drinks (Benero et al., 1974).

Although the biochemistry, the general composition, and the utilization of tamarind have been published and subsequently reviewed by Lefevre (1971), its volatile constituents have not been investigated. This paper describes the isolation and characterization of the major volatile components of tamarind by the combined technique of gas chromatography-mass spectrometry.

#### EXPERIMENTAL SECTION

**Isolation of Volatiles.** The pulp (ca. 600 g) obtained from shelled samples of tamarind was diluted with 2 l. of water and heated at 33 °C with stirring for 15 min to facilitate dispersing of the sticky mash adhered to the seeds. The seeds were removed to yield a puree by straining the pulp directly through a cheesecloth or by straining through a cheesecloth after maceration in a Waring Blendor for about 45 sec. In either case, subsequent analysis showed no significant variation in the volatile composition of the puree. An aliquot fraction (1 l.) of the resulting puree was further diluted to 2 l. and vacuum steam distilled (ca. 20 mmHg) in a Buchi Rotavapor or a Rinco Rotavaporator at 40 °C for 3 hr. Two 500-ml portions of the distillate trapped in liquid nitrogen were collected; an equal amount of water was added to the distilling flask during the steam distillation. The total distillate, having a distinct tamarind aroma, was thawed and saturated with sodium chloride prior to solvent extraction with 4 × 25 ml of Nanograde methylene chloride. The organic solution was washed with 5% sodium bicarbonate, dried over anhydrous sodium sulfate, and concentrated by slow distillation with a short fractionating column to ca. 0.5 ml for GC-MS analyses. The freshly prepared residue, still possessing the tamarind aroma, was analyzed immediately and again after being

\*Corporate Research & Development Department, The Coca-Cola Company, Atlanta, Georgia 30301.

Table I. Identity of Volatile Constituents in Tamarind

Peak no. <sup>a</sup>	Compd	Retention <sup>b</sup> index (I <sub>E</sub> ) CBW 20M	Characteristic MS data <sup>c</sup> <i>m/e</i> (relative intensity)
1	Hexanal	4.49	M <sup>+</sup> 100, 44 (100), 41 (77), 56 (75)
2	4-Methyl-3-penten-2-one	5.00	M <sup>+</sup> 98, 83 (100), 55 (88), 98 (81)
3	3-Methylbutanol	5.55	M <sup>+</sup> 88, 55 (100), 70 (88), 41 (86), 42 (85)
4	Limonene	5.79	M <sup>+</sup> 136, 68 (100), 93 (83), 67 (76)
5	Pyrazine	5.77	M <sup>+</sup> 80 (100), 53 (37), 26 (24)
6	<i>trans</i> -2-Hexenal	5.80	M <sup>+</sup> 98, 41 (100), 69 (62), 83 (48), 98 (33)
7 <sup>f</sup>	Pentanol	6.00	M <sup>+</sup> 88, 42 (100), 55 (95), 70 (63)
8	2-Methylthiazole	6.15	M <sup>+</sup> 99, 58 (100), 99 (94)
9	2-Methylpyrazine	6.41	M <sup>+</sup> 94 (100), 67 (41), 53 (16)
10	2-Ethylthiazole	6.83	M <sup>+</sup> 113, 58 (100), 113 (95), 112 (69)
11	2,5-Dimethylpyrazine	6.86	M <sup>+</sup> 108 (100), 42 (80), 81 (20)
12	2,6-Dimethylpyrazine	6.98	M <sup>+</sup> 108 (100), 42 (80), 81 (20)
13	Ethylpyrazine	7.06	M <sup>+</sup> 108, 107 (100), 108 (82), 81 (23)
14	Hexanol	7.10	M <sup>+</sup> 102, 56 (100), 43 (61), 55 (54), 42 (41), 69 (39)
15	<i>cis</i> -3-Hexen-1-ol	7.35	M <sup>+</sup> 100, 67 (100), 41 (90), 82 (65)
16	<i>trans</i> -2-Hexen-1-ol	7.50	M <sup>+</sup> 100, 57 (100), 41 (69), 82 (11)
17	<i>cis</i> -Linalool oxide (five-membered ring)	7.95	M <sup>+</sup> 154, 59 (100), 43 (92), 94 (58), 111 (4)
18	Heptanol	8.00	M <sup>+</sup> 116, 70 (100), 56 (95), 55 (90), 69 (75)
19	Furfural	8.24	M <sup>+</sup> 96, 95 (100), 96 (94), 39 (52)
20	<i>trans</i> -Linalool oxide (five-membered ring)	8.30	M <sup>+</sup> 154, 59 (100), 43 (92), 94 (58), 111 (4)
21	2-Ethylhexanol	8.37	M <sup>+</sup> 130, 57 (100), 41 (53), 43 (47), 70 (46), 83 (20)
22	2-Acetylfuran	8.70	M <sup>+</sup> 110, 95 (100), 110 (80), 39 (40), 43 (38)
23	Benzaldehyde	8.96	M <sup>+</sup> 106 (100), 105 (95), 77 (88)
24	Linalool	8.97	M <sup>+</sup> 154, 93 (100), 71 (93), 41 (65), 55 (58), 69 (50)
25	Octanol	9.04	M <sup>+</sup> 130, 55 (100), 56 (94), 41 (72), 70 (64), 69 (62), 43 (62), 84 (42), 83 (33)
26	5-Methylfurfural	9.36	M <sup>+</sup> 110 (100), 109 (89), 53 (41)
27	Terpinen-4-ol	9.65	M <sup>+</sup> 154, 71 (100), 93 (67), 111 (58), 136 (30), 154 (28)
28	Phenylacetaldehyde	10.14	M <sup>+</sup> 120, 91 (100), 120 (50), 92 (39)
29	Acetophenone	10.26	M <sup>+</sup> 120, 105 (100), 77 (74), 120 (25), 51 (20)
30	Nonanol	10.00	M <sup>+</sup> 144, 56 (100), 55 (92), 70 (77), 69 (75), 43 (75), 41 (75)
31	Neral	10.47	M <sup>+</sup> 152, 69 (100), 41 (97), 84 (48), 94 (38)
32	$\alpha$ -Terpineol	10.54	M <sup>+</sup> 154, 59 (100), 93 (92), 136 (63), 121 (60), 81 (47), 43 (45), 68 (40)
33 <sup>f</sup>	Geranial	10.95	M <sup>+</sup> 152, 69 (100), 41 (97), 84 (48), 94 (38)
34	Piperitone	11.04	M <sup>+</sup> 152, 82 (100), 110 (52)

