

Characterization of Volatiles in Rambutan Fruit (*Nephelium lappaceum* L.)

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The volatile compounds from the red-skinned cultivar of rambutan, Jitlee (*Nephelium lappaceum* L.), a tropical fruit native to Southeast Asia, were extracted using both Freon 113 and ethyl acetate solvents. Isolation and characterization of odor-active compounds present in the fruit were mediated by gas chromatography/olfactory (GC/O), chromatography, and spectrometry. Authentic standards were used to determine mass spectral, retention index, and odor match. Of over 100 volatiles detected by GC/MS, twice as many polar volatiles were detected in the ethyl acetate extract as in the nonpolar Freon extract. GC/O analysis also detected more odor-active compounds in the polar extracts. Over 60 compounds in the extracts had some odor activity. The 20 most potent odorants included β -damascenone, (*E*)-4,5-epoxy-(*E*)-2-decenal, vanillin, (*E*)-2-nonenal, phenylacetic acid, cinnamic acid, unknown 1 (sweaty), ethyl 2-methylbutyrate, and δ -decalactone. On the basis of calculated odor activity values, β -damascenone, ethyl 2-methylbutyrate, 2,6-nonadienal, (*E*)-2-nonenal, and nonanal were determined to be the main contributors to the fruit aroma. Taken together, these results indicate that the exotic aroma character of rambutan is the interaction of fruity-sweet and fatty-green odors, with the possible contribution of "civet-like"-sweaty, spicy, and woody notes.

Keywords: Aroma; GC/O; *Nephelium lappaceum*; rambutan; volatiles

INTRODUCTION

Among the many attractive and desirable attributes that create demand for fruits from the tropics and subtropics, their characteristic flavor is the most noticeable to consumers. In addition, these fruits are often inexpensive, are extremely rich in vitamins (Wills et al., 1986), and can be used in a wide range of products including beverages, dairy products, desserts, and gum.

Native to Southeast Asia, rambutan (*Nephelium lappaceum* L.) belongs in the same family (Sapindaceae) as the subtropical fruits lychee and longan and is often described as being less aromatic than the lychee. While this fruit is relatively unknown in the United States, it is an important commercial crop in Asia, where it is consumed fresh, canned, or processed and appreciated for its refreshing flavor and exotic appearance (Almeyada et al., 1979).

Various studies on the postharvest properties and marketing of this fruit have been reported without describing its flavor chemistry (Landrigan et al., 1994; Lam and Kosiyachinda, 1987). In this paper we describe the most potent odorants extracted from rambutan fruit juice detected by gas chromatography/olfactometry (GC/O) (Acree et al., 1984).

MATERIALS AND METHODS

Fruits. Rambutan fruit was obtained from the Fruit Tree Center, Primary Production Department, Singapore. The red-skinned cultivar, Jitlee, was selected for this study due to its high popularity among consumers and its longer storage life (Lye et al., 1987).

