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Evaluation of Aroma-Active Compounds in Pontianak Orange Peel Oil (*Citrus nobilis* Lour. Var. *microcarpa* Hassk.) by Gas Chromatography−Olfactometry, Aroma Reconstitution, and Omission Test

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The aroma-active compounds of Pontianak orange peel oil (*Citrus nobilis* Lour. var. *microcarpa* Hassk.) were characterized by using gas chromatography−olfactometry (GC−O) and aroma extract dilution analysis (AEDA) techniques. Forty-one compounds were found to be aroma-active, which were mainly dominated by saturated and unsaturated aldehydes. The flavor dilution (FD) factor was within the range of 2−2048, and compounds having the highest FD factor were α-pinene, β-pinene, linalool, and 2-methoxy-3-(2-methylpropyl) pyrazine, including a few unknown compounds. On the basis of GC−O results, odor activity value (OAV) and relative flavor activity (RFA) were determined for aroma model reconstitution. These resembled the original aroma of the peel oil for the green, fatty, fresh, peely, floral, and tarry attributes, with the model solution derived from OAV being the closest to Pontianak oil. Omission tests were carried out to verify the significance of (*Z*)-5-dodecenal and 1-phenylethyl mercaptan as key compounds in the aroma of Pontianak orange peel oil.

KEYWORDS: Pontianak orange; odor activity value; gas chromatography−olfactometry; aroma reconstitution; omission test

INTRODUCTION

Citrus fruits are widely produced and processed for their fruit juice and the essential oils extracted from the peel (*1*−*3*). The latter is utilized as a flavoring in the food industry and in perfume or aromatherapy applications (*4*). The history of citrus can be traced back to more than 4000 years ago, and it is believed that the fruit is native to the Southeast Asia from where it spread worldwide (*5*). There are at least 160 cultivars of citrus cultivated throughout Indonesia (*6*), and Pontianak orange (*Citrus nobilis* Lour. var. *microcarpa* Hassk.) is a preferred variety due to its high yield and pleasant organoleptic properties (*7*). The fruit has thin, fairly shiny, yellowish green-colored skin, and the juice possesses a distinct sweet taste with a slight sulfurous note. Characterization studies of the volatile compounds in Pontianak orange juice and peel oil have been undertaken (*8*−*10*), but results did not provide information on the aroma-active compounds. A recent publication by Fischer et al. (*11*) initiated the quest for identification of aroma-active compounds in Pontianak orange. However, the results are yet to be verified by aroma reconstitution, and it is the intention of the current study to investigate this subject further.

Gas chromatography−olfactometry (GC−O) is commonly used for the identification of aroma-active compounds (*12*). With the use of the human nose as the detector, a GC−O technique known as aroma extract dilution analysis (AEDA) can be employed for screening of aroma-active compounds (*13*). The result of AEDA is expressed as the flavor dilution (FD) factor, which is the highest dilution that an aroma-active compound is detectable. The significant contribution of each odorant to the characteristic flavor can be determined by two possible ways, namely, the odor activity value (OAV) and the relative flavor activity (RFA). OAV is the ratio of concentration to the odor threshold of the compound, and it is proportional to the FD factor (*14*). It is well-accepted that compounds with higher OAV contribute more to the aroma of the food (*15*). Even though the use of this value has been criticized (*16*), OAV has been used widely in determining potent odorants in foods (*17*−*21*). Alternative to OAV, RFA is obtained by the ratio of log FD factor to the square root of weight percentage of the compound. Through the years, RFA has also been used to determine the