Table I (Continued)

Peak no. <sup>a</sup>	Compd	Retention <sup>b</sup> index ( $I_E$ ) CBW 20M	Characteristic MS data <sup>c</sup> $m/e$ (relative intensity)
35 <sup>d</sup>	Naphthalene	11.15	M <sup>+</sup> 128 (100), 51 (30), 127 (30)
36	1-Phenyl-1-propanone	10.85	M <sup>+</sup> 134, 105 (100), 77 (70), 134 (50)
37	1-Phenyl-2-propanone	10.94	M <sup>+</sup> 134, 43 (100), 91 (80), 134 (55), 65 (50)
38 <sup>f</sup>	Decanol	11.00	M <sup>+</sup> 158, 56 (100), 70 (98), 69 (97), 55 (97), 83 (80)
39	Nerol	11.46	M <sup>+</sup> 154, 69 (100), 41 (97), 93 (43), 68 (31)
40	Methyl salicylate	11.56	M <sup>+</sup> 152, 120 (100), 152 (78), 92 (57)
41	Geraniol	11.85	M <sup>+</sup> 154, 69 (100), 41 (97), 93 (50)
42	Undecanol	12.00	M <sup>+</sup> 172, 43 (100), 41 (89), 55 (68), 83 (50)
43 <sup>e,f</sup>	pseudo-Ionone	12.00	M <sup>+</sup> 192, 69 (100), 41 (89), 124 (31)
44	2-Methylnaphthalene	12.10	M <sup>+</sup> 142 (100), 141 (79), 115 (26)
45 <sup>f</sup>	Benzyl alcohol	12.19	M <sup>+</sup> 108, 79 (100), 108 (88), 107 (73)
46	Safrole	12.45	M <sup>+</sup> 162 (100), 131 (71)
47	$\gamma$ -Ionone	12.50	M <sup>+</sup> 192, 93 (100), 121 (98), 192 (17)
48	1-Methylnaphthalene	12.6	M <sup>+</sup> 142 (100), 141 (79), 115 (26)
49	2-Phenylethyl alcohol	12.6	M <sup>+</sup> 122, 91 (100), 92 (74), 122 (44), 65 (25)
50	2,6-Di- <i>tert</i> -butyl- <i>p</i> -cresol	12.6	M <sup>+</sup> 220, 205 (100), 220 (37), 57 (35)
51 <sup>d,f</sup>	Benzothiazole	13.0	M <sup>+</sup> 135 (100), 108 (56), 82 (20), 63 (18)
52	$\beta$ -Ionone	13.08	M <sup>+</sup> 192, 177 (100), 43 (57), 192 (21)
53	1,7-Dimethylnaphthalene	13.30	M <sup>+</sup> 156 (100), 141 (69), 155 (51)