Materials. Linalool oxides (pyranoid and furanoid) were a gift from Dr. H. Iwabuchi (San Ei Gen, Inc., Osaka, Japan). All other authentic standards were obtained commercially. However, (*E*)-4,5-epoxy-(*E*)-2-decenal was synthesized with a modification of the method described by Schieberle and Grosch (1991). (*E,E*)-2,4-Decadienal (4 mmol) dissolved in 40 mL of methylene chloride was oxidized with 3-chloroperbenzoic acid (8 mmol) added in approximately equal portions every 10 min for an hour. The reaction mixture was stirred for 24 h at ambient temperature and extracted with 10% sodium carbonate to remove the byproduct 3-chlorobenzoic acid. The crude extract was concentrated under vacuum on a rotary evaporator almost to dryness, redissolved in 5 mL of pentane, and separated on a column (300 mm \times 10 mm i.d.) packed with \sim 30 g of silica gel, 60 Å (Aldrich, Milwaukee, WI), that was deactivated with 5% water. Five fractions were obtained by stepwise elution with 50 mL of pentane, 100 mL of 5% ether in pentane, 100 mL of 10% ether in pentane, 100 mL of 20% ether in pentane, and 150 mL of 30% ether in pentane. The last fraction, which contained the desired compound, was concentrated to 5 mL and purified using a Varian Star model 9010 HPLC solvent delivery system (Varian Associates, Inc., Walnut Creek, CA) with a UV detector (Varian, model 9065) set at 220 nm. Forty microliter portions were separated on a 380 mm \times 10 mm i.d. column filled with 5- μ m Lichrosorb SI-100 and eluted with 5% ether in pentane at 3.5 mL/min. Ninety-five milligrams of product was obtained. The EI-MS of (*E*)-4,5-epoxy-(*E*)-2-decenal gave the following: *m/z* ions (%) 68 (100), 39 (33), 41 (29), 55 (17), 81 (7), 139 (1); ¹H NMR (δ , CDCl₃) 0.91 (3H, t, $J_1 = 5.8$ Hz, $J_2 = 6.6$ Hz, H-10), 1.26–1.41 (4H, m, H-9, H-8), 1.43–1.51 (2H, m, H-7), 1.60–1.70 (2H, m, H-6), 2.97 (1H, dt, $J_1 = 2.0$ Hz, $J_2 = 5.4$ Hz, $J_3 = 5.4$ Hz, H-5), 3.33 (1H, dd, $J_1 = 2.0$ Hz, $J_2 = 6.6$ Hz, H-4), 6.39 (1H, dd, $J_1 = 7.4$ Hz, $J_2 = 15.8$ Hz, H-2), 6.56 (1H, dd, $J_1 = 6.6$ Hz, $J_2 = 15.8$ Hz, H-3), 9.57 (1H, d, $J = 7.4$ Hz, H-1).

Sample Preparation. The fruit was peeled and the flesh separated from the seed. Two batches of \approx 1.3 kg of the flesh were blended with 1.0 M CaCl₂ for 1 min to inactivate enzymes

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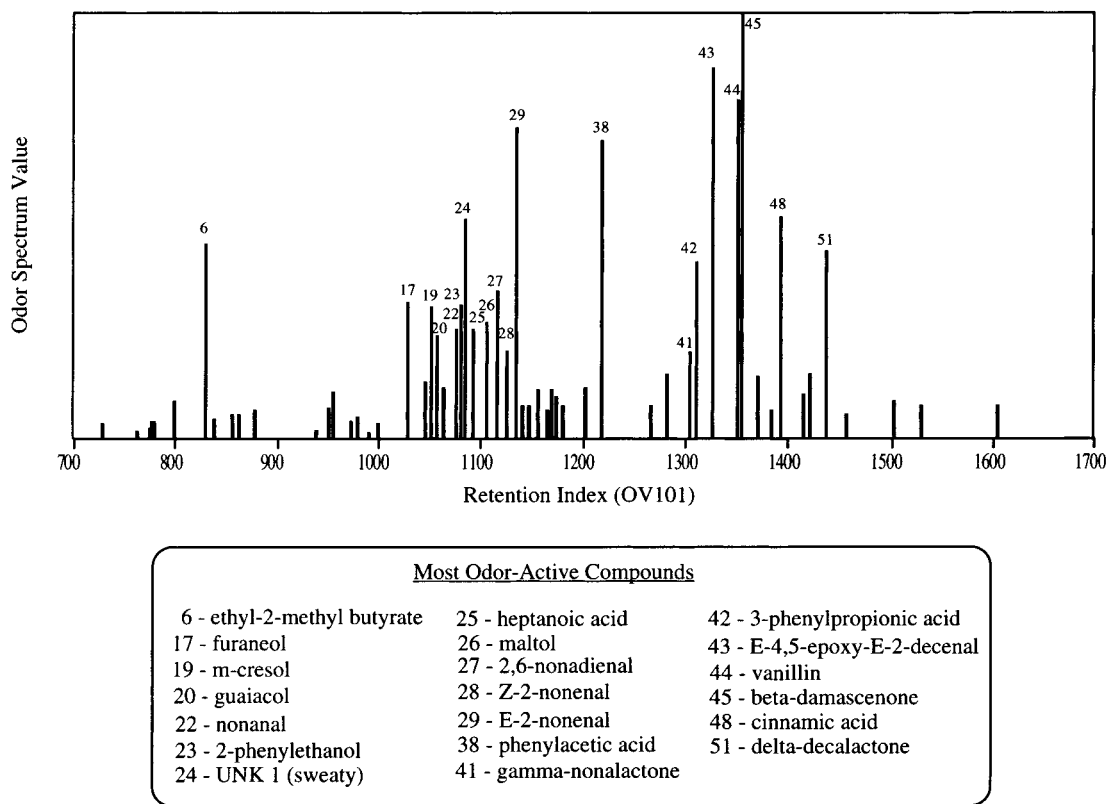


