

## Characteristic Aroma Components of the Volatile Oil of Yellow Keaw Mango Fruits Determined by Limited Odor Unit Method

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**Odor detection thresholds of optically active compounds and other volatile compounds found in the oil of yellow Keaw mangoes were determined. Odor intensity of individual components was evaluated by Lod (limited odor unit) based on data of the odor detection threshold and the concentration of individual components at the recognition threshold level of the volatile oils.  $\beta$ -Damascenone and terpinolene were found to have Lod values greater than one and were identified as the components most responsible for the characteristic aroma. Odor recognition threshold of a mixture of 15 chemicals having larger Lod values against natural Keaw mango oils was 1.8 ppm, which exceeded that of Keaw mango oils (0.62 ppm) against Ok-rong mango oils. The mixture of fifteen compounds comprising  $\beta$ -damascenone, terpinolene, ethyl hexanoate, (*E,Z*)-(2,6)-nonadienal, 2,5-dimethyl-4-methoxy-3(2*H*)-furanone, (3*R*)-(–)-linalool, ethyl butyrate, ethyl octanoate, ethanol, (1*S*)-(+)- $\delta$ -3-carene, (1*S,5S*)-(–)- $\alpha$ -pinene, *trans*-linalool oxide, (3*S*)-(+)-linalool, butyric acid, and *p*-methylacetophenone was judged to possess an aroma very similar to that of the natural Keaw mango. Thus, these fifteen compounds were the key contributors to the aroma of Keaw mango.**

Keywords: mango, *Mangifera indica* L. 'Keaw', odor detection threshold, limited odor unit, odor recognition threshold

Mango (*Mangifera indica* L.) is grown throughout the tropics, and is highly prized for its attractive flavor, delicious taste, and nutritional value (Olle *et al.*, 1998). The vitamin A content of mangoes is one of the highest among all fruits. Production of new mango varieties is increasing in the world (FAO, 1998).

Mango flavor is critical to consumer acceptability and thus to determining price of the product (Malundo *et al.*, 1997). Engel and Tressl (1983) identified 114 volatile components including lactones, a furanone, and C<sub>6</sub> aldehydes and alcohols and their esters as the volatiles in two Indian mangoes. The production and export of Thai mangoes have increased in recent years, and the volatile composition of 4 popular yellow Thai mangoes has been compared to those of other mangoes (Tamura *et al.*, 2000). Among popular Thai mango cultivars, Keaw mango is relatively cheap and suitable for processing in industrial scales. However, the compounds responsible for the characteristic mango aroma (sweet, floral, fruity and sour notes) are still not known. In recent years, GC/Olfactometry (GC/O) such as GC-sniffing (Drawert & Christoph, 1984; Yang *et al.*, 1987), AEDA (Grosch & Schieberle, 1997) and Charm analysis methods (Acree *et al.*, 1984) as well as the odor unit method (Guadagni *et al.*, 1966; Buttery *et al.*, 1990) have been adopted as tools for determining characteristic aroma components in foods. Ong and Acree (1998) reported that the odor active volatiles of lychee were four alcohols, two heterocyclic compounds, two carbonyl compounds, one ester, and one acid by using GC/O and odor spectrum value (OSV)

methods. Recently Tamura *et al.* (2001) applied the OSV method to the characterization of a green Thai mango aroma.

We introduced the "limited odor unit value (Lod; one kind of odor activity value)" method for selecting the most important aroma components and applied it to the screening of the characteristic aroma compounds in *Citrus* oils (Tamura *et al.*, 1996, 1993; Padrayuttawat *et al.*, 1997).

The objective of this work was to examine the efficacy of the Lod method for characterization of aroma profiles in mango at the level of the recognition thresholds, which is useful for evaluation of the odor-similarity of model oils to the natural oil. No one has yet reported the characteristic aroma compounds in mangoes. Therefore, those in Keaw mango are reported here for the first time using the Lod method. Characterization of the aroma quality of a common Thai mango, Keaw cultivar might accelerate and encourage the manufacturing of commercial mango products, including mango juice, candies, pudding, as well as ice cream in the near future.

### Experimental

**Materials** Two Thai mango varieties, "Keaw" and "Ok-rong" used in this experiment were obtained from a local market in Bangkok, Thailand. Both fruits were quickly frozen in a deep freezer at –30°C and ca. 20 kg of the frozen fruits was sent to Japan packed with dry-ice in 1998 and 1999. The volatile oils from Keaw and Ok-rong mangoes were separated by the method reported in a recent paper (Tamura *et al.*, 2000).