54	Phenol	13.40	M <sup>+</sup> 94 (100), 39 (29), 65 (26), 66 (24)
55 <sup>f</sup>	Biphenyl	13.5	M <sup>+</sup> 154 (100), 153 (49), 152 (36), 76 (26)
56	1,2-Dimethylnaphthalene	13.6	M <sup>+</sup> 156 (100), 141 (69), 155 (51)
57	Cinnamaldehyde	14.0	M <sup>+</sup> 132, 131 (100), 132 (78), 103 (73), 77 (62)
58	$\gamma$ -Nonalactone	14.0	M <sup>+</sup> 156, 85 (100), 128 (65)
59	<i>p</i> -Cresol	14.1	M <sup>+</sup> 108, 107 (100), 108 (88)
60 <sup>d</sup>	(Unknown)	14.3	M <sup>+</sup> 236, 57 (100), 165 (59), 180 (42), 137 (25), 221 (14), 236 (9)
61	Ethyl cinnamate	14.7	M <sup>+</sup> 176, 131 (100), 103 (42), 176 (38)
62	Fluorene	16.3	M <sup>+</sup> 166 (100), 165 (98)
63	Dimethyl phthalate	16.32	M <sup>+</sup> 194, 163 (100), 77 (38), 194 (16)
64	4-Phenyl-2-pyridone		M <sup>+</sup> 171 (100), 170 (82), 143 (36), 115 (32)
65 <sup>d</sup>	Diethyl phthalate	16.80	M <sup>+</sup> 222, 149 (100), 177 (39), 222 (13)
66 <sup>d</sup>	Dibutyl phthalate		M <sup>+</sup> 278, 149 (100), 223 (21), 104 (17)

<sup>a</sup> Refers to peak in Figure 1. <sup>b</sup> Authentic  $I_E$  values were determined on packed columns (van den Dool and Kratz, 1963), many of which were taken from the references cited in this work. <sup>c</sup> Determined with a Hitachi-RMU 6L. <sup>d</sup> Artifact. <sup>e</sup> Tentatively assigned. <sup>f</sup> The major component where overlapping occurred.

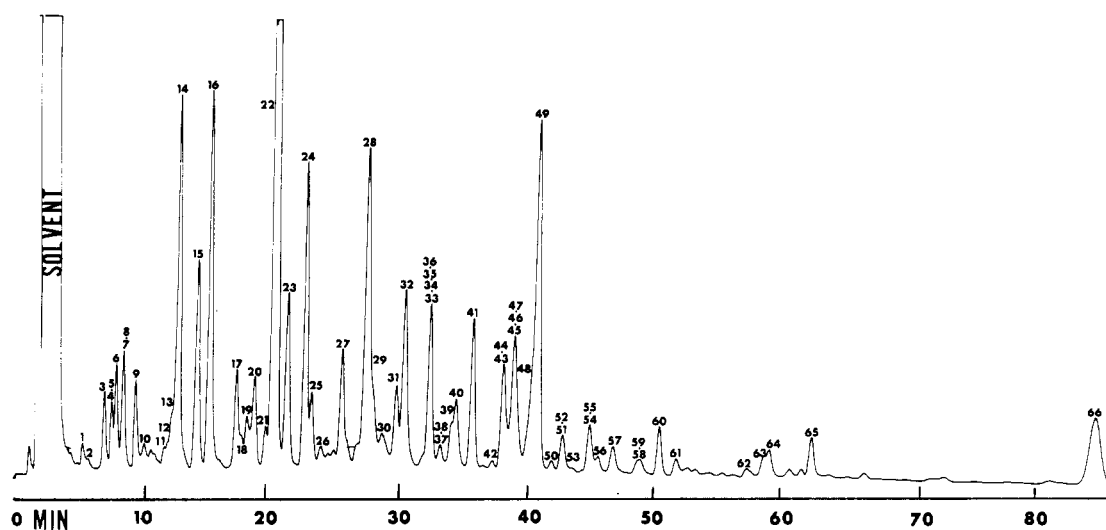


Figure 1. Gas chromatogram of tamarind volatiles.

stored under refrigeration for several days. No apparent chromatographic variation was observed in all cases.