Figure 1. Odor spectrum of rambutan fruit combined Freon and ethyl acetate extracts.

(Shure, 1992), yielding 1.3 L of the juice. The juice was sequentially extracted with Freon and ethyl acetate. Both extracts were dried with anhydrous magnesium sulfate and serially diluted or concentrated by 3-fold. The most concentrated extract was 729-fold and the least 1/27-fold.

Gas Chromatography/Olfactometry (GC/O). Extracted samples were analyzed using a GC/O system (CharmAnalysis) on a modified HP 5890 GC (Datu, Inc., Geneva, NY) and sniffed using an HP-1 (15 m \times 0.32 mm) or an HP-Innowax (15 m \times 0.32 mm) column. The oven temperature was programmed from 35 to 250 $^{\circ}$ C at 6 $^{\circ}$ C/min. All extract dilutions were sniffed twice (repeated measure) until no odor was observed (detection threshold), and the retention time of each odorant was converted to Kovats indices using 7–28-carbon normal paraffins. Caramel, floral, fruity, green, plastic, spicy, sweaty, urine, and woody were the words used to describe the most potent odorants chosen from a lexicon that also included the words coconut, citrus, earthy, fatty, gasoline, grassy, medicinal, minty, musty, mothball, and skunky. The lexicon was developed by sniffing the 729-fold sample three times and selecting the 20 most frequently used words.

Quantitative Analyses. The most odor active volatiles in rambutan as determined by GC/O were quantified using GC mass fragmentography of the most abundant ion. The concentration of each volatile was adjusted for loss during extraction by determining the percent recovery of each compound in a model system (Table 2). Odor activity values (OAVs) were then determined by dividing the concentration by its water phase detection threshold (Guadagni et al., 1966).

Capillary Gas Chromatography/Mass Spectrometry (GC/MS). An HP model 5985 MSD GC/MS was used with an HP-1 (25 m \times 0.32 mm) or HP-Innowax (25 m \times 0.32 mm) column. The oven temperature was programmed from 35 to 250 $^{\circ}$ C at 4 $^{\circ}$ C/min. Retention indices (RI) of standard compounds that matched with RI of unknowns detected by GC/O were tentatively identified. Confirmation of unknowns were based upon odor, mass spectral, and RI matches with authentic standards.

Magnetic Resonance Spectroscopy. 1 H NMR spectra was acquired on a Varian XL-200 instrument at 200 MHz.

RESULTS AND DISCUSSION

Over 100 volatiles were detected in the rambutan extract by GC/MS. Twice as many of the most polar volatiles were detected in the ethyl acetate extract as were detected in the nonpolar Freon extract. Tables 1, 3, and 4 list the 101 compounds identified by GC/MS. At least 60 of the compounds had odor activity under GC/O, including 11 unidentified compounds. More odor active compounds were also detected in the ethyl acetate extracts than in the Freon extracts (Tables 1 and 3).

The data include two different odor activity values (charm and OAV) as well as an odor intensity measure referred to as the odor spectrum value (OSV). A charm value is defined as the area of the peaks in the charm chromatogram and is proportional to the concentration of the component in the extract divided by the gas-phase detection threshold, while the OAVs are the concentration measured in the extract divided by the water-phase detection threshold reported in the literature (Table 2). Under ideal conditions, i.e. CharmAnalysis of the retronasal headspace and thresholds determined in the actual food matrix itself, the charm values and OAVs should yield the same results. The OSV is the normalized charm value modified with an approximate Stevens's law exponent (Stevens, 1958). OSVs are independent of concentration and approximate the relative importance of component odorants, while charm and OAVs as true activity measures are linear functions of concentration (Acree, 1997).