**Chemical reagents** Authentic compounds were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo), Wako Pure

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Chemical Industries, Ltd. (Osaka), Fluka Chemika-BioChemika (Tokyo) and Aldrich Chemical Co., Inc. (Milwaukee, WI). (*Z*)-3-Hexenal was supplied by Shiono Koryo Kaisha, Ltd. (Osaka). Purity and the GC-response factor of all chemicals were checked by FID-GC.

**Analytical instruments** The  $^1\text{H-NMR}$  spectrum in  $\text{CDCl}_3$  was recorded on a JEOL JNM  $\alpha$  400 NMR spectrometer using tetramethylsilane as an internal reference. Electron impact mass spectra (EI-MS) were measured on a JEOL JMX-SX102AQQ, JMA-DA 7000, using a split ratio of 1/40, a carrier gas (helium) flow rate of 0.4 ml/min and EI equal to 70 eV.

**Synthesis of 2,5-dimethyl-4-methoxy-3(2*H*)-furanone** 2,5-Dimethyl-4-methoxy-3(2*H*)-furanone was synthesized following the method described by Schieberle and Hofmann (1997). 2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone (2 mmol) and methyl iodide (6 mmol) were refluxed in acetone (8 ml) for 8 h in the presence of potassium carbonate (4 mmol). After diluting the reacted solution with deionized water (40 ml), the solutes were extracted four times with dichloromethane (total volume: 120 ml); the combined organic layer was then treated with aqueous sodium hydroxide (0.1 mol/l; 20 ml) and, after washing with brine, dried over anhydrous  $\text{Na}_2\text{SO}_4$ . The purification of the furanone was done by preparative thin layer chromatography (20×20 cm, thickness 0.5 mm, Merck, Darmstadt) with hexane : ethyl acetate equal to 1 : 9. The overall yield of this synthesis was 70% and the MS and NMR spectra were compared with those of the authentic sample. The EI-MS and  $^1\text{H-NMR}$  gave the following data: EI-MS, *m/z* (%), 142 (100), 43 (49), 71 (18), 69 (15), 55 (14), 99 (13), 70 (9), 127 (9); 400MHz- $^1\text{H-NMR}$   $\delta_{\text{H}}$  ( $\text{CDCl}_3$ ): 1.44 (3H, d, *J*=7.2Hz), 2.20 (3H, s), 3.81 (3H,  $\text{OCH}_3$ , s), 4.41 (1H, d, *J*=7.2 Hz).

**Enantiomeric ratios of optically active monoterpenes by gas chromatography and gas chromatography-mass spectrometry**

1) Preparative gas chromatography of optically active compounds eluted was performed with a Hewlett-Packard 5890 instrument equipped with a thermal conductivity detector (TCD). The sample was loaded on a semi-bore capillary column, carbo-

wax-20 M (30 m×0.53 mm i.d.; film thickness, 1.0  $\mu\text{m}$ , GL Sciences, Tokyo) to isolate individual volatile compounds. Compounds eluted were separately collected into a U-tube sunken in liquid  $\text{N}_2$  as reported by Padrayuttawat *et al.* (1997). To determine enantiomeric ratio of chiral compounds in the volatiles, enantioselective capillary gas chromatography was run with a CP-cyclodextrin- $\beta$ -2,3,6-M-19 column (30 m×0.25 mm i.d.; film thickness, 0.25  $\mu\text{m}$ , GL Sciences, Tokyo). Only  $\delta$ -3-carene only was measured by this method.

2) The enantiomeric ratio of other volatile terpenes was measured by switching the analytical column to an enantioselective capillary gas chromatograph by means of multidimensional gas chromatography-mass spectrometry (MDGC) as described in Table 1. A pre column was split between a main column and two detectors, FID and olfactometry sniffing. The odor of individual volatiles was confirmed by sniffing at the sniffing port with wet air. The heart-cut system was controlled by a control unit, MUSIC (Chrompack Co., Ltd., through GL Sciences, Tokyo). Target compounds were introduced into the main column and then the enantiomeric ratio of chiral terpenes was determined using a second chiral column,  $\beta$ -DEX 325 (30 m×0.25 mm i.d.; film thickness, 0.25  $\mu\text{m}$ , SUPELCO, Bellefonte, PA). Analytical conditions are described below.

**Pre column** HP-5890 equipped with a DB-wax semi-bore capillary column (30 m×0.53 mm i.d.; film thickness 1.0  $\mu\text{m}$ , J&W, Folsom, CA) was used for the initial analysis. Initial oven temperature was held at 50°C for 5 min and then elevated to 230°C, with a programmed rate of 3°C/min. The He carrier gas was maintained at a pressure of 10 psi under a 1 : 3 split condition.