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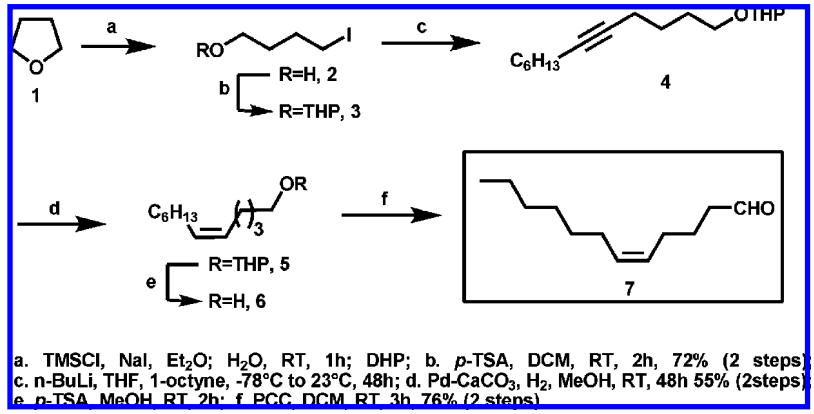
Scheme 1. Steps in the Synthesis of (*Z*)-5-Dodecenal



significant contribution of aroma-active compounds in various citrus cultivars (*22*−*25*).

In order to verify the significance of aroma-active compounds, aroma reconstitution and omission experiments are normally carried out (*13*). For aroma reconstitution, flavor compounds are mixed according to the analytical data obtained and their aroma attributes are compared with the original aroma. The importance of potent odorants is subsequently confirmed by omitting those compounds from the aroma models (*23*). In the present study, the aroma-active compounds in Pontianak orange peel oil that contributed to its aroma were investigated stepwise from GC−O sniffing to the calculation of their OAV and RFA values. Results were validated by carrying out aroma reconstitution and omission experiments.

MATERIALS AND METHODS

Materials and Chemicals. Fresh Pontianak oranges (*Citrus nobilis* Lour. var. *microcarpa* Hassk.) grown in a fruit farm in Pemangkat, West Kalimantan, Indonesia were harvested in August 2006. The hand-pressed peel oil was obtained by careful hand-squeezing of the peels of the fruits. Most of the standard chemicals were obtained from Firmenich Asia Pte. Ltd., Singapore, with the notable exception of *β*-pinene (ChromaDex, Irvine, CA), *R*-(+)-limonene, citral (mixture of neral and geranial), nonanal, decanal (Fluka, Buchs, Switzerland), carveol (Aldrich, St. Louis, MO), (*E*,*Z*)-2,6-dodecadienal (Bedoukian, Danbury, CT), and 1-phenylethyl mercaptan (Endeavor, Northamptonshire, U.K.).

Synthesis of (*Z*)-5-Dodecenal. In order to obtain pure (*Z*)-5-dodecenal (*7*), we adopted a six-step synthesis as illustrated in **Scheme 1**. Our synthesis began with Lewis acid facilitated ring-opening of tetrahydrofuran (*1*) to give the iodoalcohol **2** by in situ generation of trimethylsilyl iodide (*26*). This alcohol (*2*) was directly protected as its corresponding tetrahydropyranyl (THP) ether (*3*) in 72% yield over two steps. Acetylide addition (*27*) on **3** followed by a cis-selective hydrogenation with Lindlar's catalyst generated the (*Z*)-alkene **5** in 55% yield over two steps (*28*). THP deprotection by methanolysis of **5** followed by oxidation with pyridinium chlorochromate (PCC) afforded the targeted *cis*-alkenal **7** in 76% yield, over two steps. This synthesis was found both convenient and practical and provided (*Z*)-5-dodecenal (*7*) in sufficient quantities and high purity. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87 (3H, t, J = 7, CH_3), 1.27−1.42 (8H, m), 1.68 (2H, qen, CH_2), 1.9−2.1 (4H, m), 2.3−2.4 (2H, dt, J = 1.67, J = 5.6, CH_2CHO), 5.25−5.4 (2H, m), 9.76 (1H, t, J = 1.8, CHO). ^{13}C NMR (300 MHz, $CDCl_3$) δ 14 (CH_3), 22 (CH_2), 22.6 (CH_2), 26.4 (CH_2), 27.2 (CH_2), 28.9 (CH_2), 29.6 (CH_2), 31.7 (CH_2), 43.2 (CH_2), 128.1 (CH), 131.4 (CH), 202.5 (CHO). Mass spectrum (**Figure 1**), *m*/*z* (%) 41 (100), 43 (47), 55 (75), 54 (74), 67 (55), 68 (43), 81 (29), 82 (20), 96 (19), 98 (24).

