Research Article

Characterization of the Key Aroma Compounds in the Fruit of *Litsea pungens* Hemsl. (LPH) by GC-MS/O, OAV, and Sensory Techniques

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The key aroma compounds in the fruit of *Litsea pungens* Hemsl. (LPH) were concentrated through solvent-assisted flavor evaporation (SAFE) and characterized by gas chromatography-mass spectrometry-olfactometry (GC-MS/O), quantitative descriptive analysis (QDA), odor activity values (OAVs), and addition test. The results showed that LPH contained 31 aroma-active compounds (flavor dilution, FD = 9). Among them, 30 odorants were quantified by the standard curve method. The OAV analysis results showed that 25 odorants had OAVs ≥ 1 , which could be considered as the potent odorants. D-Limonene and 3,7-dimethyl-2,6-octadienal had the highest OAVs (OAV = 9803 and 8399), followed by (Z)-3,7-dimethylocta-2,6-dienal (OAV = 1893), β -myrcene (OAV = 1798), (E)-3-phenyl-2-propenoic acid ethyl (OAV = 1603), and β -caryophyllene (OAV = 1129). Addition experiments further confirmed that 3,7-dimethyl-2,6-octadienal, (Z)-3,7-dimethylocta-2,6-dienal, and D-limonene contributed to lemon attribute, β -myrcene contributed to green attribute, citronellal contributed to mint and fresh note, and eucalyptol contributed to eucalyptus-like note were the key odorants.

1. Introduction

Litsea pungens, as a genus belongs to Lauraceae's family, is an evergreen or deciduous tree or shrub with about 200 species distributed worldwide (mainly distributed in tropical and subtropical regions of Asia and America) [1]. In China, there are 72 species of *Litsea pungens* Hemsl. (LPH) distributed in 20 provinces (Figure 1). Among them, Yunnan Province has the most species (37), followed by Guangdong (24), Sichuan (18), Guizhou (15), and Hunan Province (12). The fruit, root, branch, and leaves of LPH have a wide range of applications in traditional Chinese medicine, fragrance industry [2, 3], cosmetics industry, and food industry due to the functional compounds existing inside, such as the aromatic compounds (essential oil), flavonoids, terpenoids, butanolides, and butenolactones steroids, lignans, amides, and alkaloids. The LPH, rich in citral, is an important raw

material for ionone and damascene [4, 5]. Due to the large and broad market demand, several decades ago, LPH species had been industrially cultivated, especially in Yunnan, Hubei, Hunan, Sichuan, Chongqing, and Guizhou provinces of China.

The therapeutic effects of LPH include removing dampness, regulating spleen deficiency, helping digestion, dysmenorrhea, expelling cold, and analgesia. All these benefits have been widely recorded in ancient Chinese medicine books such as "Guizhou folk medicine," "Chongqing Herbs," and "Hunan yaowuzhi." Modern molecular biology technologies have also elucidated that the functional compounds in the fruits, roots, branches, and leaves of LPH have anti-inflammatory activity, antimicrobial activity, hepatoprotection, antidiabetic, antiasthma activity, anticholelithiasis activity, immunomodulation, and miscellaneous bioactivities [3]. This information confirmed the

Eugenol and *p*-cresol were bought from Sigma-Aldrich (Beijing, China).