**Main column** A SUPELCO  $\beta$ -DEX 325 (30 m×0.25 mm i.d., film 0.25  $\mu\text{m}$ ) was installed in an HP G-1800 GCD. The column oven temperature for analysis was held at 50°C for 5 min and then raised up to 230°C, at a programmed rate of 2°C/min. He carrier gas was maintained at a flow rate of 1 ml/min under the splitless mode. Compounds eluted were detected with a mass detector. Ionization voltage of the GC/MS was set at 70 eV. Detailed analytical conditions of individual chiral compounds are

**Table 1.** Enantiomeric ratios of volatile compounds in the essential oil of Keaw mango.

Compound	Chiral column <sup>a)</sup>	Analytical conditions	Cutting time <sup>b)</sup> (min)	Retention time <sup>c)</sup> (min)		Enantiomeric ratio %ee <sup>d)</sup>	
				authentic compound	mango oil		
1. (1 <i>R</i> ,5 <i>R</i> )-(+)- $\alpha$ -Pinene	$\beta$ -DEX 325	50°C (10 min)	5.2–5.7	21.2		0.0	
(1 <i>S</i> ,5 <i>S</i> )-(–)- $\alpha$ -Pinene		up to 230°C; rate 2°C/min		21.6	21.7	100.0	100.0
2. (–)-Camphene	$\beta$ -DEX 325	50°C (10 min)	6.7–7.7	23.3	23.2	86.0	71.9
(+)-Camphene		up to 230°C; rate 2°C/min		23.7	24.0	14.0	
3. (1 <i>S</i> ,5 <i>S</i> )-(–)- $\beta$ -Pinene	$\beta$ -DEX 325	50°C (10 min)	8.1–9.3	25.4	25.4	75.6	51.2
(1 <i>R</i> ,5 <i>R</i> )-(+)- $\beta$ -Pinene		up to 230°C; rate 2°C/min		25.8	25.9	24.4	
4. (4 <i>S</i> )-(–)-Limonene	$\beta$ -DEX 325	70°C	12.4–12.9	13.5	13.5	20.1	
(4 <i>R</i> )-(+)-Limonene		up to 230°C; rate 2°C/min		13.8	13.8	80.0	59.9
5. (1 <i>S</i> )-(+)-3-Carene	$\beta$ -CD	40°C (100 min)	trapped <sup>e)</sup>	116.8	116.6	100.0	100.0
(1 <i>R</i> )-(–)-3-Carene		up to 100°C; rate 1°C/min				0.0	
6. (3 <i>R</i> )-(–)-Linalool	$\beta$ -DEX 325	70°C	28.2–28.8	20.9	20.9	49.5	
(3 <i>S</i> )-(+)-Linalool		up to 230°C; rate 2°C/min		21.1	21.1	50.5	0.9
7. 2,5-Dimethyl-4-methoxy-3(2 <i>H</i> )-furanone	$\beta$ -DEX 325	50°C (10 min)	31.4–31.9	u	35.7	48.5	3.0
		up to 230°C; rate 2°C/min		u	36.0	51.5	

<sup>a)</sup> $\beta$ -DEX 325 is a 2,3-di-O-methyl- $\beta$ -O-TBDMS- $\beta$ -cyclodextrin, and  $\beta$ -CD is a CP-cyclodextrin- $\alpha$ -2,3,6-M-19 column.

<sup>b)</sup>Retention time on the precolumn described in experimental section.

<sup>c)</sup>Retention time on the main column described in experimental section.

<sup>d)</sup>%ee means enantiomeric excess.

<sup>e)</sup>This compound was isolated with TCD trapping method described in experimental section. u: authentic standard compounds were not available.

listed in Table 1.

**Sensory evaluation** At least fifteen panel members, 9 females and 6 males from Kagawa University participated in the sensory judgment for the determination of odor detection threshold and odor recognition threshold. Their ages ranged from 20 to 43 years (average 25 years). Descriptive sensory evaluation was performed by a panel of 15 experienced judges.

**Determination of odor-detection threshold** The odor threshold of the individual components was determined by a two-out-of-five test for difference as described in Tamura *et al.*, (1996). Thus, two cups of five contained aqueous solutions of volatile compounds while the other three cups contained pure water. Panel members judged which two cups contained the volatile compounds. Each aqueous solution of volatiles was diluted by a factor of two until the solution was judged as odorless. The concentration at which panel members could no longer detect the compounds was defined as the odor threshold. For each volatile compound, threshold values from the panels were converted to logarithmic values. The mean of these log values was then converted back to an ordinal value. The odor detection thresholds of eighty compounds were determined with this procedure. Odor threshold of individual optical active terpenes (*R*- or *S*- form) was also determined.