Figure 1 shows the combined odor spectrum for both Freon and ethyl acetate extracts. Among the volatiles detected by GC/O, β -damascenone, (*E*)-4,5-epoxy-(*E*)-2-decenal, vanillin, (*E*)-2-nonenal, phenylacetic acid, cinnamic acid, unknown 1 (sweaty), ethyl 2-methylbutyrate, δ -decalactone, 3-phenylpropionic acid, 2,6-nonadienal, furaneol, 2-phenylethanol, *m*-cresol, maltol,

Table 1. Most Potent Odorants Found in Rambutan Fruit (above 20% OSV^a)

peak no.	compd detected	CAS Registry No.	retention indices		descriptors	charm value	odor spectrum values (OSV)	confirmed by	
			HP-1	HP-Innowax				GCO	MS
45	β -damascenone	23726-93-4	1356	1790	fruity, floral	55427	100	+	-
43	(<i>E</i>)-4,5-epoxy-(<i>E</i>)-2-decenal		1335	1913	woody	41890	87	+	+
44	vanillin	121-33	1345	2591	vanilla	34660	79	+	+
29	(<i>E</i>)-2-nonenal	18829-56-6	1130	1519	plastic, green	28158	71	+	+
38	phenylacetic acid	103-82-2	1236	2568	urine	26746	69	+	+
48	cinnamic acid	140-10-3	1394	2852	woody	14947	52	+	+
24	unknown 1		1085	2030	sweaty	14742	52	-	-
6	ethyl 2-methylbutyrate	7452-79-1	835	1041	fruity	11435	45	+	+
51	δ -decalactone	705-86-2	1437	2179	coconut	10443	43	+	+
42	3-phenylpropionic acid	501-52-0	1308	2650	balsamic	9368	41	+	+
27	2,6-nonadienal	557-48-2	1117	1553	green	5639	32	+	+
17	furaneol	3658-77-3	1029	2020	caramel	5479	31	+	+
23	2-phenylethanol	60-12-8	1078	1905	spicy	5219	31	+	+
19	<i>m</i> -cresol	108-39-4	1048	2069	medicine	5070	30	+	+
26	maltol	118-71-8	1104	1954	cotton candy	4033	27	+	+
25	heptanoic acid	111-14-8	1092	1950	sweaty	3567	25	+	+
22	nonanal	124-19-6	1080	1380	plastic, fatty	3527	25	+	+
20	guaiacol	90-05-1	1056	1848	medicine	3051	23	+	+
28	(<i>Z</i>)-2-nonenal	60784-31-8	1121	1492	plastic, green	2534	20	+	-
41	γ -nonalactone	104-61-0	1304	2008	musty	2208	20	+	+

^a Odor spectrum value (OSV) is the normalized charm value modified with an approximate Stevens's law exponent.

Table 2. Concentrations, Odor Thresholds, and Odor Activity Values (OAV) of Most Potent Odorants in Rambutan Fruit As Detected by GC/O