**Combined Gas Chromatography–Mass Spectrometry.** The components of the volatile concentrate were separated using a Varian/Aerograph Series 1200 instrument fitted with a flame ionization detector and coupled to a Hitachi RMU-6L single focusing magnetic sector mass spectrometer. A small fraction of the column effluent was bypassed through a fine metering valve into a Ryhage (single stage) glass jet separator (McFadden, 1973) leading into the ion source. Both the molecular separator and valve were maintained at 200 °C with a convection-circulated air oven.

A 2 mm (i.d.) × 3 m glass column packed with 5% Carbowax 20M on 60–80 mesh acid-washed DMCS Chromosorb W was used throughout the analyses. The column was operated at a programmed rate of 2 °C/min from 60 to 190 °C with a helium flow of 25 ml/min. Both the injector and detector temperatures were maintained at 230 °C. The retention indices ( $I_E$ ) were determined using a mixture of ethyl esters ( $C_{11}$ – $C_{18}$ ) as internal standards by the method of van den Dool and Kratz (1963). All mass spectra were obtained at 90 eV and 80  $\mu$ A with an ion source temperature of 200 °C.

## RESULTS AND DISCUSSION

A typical chromatogram of the methylene chloride concentrate of tamarind volatiles is shown in Figure 1. Table I lists 66 compounds together with their determined gas chromatographic retention indices ( $I_E$ ) and observed characteristic mass spectral data. The identification of these compounds was based on comparison of their respective mass spectral and  $I_E$  data with those of authentic compounds and with that published in the literature (MSDC, 1970). It is readily seen from Figure 1 that considerable overlapping occurred among some of the compounds. In such cases identification was accomplished solely from their mass spectral profiles since the retention indices of unresolved components were observed to deviate slightly from those expected for single compounds.

The occurrence of the five simple pyrazines and two lower alkylthiazoles in tamarind was somewhat unexpected. These compounds are normally formed during roasting and frying of a variety of foods such as potato chips (Buttery et al., 1971), roasted barley (Wang et al., 1969), coffee aroma (Bondarovich et al., 1967), roasted peanuts (Johnson et al., 1971), roasted filberts (Kinlin et al., 1972), popcorn (Waldradt et al., 1970), and cooked beef (Wilson et al., 1973). The mass spectral data and  $I_E$  values of the pyra-

zines and thiazoles were consistent with those reported for coffee aroma and roasted filberts. The flavor properties of these types of compounds were evaluated by Pittet and Hruza (1974), in which the mono- and dialkylpyrazines were described as green, nutty, and roasted, and the lower 2-alkylthiazoles as green and vegetable like.

Benzothiazole has been reported in numerous foods including cocoa concentrate (Flament et al., 1967), popcorn, roasted peanuts, soybean milk (Wilkins and Lin, 1970), shallow-fried beef (Wanatabe and Sato, 1972), and sterilized concentrated milk (Arnold et al., 1966). Its presence in nonfat dry milk was attributed to an artifact by Ferretti and Flanagan (1973). To determine whether benzothiazole is indeed an artifact in the present work, a vacuum steam distillation using water alone was carried out in the same apparatus used for the isolation of the tamarind volatiles. Subsequent GC–MS analyses of the methylene chloride extract of the water thus processed definitely established the presence of benzothiazole.

The observed mass spectra of the two linalool oxides agreed with those previously reported by Felix et al. (1963) and together with linalool imparted a slight rosy aroma to tamarind. The most abundant constituent, 2-acetylfuran, which has a balsamic-cinnamic note, coupled with furfural and 5-methylfurfural (both present in minute amounts and possessing a sweet, caramel-like flavor) appeared to contribute significantly to the overall aroma of tamarind.