Gas Chromatography−Olfactometry. The GC−O instrument comprises a Shimadzu GC−MS QP5000 with the olfactometer ODO II (SGE, Ringwood, Australia) attached to it. The column used was DB-5MS (5% phenyl/95% methyl polysiloxane−60 m × 0.32 mm, 1 μm film thickness; J&W Scientific, Folsom, CA) while both injector and MS interface temperatures were set at 270 °C. The electron ionization (EI) method was used for the MS at the ionization energy of 70 eV with the scan range of 40−300 *m*/*z*. The compounds were identified by comparison of mass spectra of the target compounds with those of the NIST (National Institute of Standards and Technology) library and verified by the retention indices of pure standard compounds. A volume of 2 μL of Pontianak orange peel oil was injected, and the temperature program was set from 120 to 240 °C at the rate of 2 °C/min and increased to 270 at 10 °C/min with a 2 min final temperature hold. The flow rate of helium carrier gas was 2.3 mL/min, and humid air was constantly added to the effluent at the sniffing port. Four flavorists (two females and two males) from Firmenich Singapore were the panelists for sniffing the oil. Sniffing of the compounds eluted from the sniffing port was divided into four sessions of 15 min with a break of 15 min in between. The panelists were asked to describe the odor perceived, and the detection of an odorous compound at the sniffing port by fewer than three assessors was considered to be noise.

Aroma Extract Dilution Analysis, Relative Flavor Activity, and Odor Activity Value. For AEDA, the peel oil was diluted stepwise 2-fold with diethyl ether by volume to obtain dilutions of 1:2, 1:4, 1:8, 1:16 and so on, with dilutions being injected into the GC−O. The highest dilution in which an aroma-active compound was detectable is then referred as the FD factor of that compound. On the basis of AEDA results, RFA of each aroma-active compound was obtained by using the equation RFA = log $2^n/S^{0.5}$ (*22*), where 2^n is the FD factor and *S*

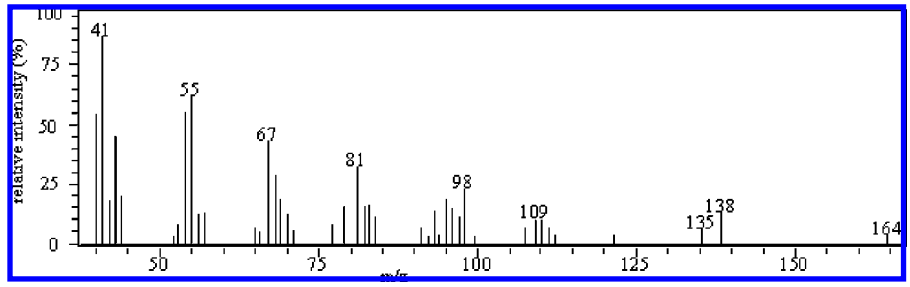


Figure 1. Mass spectrum obtained for (*Z*)-5-dodecenal.

is the weight percent of a compound. In order to obtain the OAV, the absolute concentration of the compound was derived from its GC−MS calibration curve, while its odor threshold in water was obtained from the literature (*29*−*33*). The threshold of each compound was determined by using a two-out-of-five sensory evaluation test, whereby each aqueous solution was diluted by a factor of 2 until the solution was judged to be odorless. The concentration at which the odor of compounds could not be detected by panelists was defined as its odor threshold (*29*).