2.3. Sensory Evaluation. Quantitative descriptive analysis (QDA) was used to evaluate the aroma profiles of LPH's fruit. Twelve panelists with no rhinitis and no smoking (6 females and 6 males, age of 22–30) were recruited from our laboratory. The sensory evaluation room temperature was 23–25°C, the humidity was 50–55%, and filament lamp (36 W) was used. All panelists were informed of the aim, detailed experimental steps, and requirements of sensory evaluation before participating in this experiment. They were trained for 3 weeks before the QDA analysis: (1) all panelists were requested to sniff and describe the aroma characteristics of 54-aroma kit (Le Nez du Vin®, France) with 3 times a week (each training lasted for 30 min); (2) then, panelists were requested to analyze the aroma profiles of LPH's fruit descriptively. The final 6 aroma attributes (lemon, floral and sweet, mint and fresh, green, eucalyptus-like, and sour) were determined according to the frequency of descriptors, and their corresponding referenced standards were D-limonene, nerol, menthol, 1-hexanol, 1,8-cineole, and propionic acid, respectively; (3) finally, 12 panelists were qualified to score the intensity of 6 aroma attributes on a scale from 1 to 9 (1–3, weak; 4–6, medium; 7–9, strong). The LPH's fruit sample (4.00 g) loaded in 200 mL transparent glasses was presented to the panelist.

2.4. Isolation of the Volatile Compounds by SAFE. Dried LPH's fruit (20.00 ± 0.20 g) and dichloromethane solvent (80 mL) were loaded in a conical flask (250 mL) and extracted for 15 min by ultrasonication (KH-500 DE, Jiangsu, China) in 500 W at 10°C. Then, the organic phase was collected after filtration. After 3 extractions, the collected solvents were combined and submitted to the SAFE apparatus for volatile isolation. Isolation of the volatile compounds from the solvents was reference from our previous work with some modifications [25, 26]. The recycled water in SAFE apparatus was $(40 \pm 1)^\circ\text{C}$; the distillation flask was bath at $(40 \pm 1)^\circ\text{C}$; the collection flask was immersed in liquid nitrogen; the extraction system was operated under vacuum (10^{-5} – 10^{-6} Pa) via molecular turbine pump (Edwards, England), and the filtrate was added dropwise to the distillation flask. Then, the extract was concentrated to 1–2 mL with rotary evaporation instrument (EYELA N-1100, Tokyo Physical and Chemical Equipment Co., Ltd, Japan) after drying with anhydrous sodium sulfate. Finally, the concentrate was reduced to 1.00 mL by nitrogen (99.99%) before GC-MS and GC-MS/O analysis. All analyses were repeated in triplicate.

2.5. GC-MS and GC-MS/O Analysis. The identification and quantification of the aroma compounds were conducted by a single quadrupole gas chromatograph-mass spectrometer (GC-MS) (Thermo Fisher Trace 1310, Thermo Fisher Technology Co., Ltd, USA) in a split ratio of 50:1 (optimized in our lab). The aroma-active compounds were screened by

GC-MS equipped with a sniffing port (ODP3, Gerstel, Germany) (GC-MS/O). The temperature of sniffing port was 220°C, and the humidifier with flow rate of 10 mL/min (nitrogen, 99.999%) was used to humidify the air at sniffing port. The GC effluent was split at a ratio of 1:1 between the MS and sniffing port for the GC-MS/O's special structure in splitless injection. Separation of the aroma compounds in LPH's fruit extract was achieved on TG-5MS and TG-WAX columns (both $30\text{ m} \times 0.25\text{ mm i.d.} \times 0.25\text{ }\mu\text{m}$, Thermo Fisher). Helium (99.999%) was the carrier gas, and the carrier gas flow rate was constant at 1.200 mL/min and 2.000 mL/min in GC-MS and GC-MS/O, respectively.

The oven temperature of TG-WAX column analyzer was initially held at 40°C for 2 min, increased to 100°C (temperature rise rate, 4°C/min) and held for 1 min, and then increased to 175°C (temperature rise rate, 2°C/min) and held for 1 min, finally increased to 230°C (temperature rise rate, 5°C/min). The oven temperature of TG-5MS column analysis was initially held at 40°C, then increased to 100°C (temperature rise rate, 3°C/min), increased to 170°C (temperature rise rate, 1°C/min) and held for 1 min, and finally increased to 230°C (temperature rise rate, 5°C/min). The temperature of the sniffing port was kept at 230°C. The injector temperature was 250°C, and the ion source temperature was 280°C. The electronic-impact mass spectra ionization mode with ionization energy of 70 eV was used. The full scan mode (m/z range from 40 to 350 amu) was used.