peak no.	compd	% recovery	concn (μ g/L of juice)	threshold values ^a (ppb in water)	OAV
45	β -damascenone	115	2.27	0.01 (a)	226.69
43	(<i>E</i>)-4,5-epoxy-(<i>E</i>)-2-decenal	92	14.92	5 (b)	2.98
44	vanillin	116	21.10	200 (c)	0.13
29	(<i>E</i>)-2-nonenal	95	7.03	0.08 (d)	87.83
38	phenylacetic acid	105	131.67	10000 (c)	0.02
48	cinnamic acid	114	1340.15	5000 (b)	0.27
6	ethyl 2-methylbutyrate	105	15.13	0.1 (d)	151.30
51	δ -decalactone	105	9.77	100 (e)	0.12
42	3-phenylpropionic acid	104	363.09	25000 (c)	0.02
27	2,6-nonadienal	92	1.22	0.01 (d)	121.50
17	furaneol	90	240.15	25 (f)	9.61
23	2-phenylethanol	95	107.78	17 (c)	8.78
19	<i>m</i> -cresol	85	5.65	650 (c)	0.01
26	maltol	90	53.79	10000 (b)	0.01
25	heptanoic acid	99	30.43	500 (c)	0.08
22	nonanal	101	51.90	1 (c)	62.64
20	guaiacol	104	11.99	21 (c)	0.61
28	(<i>Z</i>)-2-nonenal	95	nd ^b		
41	γ -nonalactone	118	29.43	1 (g)	35.90

^a (a) Ohloff (1978); (b) determined using method as described by Takeoka et al. (1990); (c) Fazzalari (1978); (d) Buttery (1981); (e) Takeoka et al. (1990); (f) Guth and Grosch (1994); (g) Stahl, 1973. ^b nd, not detected.

heptanoic acid, nonanal, guaiacol, (*Z*)-2-nonenal, and γ -nonalactone were identified as being the most potent odorants in rambutan extract (Table 1). Of the 20 most potent odorants, 7 were polar in nature, indicating the value of solvent extraction with polar solvents. An odor spectrum that would more accurately reflect the sensory perception of the fruit could be made from the GC/O of the retronasal headspace (Guth and Grosch, 1994; Roberts, 1996; Linforth and Taylor, 1993). However, an approximation of the retronasal headspace odor spectrum is the list of OAVs shown in Table 2, indicating a lower contribution to the perceived odor expected for compounds such as vanillin, maltol, and cinnamic acid.

The concentrations of the most potent odorants in rambutan determined by GC/MS are presented in Table 2. These values were used to determine the OAVs of each volatile. On the basis of OAVs, β -damascenone, ethyl 2-methylbutyrate, 2,6-nonadienal, (*E*)-2-nonenal, and nonanal had values over 60, indicating their significant contribution to the aroma of this fruit in

water-based media. Compounds that were acidic in nature, such as phenylacetic acid and cinnamic acid, had very low OAVs due to their very high thresholds reported in water. The exact role of these compounds is not clear, but they should be tested for their contribution in mixture experiments.

β -Damascenone, with a characteristic fruity-floral aroma, had the largest odor activity by all three measures. This compound was found using GC/O to be a potent aroma compound in many other fruits such as apples, grapes, and tomatoes (Braell et al., 1986; Cunningham et al., 1986; Buttery et al., 1990). (*E*)-4,5-Epoxy-(*E*)-2-decenal, previously reported in puff pastries (Gassenmeier and Schieberle, 1994), soybean oil (Guth and Grosch, 1990), and wheat bread crumbs (Schieberle and Grosch, 1991), has been described as having a metallic odor. However, this compound was described by the authors as having more of a woody note at lower concentrations. Other odor descriptors provided by an informal sensory panel included green, chalky, fatty, and rust-like. The odor of this compound, the authors

Table 3. Additional Potent Odorants in Rambutan Fruit (below 20% OSV^a)