Aroma Reconstitution and Omission Test. The synthetic blends of odorants (aroma models) were prepared based on the analytical data of the concentration of aroma-active compounds in the original peel-oil extract, which was obtained by plotting calibration curve of each reference compound. Three different sets of aroma models were prepared. The first model contained the 33 compounds identified by GC−MS. The second and third models were prepared based on the upper range of OAV and RFA obtained from the original set of compounds, as shown in **Tables 3** and **4**, respectively. Sensory evaluation was carried out by seven trained assessors (two males and five females). They were asked to rate given odor qualities (green, fatty, fresh, peely, floral, and tarry) of the original Pontianak orange peel oil and the three aroma models using a seven-point intensity scale ranging from 0.0 to 3.0 at intervals of 0.5. Omission tests were carried out to verify the findings by omitting from the aroma models (*Z*)-5-dodecenal and 1-phenylethyl mercaptan, which were deemed to be important contributors of Pontianak orange oil aroma. Assessors were asked to rate the degree of similarity between the original Pontianak orange oil and the aroma models with omitted compounds. The score assigned was from 1 being extremely different to 9 being extremely similar, and the statistical analysis of the sensory evaluation was performed using *t* test.

RESULTS AND DISCUSSION

Aroma-Active Compounds of Pontianak Orange Peel Oil. Forty-one aroma-active volatiles were detected from the GC−O analysis of Pontianak orange peel oil, as shown in **Table 1**. The AEDA method was performed to categorize the compounds according to their odor potency. The FD factors of the compounds were detected to fall within the range of 2−2048 (**Table 1**). The compounds having the highest FD factor (2048) were α-pinene, *β*-pinene, linalool, 2-methoxy-3-(2-methylpropyl) pyrazine, and few unknown compounds that exhibited woody, metallic, green, earthy, sulfury, and citrus-like odor quality. Limonene, which is the most abundant compound of Pontianak orange peel oil, exhibited an FD factor of 1024. The aroma-active compounds found in Pontianak orange peel oil were dominated by saturated and unsaturated aldehydes (FD factor from 16 to 512), such as nonanal, decanal, undecanal, dodecanal, (*E*)-2-nonenal, (*E*)-2-decenal, (*E*)-2-dodecenal, (*Z*)-5-dodecenal, (*E,E*)-2,4-decadienal, and (*E,Z*)-2,6-dodecadienal. From these compounds, (*Z*)-5-dodecenal has rarely been reported in citrus fruits (*34*), and no significance in contributing to the aroma of the oil was assigned. The compound has been found to be one of the major volatiles in insect pheromones (*35*). In addition, 1-phenylethyl mercaptan (*11, 36*), which was also detected in the present study with an FD factor of 256, was reported to be part of the composition of the Asian Pontianak orange peel oil. Its odor was described as sulfurous and resinous resembling that of the whole fruit (*11*). Both (*Z*)-5-dodecanal and 1-phenylethyl mercaptan were documented presently to have unique characteristics contributing to the flavor of Pontianak orange oil.

Odor Activity Value and Relative Flavor Activity. In order to determine the relative contribution of each compound to the aroma of Pontianak orange peel oil, OAV and RFA have been employed (**Table 2**). OAV was obtained by taking into account the concentration and odor threshold of each compound, whereas RFA utilized the FD factor and weight percentage of the compound. **Table 3** displays 18 compounds that have the highest OAV in descending order. Due to the unavailability of odor threshold data in the literature or the below-detection peak intensity, the OAV of 1-phenylethyl mercaptan, 2-methoxy-3-(2-methylpropyl) pyrazine, (*Z*)-5-dodecenal, and (*E,Z*)-2,6-dodecadienal was not determined. Results in **Table 3** indicate that limonene has the highest OAV followed by (*E*)-2-nonenal, linalool, (*E*)-2-dodecenal, (*E,Z*)-2,6-nonadien-1-ol and myrcene. Compounds like camphor, 4-terpineol, *trans*-carveol, and neryl acetate were among those with the lowest OAV (below 100). In general, compounds that had high FD factor also had high OAV, which confirms the positive relationship between FD factor and OAV (*17*).