**Determination of odor recognition threshold** Recognition threshold is the concentration at which panelists recognize the two odor differences of the concentration and the odor quality in two kinds of oils presented. One series of the sensory test was composed of 8 samples, including 4 sample solutions of a target oil at 4 different concentrations, 3 sample solutions of a reference oil at 3 different concentrations and 1 of odorless water (Table 2). The panel members would distinguish which 4 cups contained the target oil, and which 3 cups contained the reference oil. They would then have to arrange the 4 cups of the target oil's solutions in the order from the highest concentration to the lowest. The minimum concentration at which the panel members correctly distinguished the characteristic aroma of the target oil was defined as the recognition threshold (Tamura *et al.*, 1996). The average of the recognition threshold values was calculated from the logarithmic values of the individual recognition threshold values determined by the 15 or more panel members.

**Determination of limited odor unit values (Lod values) of identified compounds in essential oil of Keaw mango** The Lod value of each volatile component in Keaw mango oils was calculated by the following equation (Tamura *et al.*, 1996),

$$\text{Lod} = \frac{Cr}{Td}$$

*Cr*: concentration of the components at the recognition threshold of the mango oils against the reference oil, *Td*: concentration of its odor detection threshold.

The concentration of compounds isolated was calculated from the area percentages and GC-response factors of individual compounds. Concentration of individual components in Keaw mango oils at the recognition threshold level was calculated against Ok-rong mango oils as reference.

## Results and Discussion

**Enantiomeric separation of chiral volatile compounds in Keaw mango oils** The enantiomeric ratio of individual com-

**Table 2.** Three series of concentrations of Keaw and Ok-rong oils for determining the recognition thresholds.

Sensory test	Keaw mango oils <sup>a)</sup>					Ok-rong mango oils <sup>a,b)</sup>		
1st trial	0.01	0.1	1	10	W	0.03	0.03	3
2nd trial	0.03	0.3	3	30	W	0.03	0.3	3
3rd trial	0.05	0.5	5	50	W	0.03	0.3	3

<sup>a)</sup>concentration in water (ppm)

<sup>b)</sup>reference

W: water

pounds is shown in Table 1. As each one of a pair of enantiomers has its individual detection threshold and their odor quality may be different from each other in some cases (Padrayuttawat *et al.*, 1997), we must know the accurate enantiomeric ratio and concentration of the optically active chemicals in natural oils.  $\delta$ -3-Carene trapped by a U-tube from the Keaw mango oils was injected into a CP-cyclodextrin- $\beta$ -2,3,6-M-19 column and the purity of the (+) form was found to be 100%. Bernreuther *et al.* (1989) determined the enantiomeric excess of linalool in several fruit extracts including mango extracts and reported the *S*-(+)-enantiomer to be dominant in mango. However, a racemic mixture of linalool was found in the essential oil of Keaw mango. The *R/S* forms of 2,5-dimethyl-4-methoxy-3(2*H*)-furanone was found in nearly equal amounts. The asymmetric center (carbon-2) of the furanone can be easily racemized through the enol form, and stabilized by the aromatization as suggested by Fisher and Hammerschmidt (1992). (–)-Camphene, (+)-limonene and (–)- $\beta$ -pinene were more abundant than the other optical isomers.  $\alpha$ -Pinene was present only as the pure (1*S*,5*S*) enantiomer whereas  $\delta$ -3-carene was present in the (1*S*)-(+)-configuration.

**Recognition threshold of the odors of Keaw mango oil against those of Ok-rong mango oil** As the volatile compositions of Keaw mango oil and Ok-rong mango oil are very similar (Tamura *et al.*, 2000), the latter was selected as the reference for determining the recognition threshold of Keaw mango oil. The entire set of this experiment consisted of 3 series of tests as shown in Table 2. Thus, the concentration of Keaw mango oil was prepared at 0.01 ppm to 50 ppm in 12 steps. Panel members had to distinguish 10 times the difference in concentration of Keaw mango oils in the series according to the Weber-Fechner law. Consequently, Keaw mango oil was distinguished at and over 0.62 ppm by the aroma of Ok-rong mango oil. This is the recognition threshold value of Keaw mango oil against Ok-rong mango oil, and refers to the similarity of the aroma at and under 0.62 ppm against the reference oil. This is the first time the degree of similarity of an aroma has been shown by a numerical value of the recognition threshold. The value is semi-quantitative and provides a basis for comparison of odor difference and odor similarity. An odor description such as “mango-like,” “sweet mango-like,” and “green mango-like” aromas gives only a qualitative meaning.