compd detected	CAS Registry No.	retention indices		descriptors	charm value	odor spectrum values (OSV)	confirmed by	
		HP-1	HP-Innowax				GCO	MS
(<i>E,E</i>)-2,4-decadienal	25152-84-5	1284	1778	citrus	1187	15	+	+
ethyl cinnamate	103-36-6	1426	2102	musty	1171	15	+	+
unknown		1372		mothball-like	1136	14	+	-
2-acetyl-2-thiazoline	29926-41-8	1053	1725	caramel	836	12	+	+
(<i>E</i>)-furan linalool oxide	34995-77-2	1065	1453	green	721	11	+	+
carvone	6485-40-1	1208	1705	minty	711	11	+	+
unknown		1170		earthy	664	11	+	-
(<i>E,Z</i>)-2,4-nonadienal	5910-87-2	1165	1644	vegetative	637	11	+	+
1-octen-3-ol	3391-86-4	954	1445	earthy	572	10	+	+
γ -decalactone	706-14-9	1418	2109	coconut	496	9	+	+
(<i>E,E</i>)-2,4-nonadienal	5910-87-2	1180	1680	green	446	9	+	+
unknown		1505		plastic	370	8	+	-
furfural	98-01-1	798	1410	burnt	347	8	+	+
unknown		1605		plastic	286	7	+	-
unknown		1141		minty	281	7	+	-
benzothiazole	95-16-9	1180	1909	musty	269	7	+	+
unknown		1149		spicy	266	7	+	-
(<i>E,Z</i>)-2,4-decadienal	25152-83-4	1267	1750	fatty, green	251	7	+	+
γ -undecalactone	104-67-6	1529	2235	fruity	247	7	+	+
3-methyl(thio)propanol	505-10-2	950	1708	earthy	214	6	+	+
<i>endo</i> -isocamphone ^b	3767-44-0	1165		musty	197	6	-	+
unknown		1385		musty	179	6	+	-
unknown		878		musty	174	6	+	-
α -humulene	6753-98-6	1454	1663	woody	138	5	+	+
2-heptanone	110-43-0	865	1151	musty	125	5	+	+
isoamyl acetate	123-92-2	860	1101	floral	110	4	+	+
2-amylfuran	3777-69-3	980	1221	balsamic	94	4	+	+
2-methylbutyric acid	600-07-7	846	1665	sour	76	4	+	+
2-acetylthiazole	24295-03-2	973	1623	popcorn	59	3	+	+
ethyl butyrate	105-54-4	780	1029	fruity	46	3	+	+
hexanal	66-25-1	778	1071	grassy	46	3	+	+
hexanoic acid	142-62-1	1005	1836	sweaty	43	3	+	+
unknown		720		fruity	28	2	+	-
unknown		775		skunky	16	2	+	-
hexyl acetate	142-92-7	994	1267	grassy	<5	<1	+	+
5-methylfurfural	620-02-0	931	1558	musty	<5	<1	+	+
isobutyl acetate	110-19-0	760	1009	fruity	<5	<1	+	+
1,2-dimethoxybenzene	91-16-7	1112	1716	plastic	<5	<1	+	+
isobutyric acid	79-31-2		1551	rancid	<5	<1	+	+
octanoic acid	124-07-2	1165	2040	sweaty	<5	<1	+	+
butyric acid	107-92-6		1630	vomit/rancid	<5	<1	+	+

^a Odor spectrum value (OSV) is the normalized charm value modified with an approximate Stevens's law exponent. ^b Tentatively identified by GC/MS.