Since OAV often depends on concentration and may not always reveal the characteristic odorants, the concept of RFA

Table 1. Aroma-Active Compounds (FD ≥ 2) in Pontianak Orange Peel Oil

no.	RI[a]	compd[b]	odor quality[c]	FD[d]
1	963	α-pinene	woody, piney, citrusy	2048
2	1003	*β*-myrcene	sulfury, mango-like, metallic	128
3	1014	*β*-pinene	metallic, citrusy, woody	2048
4	1059	limonene	orange-like, fruity	1024
5	1114	linalool	floral, green	2048
6	1120	nonanal	soapy, aldehydic	128
7	1160	1-phenylethyl mercaptan[e]	tarry, sulfury	256
8	1166	citronellal	lemongrass-like	128
9	1170	(*E,Z*)-2,6-nonadien-1-ol	cucumber-like	64
10	1174	(*E*)-2-nonenal	melon-like, fatty	256
11	1181	1-nonanol	fresh, green	4
12	1187	camphor	fruity, green, grassy	128
13	1193	2-methoxy-3-(2-methylpropyl) pyrazine[e]	chili-like, peppery	2048
14	1203	unknown	earthy, tarry, sulfury	2048
15	1208	unknown	citrusy, soapy	512
16	1211	4-terpineol	floral, fresh	8
17	1218	decanal	soapy, aldehydic	512
18	1228	unknown	sweet, floral	128
19	1229	unknown	earthy	2048
20	1236	citronellol	citrusy, fruity	64
21	1240	nerol	citrusy, roselike	8
22	1246	*trans*-carveol	sulfury, fruity	2
23	1255	neral	floral, green	2
24	1260	geraniol	fruity, citrusy	128
25	1271	L-carvone	minty	256
26	1276	(*E*)-2-decenal	fatty, aldehydic	16
27	1279	1-decanol	fresh, floral	2
28	1284	geranial	lemon-like	8
29	1309	perilla aldehyde	almond-like, floral	64
30	1319	undecanal	soapy, aldehydic	128
31	1335	(*E,E*)-2,4-decadienal	fatty, oily	256
32	1344	unknown	earthy	64
33	1353	unknown	floral, green	2048
34	1365	neryl acetate	green, citrusy	4
35	1379	unknown	floral	256
36	1393	geranyl acetate	citrusy	2
37	1405	(*Z*)-5-dodecenal	soapy, citrusy	128
38	1421	dodecanal	soapy, aldehydic	128
39	1465	(*E,Z*)-2,6-dodecadienal	fruity, soapy	256
40	1482	(*E*)-2-dodecenal	fatty, citrusy	512
41	1490	unknown	oily, creamy	128

[a] Experimental linear retention index on a DB5-MS column. [b] The compound was identified by comparing its retention time and mass spectrum with the reference standard. [c] Odor quality perceived through the sniffing port. [d] FD factor of the odor-active compound. [e] The MS signal was too weak for interpretation. The compound was identified based on the similarity odor quality perceived at the sniffing port and the retention time of its odor detection with the standard compound.

Table 2. Odor Activity Values (OAV) and Relative Flavor Activity (RFA) of Aroma-Active Compounds in Pontianak Orange Peel Oil