**Odor detection thresholds and concentration of identified compounds in essential oil of Keaw mango** The odor detection threshold of seventy-eight compounds found in Keaw oils was determined under the same conditions mentioned in experimental (Table 3. Some of the thresholds were cited from papers). Detection thresholds of  $\beta$ -damascenone, ethyl butyrate, (*E,Z*)-(2,6)-nonadienal and (*Z*)-3-hexenal were 1.3 ppt, 200 ppt, 140 ppt and 1.7 ppb, respectively. Ohloff (1978) reported the thresh-

**Table 3.** Volatile aroma compounds in yellow Keaw mango.

No.	R. Index (DB-wax)	Compound	Concentration (ppm)	Odor threshold (ppm)
1	703	Acetaldehyde	0.94	0.015 <sup>a)</sup>
2	887	Ethyl acetate	1.23	3.28
3	913	2-Butanone	0.0026	
4	942	Ethanol	171.90	4.51
5	963	Ethyl propionate	0.0047	
6	977	2,3-Butanedione	0.0039	0.0011
7	990	2-Pentanone	0.019	1.38
8	1025	2-Butanol	0.017	3.30
9	1036	(1 <i>S</i> ,5 <i>S</i> )-(-)- $\alpha$ -Pinene	1.50	0.10
10	1039	2-Methyl-3-buten-2-ol	0.010	1.14
11	1042	Ethyl butyrate	0.011	0.00018
12	1106	2-Hexanone	0.010	0.56
13	1117	(1 <i>S</i> ,5 <i>S</i> )-(-)- $\beta$ -Pinene	0.0020	4.16
14	1117	(1 <i>R</i> ,5 <i>R</i> )-(+)- $\beta$ -Pinene	0.00065	2.54
15	1125	Sabinene	0.017	0.98
16	1129	Propyl butyrate	0.076	0.16
17	1139	3-Penten-2-one	0.34	1.20
18	1144	2-Carene	0.037	
19	1147	1-Butanol	0.053	4.33
20	1150	( <i>Z</i> )-3-Hexenal	0.011	0.0017
21	1161	1-Penten-3-ol	0.047	0.4 <sup>b)</sup>
22	1162	(1 <i>S</i> )-(+)-3-Carene	1.26	0.044
23	1170	Myrcene	0.28	0.099
24	1178	$\alpha$ -Phellandrene	0.16	0.16
25	1192	$\alpha$ -Terpinene	0.53	0.085
26	1210	(4 <i>S</i> )-(-)-Limonene	0.11	1.04
27	1210	(4 <i>R</i> )-(+)-Limonene	0.45	1.20
28	1212	3-Methyl-1-butanol	0.019	3.06
29	1225	$\beta$ -Phellandrene	0.40	
30	1226	Butyl butyrate	0.0061	0.11
31	1231	( <i>E</i> )-2-Hexenal	0.24	0.082
32	1240	Ethyl hexanoate	0.28	0.0010
33	1254	$\gamma$ -Terpinene	0.20	0.26
34	1274	1-Pentanol	0.042	0.12
35	1281	<i>p</i> -Cymene	0.019	0.12
36	1293	Terpinolene	23.56	0.041
37	1299	3-hydroxy-2-butanone	0.010	0.014
38	1327	( <i>Z</i> )-2-Penten-1-ol	0.030	0.72
39	1329	3-Methyl-2-buten-1-ol	0.023	0.25
40	1361	1-Hexanol	0.032	1.62
41	1372	( <i>E</i> )-3-Hexen-1-ol	0.0059	0.11
42	1393	( <i>Z</i> )-3-Hexen-1-ol	0.43	0.91
43	1396	Methyl octanoate	0.0023	
44	1443	Ethyl octanoate	0.61	0.015
45	1447	$\alpha$ , <i>p</i> -Dimethylstyrene	2.34	
46	1456	<i>cis</i> -Linalool oxide (furanoid)	0.52	0.10
47	1457	Acetic acid	1.28	25.59
48	1469	( <i>Z</i> )-3-Hexenyl butyrate	0.47	0.50
49	1472	Furfural	0.50	0.77
50	1484	<i>trans</i> -Linalool oxide (furanoid)	1.19	0.19
51	1554	(3 <i>S</i> )-(+)-Linalool	0.080	0.0074
52	1554	(3 <i>R</i> )-(-)-Linalool	0.080	0.00080
53	1567	8,9-Limonene oxide	0.059	0.20
54	1582	5-Methylfurfural	0.0026	1.11
55	1594	( <i>E,Z</i> )-(2,6)-Nonadienal	0.019	0.00014
56	1605	2,5-Dimethyl-4-methoxy-3(2 <i>H</i> )-furanone	1.66	0.016
57	1610	$\beta$ -Caryophyllene	0.029	1.54
58	1624	Octyl butyrate	0.030	0.25
59	1632	Butyric acid	12.33	1.40
60	1644	Ethyl decanoate	0.14	0.023
61	1677	3-Methylbutyric acid	0.16	0.25 <sup>c)</sup>
62	1681	Humulene	0.14	0.39
63	1695	Neral	0.23	0.053
64	1705	$\alpha$ -Terpineol	0.020	5.00
65	1714	4-Hexanolide	0.039	0.26
66	1720	Germacrene D	0.019	
67	1733	$\beta$ -Selinene	0.98	
68	1737	$\alpha$ -Selinene	0.093	
69	1746	Pentanoic acid	0.0063	0.28
70	1812	Perillaldehyde	0.021	0.030
71	1814	<i>p</i> -Methylacetophenone	0.14	0.021
72	1833	$\beta$ -Damascenone	0.0079	0.0000013
73	1847	<i>trans</i> -Carveol	0.0080	0.25
74	1850	Ethyl dodecanoate	0.80	0.40
75	1851	Hexanoic acid	2.032	1.84
76	1872	1-Undecanol	0.035	
77	1882	<i>p</i> -Cymen-8-ol	1.75	