The citrus note in tamarind was attributed to the monoterpenoids limonene, terpinen-4-ol, neral,  $\alpha$ -terpineol, geraniol, and geraniol. Piperitone, with its fresh, minty, camphoraceous odor, was identified from the multicomponent peak 33–36 by its characteristic fragmentation pattern which is consistent with that reported by von Sydow (1964). The compounds methyl salicylate, safrole, the ionones, cinnamaldehyde, and ethyl cinnamate undoubtedly contribute the warm spice-like notes to tamarind.

The trace components 1-phenyl-1-propanone and 1-phenyl-2-propanone were identified by their characteristic fragmentation pattern and  $I_E$  values, which are in agreement with that reported by Kinlin et al. (1972). The assignment of 4-phenyl-2-pyridone was confirmed by comparison of its mass spectrum with that of an authentic sample. The phthalates, naphthalenes, and fluorene found in tamarind have been reported in coffee aroma (Stoll et al., 1967), green tea (Yamanishi et al., 1970), grapes (Stevens et al., 1966), and cranberry (Croteau and Fagerson, 1968). As was the case with benzothiazole, the compounds naphthalene, diethyl phthalate, dibutyl phthalate, and component no. 60 are artifacts; they were also identified as constituents in

the experiment with water described above. The possibility that the remaining naphthalenes, which appear here in trace amounts, are also artifacts cannot be ruled out.

As can be seen from Figure 1, a number of trace components have remained unidentified. However, it is believed that the aroma compounds identified in the present work in tamarind adequately account for the citrus, the warm spice-like flavors, and the roasted notes which are characteristic of tamarind.

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## Quantitative Determination of Limonin in Grapefruit Juice by High-Pressure Liquid Chromatography

James F. Fisher

Microgram quantities of limonin were resolved from a chloroform extract of grapefruit juice by high-pressure liquid chromatography using a mi-

croporasil column and eluting with a chloroform-acetonitrile system. The limonin was detected with a refractometer.

Several analytical methods (Chandler and Kefford, 1966; Wilson and Crutchfield, 1968; Maier and Beverly, 1968; Maier and Grant, 1970; Chandler, 1971; Kruger and Colter, 1972; Tatum and Berry, 1973; Fisher, 1973; Beisel, 1974) for limonin, a bitter constituent of grapefruit juice (Maier and Dreyer, 1965), appear in the literature. However, a simplification of the parameters associated with these methods such as time, sample preparation, detection, and objectivity would be advantageous.

The important role limonin plays in grapefruit juice bitterness and its correlation with grapefruit flavor (Fisher, 1975) required the development of an improved objective method for the quantitation of limonin in grapefruit juice.

The object of this work was to design such an assay. The following procedure was developed and has been used in this laboratory.

#### MATERIALS

**Apparatus.** A Model ALC202 high-pressure liquid chromatograph (HPLC) with a Model 6000 pump, U6K injector, and R401 differential refractometer (Waters Asso-

ciates, Milford, Mass.) was used. A Precision Scientific Company constant temperature water circulating system (Model 66600) was used. The recorder was a Texas Instrument servo/riter II 2-pen. A Model BB Burrell wrist action shaker (Burrell Corp., Pittsburgh, Pa.) was used. A Waters Associates Sample Clarification Kit with 1.0 or 0.5  $\mu$ m Millipore-fluoropore filter was used for filtering.

**Column.** A Waters Associates 30 cm  $\times$  4 mm i.d.  $\mu$  porasil column (10  $\mu$  porous silica packing) was used. The column was contained within an insulated water jacket.

**Reagents.** The eluting system was chloroform-acetonitrile (95:5) (Burdick and Jackson Laboratories, Muskegon, Mich.).

#### PROCEDURE

**Sample Preparation.** A 10-g sample of processed single strength grapefruit juice or reconstituted concentrate was extracted with 3  $\times$  10 ml of chloroform for 2 min on the Burrell shaker set for maximum agitation. A centrifuge was used to separate the layers. The combined chloroform layers were evaporated to approximately 1.0 ml under nitrogen at 40°C. The resulting solution was filtered and the filtering system well rinsed. The filtrate was evaporated to dryness under nitrogen and redissolved in 50 to 100  $\mu$ l of the eluting system, depending upon the expected limonin concentration.

Florida Department of Citrus, University of Florida, IFAS, Agricultural Research and Education Center, Lake Alfred, Florida 33850.