no.	RI	compd	concn (ppm)[a]	odor threshold in water (ppm)[b]	OAV[c]	% weight[d]	RFA[e]	ref source[f]
1	963	α-pinene	3526	0.19 (*30*)	18 560	0.39	5.3	Firmenich
2	1003	β-myrcene	19 640	0.67 (*31*)	29 310	2.20	1.4	Firmenich
3	1014	β-pinene	3440	1.5 (*30*)	2290	0.38	5.4	ChromaDex
4	1059	limonene	874 500	0.2 (*30*)	4 372 500	95.70	0.3	Fluka
5	1114	linalool	4067	0.028	145 240	0.45	5.0	Firmenich
6	1120	nonanal	1587	0.1	15 870	0.17	5.1	Fluka
7	1160	1-phenylethyl mercaptan	nd[g]	N/A[h]				Endeavor
8	1166	citronellal	818	0.046	17 770	0.09	7.0	Firmenich
9	1170	(*E*,*Z*)-2,6-nonadien-1-ol	38	0.001 (*32*)	38 010	0.004	28.0	Firmenich
10	1174	(*E*)-2-nonenal	73	0.0004 (*30*)	182 570	0.01	26.9	Firmenich
11	1181	1-nonanol	109	1.0	109	0.01	5.5	Firmenich
12	1187	camphor	5.7	4.6	1	0.001	84.2	Firmenich
13	1193	2-methoxy-3-(2-methylpropyl) pyrazine	nd[g]	0.000 045 (*33*)				Firmenich
14	1211	4-terpineol	39	6.4	6	0.004	13.8	Firmenich
15	1218	decanal	1673	0.07	23 890	0.18	6.3	Fluka
16	1236	citronellol	459	0.062	7400	0.05	8.1	Firmenich
17	1240	nerol	815	0.68	1200	0.09	3.0	Firmenich
18	1246	*trans*-carveol	104	4	26	0.01	2.8	Aldrich
19	1255	neral	133	0.1	1330	0.02	2.5	Fluka
20	1260	geraniol	55.7	0.01	5570	0.01	27.0	Firmenich
21	1271	L-carvone	108	0.067	1620	0.01	22.1	Firmenich
22	1276	(*E*)-2-decenal	134	0.017	7860	0.02	10.0	Firmenich
23	1279	1-decanol	78.7	0.775	102	0.01	3.2	Firmenich
24	1284	geranial	171	0.1	1710	0.02	6.6	Fluka
25	1309	perilla aldehyde	214	0.062	3460	0.02	11.8	Firmenich
26	1319	undecanal	636	0.04	15 900	0.07	8.0	Firmenich
27	1335	(*E*,*E*)-2,4-decadienal	83	0.01	8300	0.01	25.3	Firmenich
28	1365	neryl acetate	101	2	51	0.01	5.7	Firmenich
29	1393	geranyl acetate	93.5	0.15	623	0.01	3.0	Firmenich
30	1405	(*Z*)-5-dodecenal	110	N/A[h]		0.01	19.2	Firmenich
31	1421	dodecanal	647	0.055	11 750	0.07	7.9	Firmenich
32	1465	(*E*,*Z*)-2,6-dodecadienal	195	N/A[h]		0.02	16.5	Bedoukian
33	1482	(*E*)-2-dodecenal	200	0.0014	143 000	0.02	18.3	Firmenich

[a] The concentration of all aroma-active compounds was obtained by plotting the calibration curve of the reference substances, and these were used to calculate their OAVs. [b] Odor thresholds reported in ref *29* or stated by the number in parentheses as given in "Literature Cited". [c] The OAV was obtained by dividing the concentrations of the odorants by their reported thresholds in water. [d] Weight percentage of each compound based on its concentration relative to the total concentration of all compounds. [e] RFA = log $2^n/S^{0.5}$, where 2^n is the FD factor and S is the wt % of a compound. [f] Source of reference volatiles. [g] nd, not determined. [h] Data not available.

Table 3. Potent Odorants in Pontianak Orange Peel Oil Based on Their Odor Activity Values (OAV > 2000)

no.	compd	OAV[a]
1	limonene	4 372 500
2	(*E*)-2-nonenal	182 570
3	linalool	145 240
4	(*E*)-2-dodecenal	143 000
5	(*E*,*Z*)-2,6-nonadien-1-ol	38 010
6	β-myrcene	29 310
7	decanal	23 890
8	α-pinene	18 560
9	citronellal	17 770
10	undecanal	15 900
11	nonanal	15 870
12	dodecanal	11 750
13	(*E*,*E*)-2,4-decadienal	8300
14	(*E*)-2-decenal	7860
15	citronellol	7400
16	geraniol	5570
17	perilla aldehyde	3460
18	β-pinene	2290

[a] The OAV was obtained by dividing the concentrations of the odorants by their reported thresholds in water.