**Table 3.** (continued).

No.	R. Index (DB-wax)	Compound	Concentration (ppm)	Odor threshold (ppm)
78	1930	4-Octanolide	0.0092	0.014
79	1952	$\beta$ -Ionone	0.027	0.0059
80	1962	Heptanoic acid	0.052	0.5 <sup>d</sup>
81	1975	1-Dodecanol	0.070	0.016
82	2014	Methyl tetradecanoate	0.012	
83	2053	Ethyl tetradecanoate	0.36	0.18
84	2067	Octanoic acid	0.72	3 <sup>d</sup>
85	2078	1-Tridecanol	0.41	
86	2224	Methyl hexadecanoate	0.018	4000 <sup>d</sup>
87	2260	Ethyl hexadecanoate	0.35	24500 <sup>d</sup>
88	2282	Decanoic acid	0.34	0.13
89	2385	1-Hexadecanol	0.48	
90	2465	Ethyl octadecanoate	0.026	15000 <sup>d</sup>
91	2497	Dodecanoic acid	0.58	10 <sup>d</sup>
92	>2600	Tetradecanoic acid	1.25	10 <sup>d</sup>

<sup>a</sup>Flath *et al.* (1967), <sup>b</sup>Buttery *et al.* (1998), <sup>c</sup>Buttery *et al.* (1990).

<sup>d</sup>Compilation of Odor and Taste Threshold Values Data-Ds48A, edited by F.A. Fazzalari, 1978, Astm Data Series Publication, Philadelphia, pp 1–165.

**Table 4.** Reconstitution of volatile models for characteristic Keaw mango aroma based on Lod values.

No	Compound	Lod 5	Lod 10	Lod 15	Odor description	GC-Response Factor	Lod
1	$\beta$ -Damascenone	○	○	○	sweet, fruity	1.20	14.40
2	Terpinolene	○	○	○	floral sweet, slightly green mango, sour	1.00	1.39
3	Ethyl hexanoate	○	○	○	fruity	0.82	0.67
4	( <i>E,Z</i> )-(2,6)-Nonadienal	○	○	○	waxy, fatty, cucumber-like	0.68	0.32
5	2,5-Dimethyl-4-methoxy-3(2 <i>H</i> )-furanone	○	○	○	sweet, candy-like, caramel-like	0.59	0.25
6	(3 <i>R</i> )-(-)-Linalool		○	○	floral, woody	0.99	0.24
7	Ethyl butyrate		○	○	sweet, fragrant, estery	0.87	0.15
8	Ethyl octanoate		○	○	fruity	0.82	0.10
9	Ethanol		○	○	sweet alcohol	0.50	0.09
10	(1 <i>S</i> )-(+)-3-Carene		○	○	mango leaf-like, sweet, green	1.06	0.07
11	(1 <i>S</i> ,5 <i>S</i> )-(-)- $\alpha$ -Pinene			○	terpene-like	1.09	0.04
12	<i>trans</i> -Linalool oxide (furanoid)			○	sweet, fruity	0.89	0.03
13	(3 <i>S</i> )-(+)-Linalool			○	fresh flowery, sweet	0.99	0.03
14	Butyric acid			○	sweat, rancid	0.71	0.02
15	<i>p</i> -Methylacetophenone			○	intense green	1.12	0.02