Table 4. Volatile Compounds Not Odor Active in Rambutan Fruit

compd detected by GC/MS	CAS Registry No.	retention indices		compd detected by GC/MS	CAS Registry No.	retention indices	
		obsd	std			obsd	std
butyl acetate	123-86-4	798	799	δ -octalactone	698-76-0	1230	1228
ethyl crotonate	623-70-1	823	823	nonanoic acid	112-05-0	1273	1271
(<i>E</i>)-2-hexenal	6728-26-3	824	826	1,2,3-trimethoxybenzene	634-36-6	1276	1276
(<i>E</i>)-2-hexen-1-ol	928-95-0	850	850	farnesane	3891-98-3	1286	1286
1-hexanol	111-27-3	854	854	ethyl 3-hydroxyoctanoate	7367-90-0	1310	1310
γ -butyrolactone	96-48-0	856	855	butyl benzoate	136-60-7	1348	1348
amyl acetate	628-63-7	897	897	decanoic acid	334-48-5	1353	1350
ethyl 3-hydroxybutyrate	5405-41-4	910	910	tetradecane	629-59-4	1398	1400
benzaldehyde	100-52-7	926	926	<i>trans</i> -isoeugenol	97-54-1	1420	1420
ethyl 3-hydroxy-3-methylbutyrate	18267-36-2	929	930	β -caryophyllene	87-44-5	1421	1430
ethyl hexanoate	123-66-0	982	984	2-methoxybenzene	100-66-3	1425	1425
benzyl alcohol	100-51-6	1008	1003	geranyl acetone	3796-70-1	1430	1431
limonene	5989-27-5	1021	1022	β -ionone	14901-07-6	1448	1448
acetophenone	98-86-2	1030	1030	methyl vanillate	3943-74-6	1476	1476
ethyl 2-hydroxycaprate	124439-28-7	1042	1041	<i>o</i> -phenylphenol	90-43-7	1483	1484
camphor-L	76-22-2	1120	1120	ethyl 4-hydroxybenzoate	120-47-8	1488	1485
DDMP ^b	28564-83-2	1124	1124	vanillic acid	121-34-6	1541	1540
benzyl acetate	140-11-4	1134	1134	ethyl vanillate	617-05-0	1547	1548
octyl acetate	1112-14-1	1136	1137	lauric acid	143-07-7	1550	1550
ethyl benzoate	93-89-0	1146	1146	lauryl acetate	112-66-3	1592	1591
(<i>Z</i>)-pyran linalool oxide	14009-71-3	1150	1148	δ -undecalactone	710-64-3	1617	1617
nonyl alcohol	143-08-8	1155	1155	myristic acid	544-63-8	1742	1741
(<i>E</i>)-pyran linalool oxide	39028-58-5	1157	1155	ethyl myristate	124-06-1	1777	1775
benzoic acid	65-85-0	1170	1167	palmitic acid	57-10-3	1954	1952
ethyl phenylacetate	101-97-3	1213	1213	ethyl palmitate	628-97-7	1987	1986
phenylethyl acetate	103-45-7	1227	1226				

^a Retention indices on HP-1 column. ^b 2,3-Dihydroxy-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one.

believe, is a detectable attribute of rambutan fruit. The fatty and green notes, which were exhibited by (*E*)- and (*Z*)-2-nonenal, nonanal, and 2,6-nonadienal, were all probably derived from lipid oxidation. The presence of the alkadienals (C9 and C10) has been shown to be derived from the hydroperoxidation of unsaturated fatty acids typically dictated by a lipogenase (Lindsay, 1985). The civet-like and sweaty notes were due to phenylacetic acid, heptanoic acid, and unknown 1, which may contribute to the underlying exotic aroma of the fruit. However, as noted in their low OAVs, the contribution of these compounds found even at relatively high concentrations may be limited.

Table 3 lists odorants that had OSVs below 20% of the most potent odorant identified— β -damascenone. These compounds have all been reported in various fruits and vegetables. Although these compounds seem to contribute little to the odor character of rambutan, they should be tested for their contribution to the complexity of rambutan flavor.

Listed in Table 4 are the remaining rambutan volatiles that were detected by GC/MS. Over 50 volatiles were identified, including alcohols, aldehydes, esters, ketones, terpenes, and other miscellaneous compounds. Most of these volatiles had no detectable odor when analyzed by GC/O, thus indicating these compounds had no odor activity or were below their respective thresholds in this fruit.

Analysis of the rambutan extracts on a polar HP-Innowax column was useful in the analysis of the ethyl acetate extracts, providing better chromatographic separation of the acidic compounds. Although unknown 1 is presently unidentified, it is suspected that the sweat-like odor [RI = 1085 (HP-1) and 2030 (Innowax)] is an unsaturated acid. These acids have been identified as the chemicals responsible for the characteristic human axillary odors (Zeng et al., 1991) and are likely suspects since the odor detected in the fruit had an odor similar to human sweat.

Taken together, these results indicate that the exotic aroma character of rambutan is the interaction of fruity-sweet (β -damascenone, ethyl 2-methylbutyrate, fura-neol) and fatty-green (2-nonenal, nonanal, 2,6-nonadienal) notes, with sweaty (phenylacetic acid, heptanoic acid, unknown 1), woody [(*E*)-4,5-epoxy-(*E*)-2-decenal, lactones, vanillin], and spicy (cinnamic acid, guaiacol, etc.) notes contributing to the complexity of the flavor.

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