Table 4. Potent Odorants in Pontianak Orange Peel Oil Based on Their Relative Flavor Activity (RFA > 6.5)

no.	compd	RFA[a]
1	camphor	84.2
2	(*E*,*Z*)-2,6-nonadien-1-ol	28.0
3	geraniol	27.0
4	(*E*)-2-nonenal	26.9
5	(*E*,*E*)-2,4-decadienal	25.3
6	L-carvone	22.1
7	(*Z*)-5-dodecenal	19.2
8	(*E*)-2-dodecenal	18.3
9	(*E*,*Z*)-2,6-dodecadienal	16.5
10	4-terpineol	13.8
11	perilla aldehyde	11.8
12	(*E*)-2-decenal	10.0
13	citronellol	8.1
14	undecanal	8.0
15	dodecanal	7.9
16	citronellal	7.0
17	geranial	6.6

[a] RFA = log $2^n/S^{0.5}$, where 2^n is the FD factor and S is the weight percent of a compound.

could be used as an alternative to identify potent aroma-active compounds (*24*). RFA is calculated by using FD factors instead of the odor threshold values, and the concept of RFA was created to compensate for the inability of OAV to be invariably correct in judging the important aroma-active compounds. To demonstrate this, a compound like limonene may have high OAV but a relatively low RFA value and it is not the most important contributor to aroma of citrus fruits.

Table 4 reproduces 17 compounds with the highest RFA. This was not calculated for 1-phenylethyl mercaptan and 2-methoxy-3-(2-methylpropyl) pyrazine because their concentrations could not be determined. Results indicated that camphor is the highest RFA compound followed by (*E*,*Z*)-2,6-nonadien-1-ol, geraniol, (*E*)-2-nonenal, (*E*,*E*)-2,4-decadienal, and L-

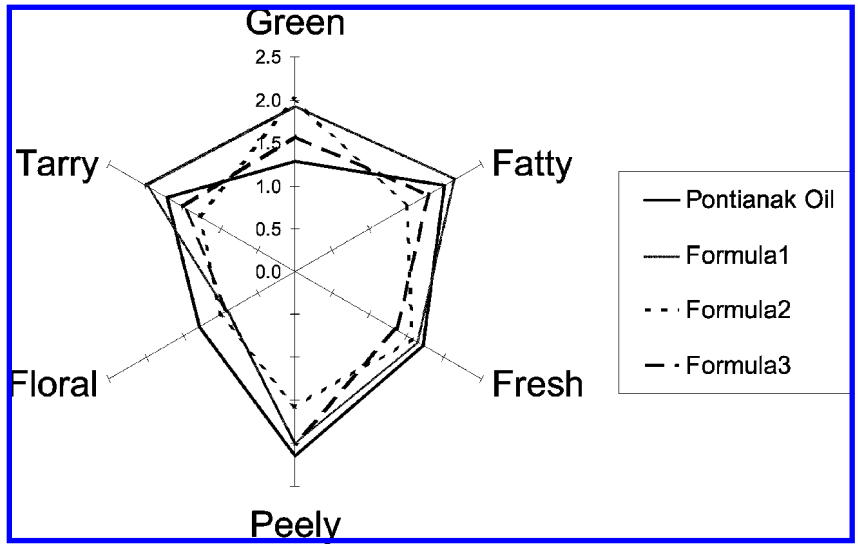


Figure 2. Comparative flavor profile analysis of Pontianak orange peel oil and the reconstituted aroma model solutions based on all available compounds (formula 1), relative flavor activity (RFA, formula 2), and odor activity value (OAV, formula 3).

carvone. Compounds having the lowest RFA were limonene, myrcene, and neral. In general, compounds that have high OAV are low in their RFA and vice versa, with limonene obeying this trend and camphor exhibiting low OAV/high RFA. Notable exceptions were (*E*)-2-nonenal and (*E*,*Z*)-2,6-nonadien-1-ol with high OAV and RFA owing to very low concentrations in peel oil and similarly low odor thresholds that relate to relatively high FD factors.