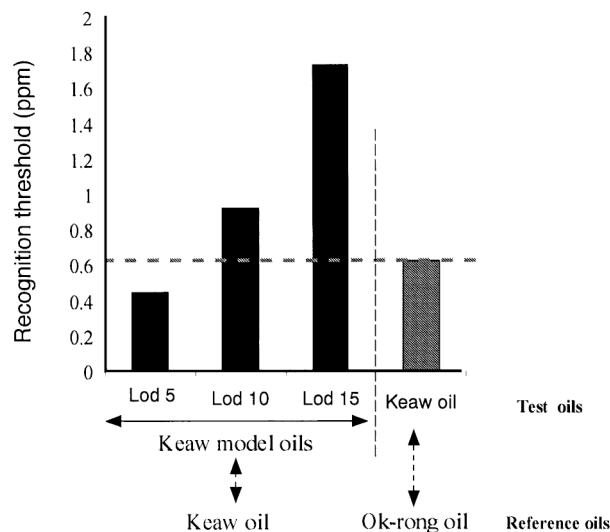
old value of  $\beta$ -damascenone was 10 ppt, while Buttery *et al.* (1990) determined the detection threshold of  $\beta$ -damascenone was 2 ppt. The threshold value of this compound in water was at 1.3 ppt, this value was almost consistent with their data. Buttery *et al.* (1981) and Forss *et al.* (1962) also reported the threshold value of (2,6)-nonadienal was 100 ppt. Detection threshold value of the C9 aldehyde was approximately equal to that of their papers. (*Z*)-3-Hexenal showed the odor detection threshold at 1.7 ppb. This value was completely coincident with the data reported by Reiners and Grosch (1998). However, the detection threshold value of ethyl butyrate (56 ppt) was lower than that in published papers. Flath *et al.* (1967) and Buttery *et al.* (1982) determined that the detection threshold of ethyl butyrate was 1 ppb. Preininger and Grosch (1994) reported the threshold was 28 ppb in refined sunflower oil. As the threshold values of the four compounds,  $\beta$ -damascenone, ethyl butyrate, (*E,Z*)-(2,6)-nonadienal and (*Z*)-3-hexenal, were the lowest in all of the volatile components in Keaw mango, contribution of these compounds for Keaw mango aroma, therefore, may be high despite trace amounts in the oils. Furthermore, ( $\pm$ )-2,5-dimethyl-4-methoxy-3(2*H*)-furanone has an interesting sweet caramel-like note. The odor detection threshold and the concentration in the mango volatiles were 15.8 ppb and 4.0 ppb, respectively. The caramel-like aroma may contribute to the sweet aroma in the mango oil (Wilson *et al.*, 1990). On the other hand, although terpinolene is a

hydrocarbon having a fairly larger detection threshold (0.26 ppm), it was one of the major aroma contributors for Keaw mango because of the higher concentration (Tamura *et al.*, 2000; MacLeod & Pieris, 1984). This may contribute to the sweet green aroma for the Thai mango. Sulfur containing compounds such as dimethyl sulfide, (methylthio)phenylacetaldehyde and benzothiazol identified by Engel and Tressl (1983), and MacLeod and Pieris (1984) were not detected by high resolution GC/MS.

*Limited odor unit values (Lod values) of identified compounds in essential oil of Keaw mango* Concentration of individual components in Keaw mango oils was calculated at 0.62 ppm of the total oils because the recognition threshold level against Ok-rong mango oils was 0.62 ppm as mentioned above. Lod values of 78 compounds were calculated and the compounds having larger Lod values are listed in Table 4. Lod values of  $\beta$ -damascenone, and terpinolene were greater than one. Therefore,  $\beta$ -damascenone, (Lod=14.4) and terpinolene (Lod=1.4) should contribute to the base note of Keaw mango aroma. Actually,  $\beta$ -damascenone had a sweet and fruity aroma and terpinolene had the typical aroma of the Keaw mango. All panelists (15 persons) pointed out that terpinolene has a typical green Thai mango-like aroma, whereas  $\delta$ -3-carene has a weak green-like mango aroma (Sakho *et al.*, 1985; Engel & Tressl, 1983). Others such as ethyl hexanoate, (*E,Z*)-(2,6)-nonadienal,