Aroma Reconstitution. In order to verify the contribution of aroma-active compounds to the flavor profile of Pontianak orange peel oil, synthetic blends were made based on the aforementioned findings. Three formulas were prepared according to the results shown in **Tables 2**−**4**. Furthermore, 1-phenylethyl mercaptan, 2-methoxy-3-(2-methylpropyl) pyrazine, (*Z*)-5-dodecenal, and (*E*,*Z*)-2,6-dodecadienal were included in each aroma model, since these are relatively rare compounds in citrus but found in Pontianak oil and hence may contribute considerably to its overall flavor. As the concentrations of 1-phenylethyl mercaptan and 2-methoxy-3-(2-methylpropyl) pyrazine could not be determined due to a weak MS signal, trials were carried out to determine optimum reconstitution levels. Results indicated that additions of 0.001% w/w from each compound produced a reconstituted aroma blend that matched best that of the natural material. Thus, we prepared three model solutions, as follows: model solution 1 included all compounds in **Table 2** (33 compounds), model solution 2 included all compounds in **Table 3** (18 compounds plus 1-phenylethyl mercaptan, 2-methoxy-3-(2-methylpropyl) pyrazine, (*Z*)-5-dodecenal, and (*E*,*Z*)-2,6-dodecadienal that the OAV could not be obtained), and model solution 3 included all compounds in **Table 4** (17 compounds plus 1-phenylethyl mercaptan and 2-methoxy-3-(2-methylpropyl) pyrazine that the RFA could not be obtained and limonene).

Six sensory properties, namely, green, fatty, fresh, peely, floral, and tarry, were selected to be the major characteristics of Pontianak orange oil following sensory trials and consensus among the flavorists. The aroma of the Pontianak peel oil was then compared with the sensory characteristics of the aroma models. **Figure 2** showed that the intensities of floral, peely, and fresh were rated slightly higher in the peel oil than the models, whereas the green attribute of the oil was rated slightly lower than the models. Overall for the examined attributes, the aroma of all models was found to be comparable to the original

Table 5. Sensory Evaluation for the Aroma Model of the Pontianak Orange Peel Oil As Affected by the Omission of Compounds

no.	compd(s) omitted	av score[a]
1	none (Pontianak orange aroma model)	6.8 a
2	(*Z*)-5-dodecenal	5.4 a
3	1-phenylethyl mercaptan	5.4 a
4	(*Z*)-5-dodecenal and 1-phenylethyl mercaptan	4.3 b

[a] The average score of seven panelists with a scale of 1 (extremely different from) to 9 (extremely similar to) Pontianak orange peel oil. The difference between levels with the same letter is not significant ($p < 0.05$).

Pontianak oil, as there was no significant statistical difference ($p < 0.5$). Nevertheless, the panelists felt that the OAV-based aroma model was closer to the aroma of Pontianak orange. This is in agreement with previous findings that OAV short-lists effectively potent aroma compounds, whereas RFA may not always correlate directly to the characteristic aroma compounds in food (*13*, *23*).

Omission Experiments. Results of the various aspects of this work (GC−O, aroma reconstitution, and sensory trials) on all available compounds indicated that (*Z*)-5-dodecenal and 1-phenylethyl mercaptan played a major role in the aroma of Pontianak orange peel oil, and work in this section was carried out to confirm this. Individual and binary omissions of those two compounds from the OAV-based model system were prepared and evaluated orthonasally by the panelists. There was no significant difference ($p < 0.05$) in the average score of ratings in model solutions when either compound was omitted and compared to the complete aroma model (**Table 5**). However, the binary omission resulted in significant difference ($p < 0.05$) in relation to the complete model mixture, an outcome which suggests the importance of both (*Z*)-5-dodecenal and 1-phenylethyl mercaptan to the aroma of Pontianak peel oil.

From this work, it was concluded that extensive experimentation combining instrumental GC−O with sensory evaluation of reconstituted aroma formulations and omission tests are essential for the evaluation of the aroma-active compounds of Pontianak orange peel oil. Aroma extract dilution analysis was utilized to obtain the FD factor, which was instrumental in describing the OAV and RFA characteristics of the peel-oil compounds. The approach was successful in the identification of potent odorants

and short-listed (*Z*)-5-dodecenal and 1-phenylethyl mercaptan as essential contributors to the aroma of Pontianak orange peel oil.

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