**Table 5.** Mean<sup>1</sup> panel scores<sup>2</sup> for attributes of mango model oils.

Sample	Sensory scores <sup>3</sup>					
	Woody	Floral	Fruity	Sour	Green	Sweet
Lod 5	5.27 a	3.12	4.88	4.38	5.40 a	4.40 a
Lod 10	3.73 b	3.94	4.94	3.75	4.13 ab	5.00 a
Lod 15	3.47 b	4.31	5.50	4.25	3.27 b	5.60 ab
mango oil	2.87 b	4.13	6.13	2.69	2.93 b	6.33 b
F-test	*	ns	ns	ns	*	**

<sup>1</sup>n=15<sup>2</sup>Range of scoring: 1 to 9 with 1 denoting weak and 9 denoting intense for each attribute.\*, \*\*Significant difference at 5% ( $p < 0.05$ ) or 1% ( $p < 0.01$ ) level of F.<sup>3</sup>Means followed by the same letter within an attribute do not differ significantly ( $p < 0.05$ ) from each other by DMRT.**Fig. 1.** Recognition thresholds of the three model oils against Keaw oil and that of Keaw oil against Ok-rong oil.

2,5-dimethyl-4-methoxy-3(2H)-furanone, (3R)-(-)-linalool, ethyl butyrate, ethyl octanoate, ethanol, (1S)-(+)- $\delta$ -3-carene, (1S,5S)-(-)- $\alpha$ -pinene, *trans*-linalool oxide, (3S)-(+)-linalool, butyric acid, *p*-methylacetophenone, having Lod values lower than 1, would serve to increase a more yellow Keaw mango-like aroma.

*Characterization of the odors of Keaw mango oil on the basis of recognition thresholds between the oils of model mixtures and natural Keaw mango* In many volatile components sniffed, there was no single compound having the typical Thai mango odor. Consequently, the characteristic aroma of the fresh mango might be expressed by a mixture of the volatiles in certain proportions. For the selection of mango aroma components, model mixtures of Keaw mango oil were prepared by the compounds at the top five, top ten and top fifteen Lods (Lod5, Lod10, and Lod15), as shown in Table 4. Recognition threshold values of three model mixtures of Keaw mangoes were determined by comparing the aromas with that of natural Keaw mango. The recognition threshold values of Lod 5, Lod 10, and Lod 15 against natural Keaw mango oils were determined along with the same procedure as the recognition threshold value of Keaw mango oil against Ok-rong mango oil mentioned above. Consequently, these were at 0.4 ppm for Lod 5, 0.92 ppm for Lod 10 and 1.8 ppm for Lod 15, respectively. These values are the similarity

indices of the aromas of the three mixtures against that of natural Keaw mango oil. Top 5 compounds emitted a slightly sweet and strong green aroma as the base note (Table 5). When the number of compounds for the model mixtures was increased, the aroma became sweeter and closer to the yellow Keaw mango oil (Table 5). The model aqueous solution of Lod 15 showed a similar aroma to that of the natural oil. Five compounds from 11th to 15th number of the larger Lod values:  $\delta$ -3-carene (sweet, green mango leaf-like), (1S,5S)-(-)- $\alpha$ -pinene, *trans*-linalool oxide, (3S)-(+)-linalool and butyric acid increased the positive sensation and provided an additive effect (Guadagni *et al.*, 1963) on the mango aroma character.

As the recognition threshold of the Keaw mango oil against Ok-rong mango oil was at 0.62 ppm (Fig. 1), this concentration (0.62 ppm) will be a criterion of the similarity of the three mango model oils to the natural one.

Recognition threshold value of Lod 15 oil (1.8 ppm) against Keaw oil was higher than that of Keaw oil (0.62 ppm) against Ok-rong oil. In the two cases, the reference oils were different from each other. However, the recognition threshold value of the model mixture composed of 15 compounds means that the aroma of the mixture has great similarity to natural Keaw mango. Thus, this reconstituted model composed of  $\beta$ -damascenone, terpinolene, ethyl hexanoate, (*E,Z*)-(2,6)-nonadienal, 2,5-dimethyl-4-methoxy-3(2H)-furanone, (3R)-(-)-linalool, ethyl butyrate, ethyl octanoate, ethanol, (1S)-(+)- $\delta$ -3-carene, (1S,5S)-(-)- $\alpha$ -pinene, *trans*-linalool oxide, (3S)-(+)-linalool, butyric acid, *p*-methylacetophenone should have a representative character of Keaw mango-aroma. The sweet mango aroma may be formed by  $\beta$ -damascenone, ethyl hexanoate, ethyl butyrate, 2,5-dimethyl-4-methoxy-3(2H)-furanone, and ethyl octanoate.

We propose that these are essentially the key components for yellow Keaw mango aroma. The compositional data will be helpful for industrial applications such as mango juice, dehydrated mangoes and so forth.

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