2.6. Gas Chromatography-Olfactometric (GC-O) Analysis. The aroma frequency, combined with the aroma dilution method, was used in the GC-O study. Firstly, the concentrated organic extract was diluted to 1:9 with dichloromethane solvent. The diluted sample was then submitted to the GC-MS/O with the TG-WAX column to screen the aroma-active compounds with flavor dilution (FD) factor over 9. The diluted sample was repeated 3 times by 3 trained panelists. Only the aroma compounds detected over 5 of 9 were recorded. Panelists underwent GC-MS/O training by sniffing 31 standards aroma compounds in dichloromethane solvent (1,000 $\mu\text{g/L}$) three times before this experiment.

2.7. Identification and Quantification. The identification of the aroma compounds was based on comparing the mass spectra (MS) database NIST 2020, with retention indexes (RIs, on nonpolar and polar GC columns), pure standards (S), and the odor characteristics (O). All quantifications of key odorants were performed by constructing standard curves. The abscissa was referred to the ratio of the peak area of each compound to the three internal standards (1,2-dichlorobenzene, 2,500 $\mu\text{g/mL}$; 2-octanol, 2,900 $\mu\text{g/mL}$; 3-methylacetophenone, 3,000 $\mu\text{g/mL}$) that are obtained by GC-MS and the ordinate was the concentration ratio of aroma compounds to the three internal standards [29]. Each quantified aroma compound referenced from the specifically internal standard was labeled in Table 1. Each of the internal standards (100 μL) was added when LPH's fruit was extracted by dichloromethane solvent.

TABLE 1: Odor activity values (OAVs) of aroma-active compounds (FD = 9) in *Litsea pungens* Hemsl. fruit.

No.	Compounds	CAS	Concentration ($\mu\text{g/kg}$)	Threshold ($\mu\text{g/kg}$)	OAV	Standard curves	R^2
5	D-Limonene ^c	5989-27-5	333,321.19 $\pm$ 1,339.09	34 [30]	9803	$y = 0.0539x - 0.0014$	0.9932
20	3,7-Dimethyl-2,6-octadienal ^a	5392-40-5	251,978.13 $\pm$ 683.24	30 [31]	8399	$y = 0.0851x + 0.0288$	0.9936
17	(Z)-3,7-Dimethylocta-2,6-dienal ^a	106-26-3	189,366.87 $\pm$ 7,528.88	100 [31]	1893	$y = 0.074x + 0.0085$	0.9958
3	β -Myrcene ^a	123-35-3	179,800.17 $\pm$ 4,978.31	100 [32]	1798	$y = 0.0896x - 0.0085$	0.9975
28	(E)-3-Phenyl-2-propenoic acid ethyl ^b	4192-77-2	6,913.31 $\pm$ 913.99	4 [31]	1603	$y = 0.1055x + 0.0146$	0.9975
26	β -Caryophyllene ^b	87-44-5	76,771.87 $\pm$ 1,296.43	64 [30]	1129	$y = 0.068x + 0.0212$	0.9837
12	Citronellal ^a	106-23-0	25,397.45 $\pm$ 1,483.47	31 [30]	819	$y = 0.0487x + 0.008$	0.9972
7	Linalool ^a	78-70-6	360,841.19 $\pm$ 7,836.63	500 [32]	722	$y = 0.073x + 0.0067$	0.9944
4	6-Methyl-5-hepten-2-one ^a	110-93-0	29,118.53 $\pm$ 9,49.40	50 [32]	582	$y = 0.094x - 0.0003$	0.9999
2	Methional ^a	3268-49-3	890.48 $\pm$ 177.02	1.8 [33]	494	$y = 0.4585x + 0.004$	0.995
13	Estragole ^b	140-67-0	13,376.54 $\pm$ 3,255.45	35 [31]	382	$y = 0.0875x - 0.004$	0.9964
6	Eucalyptol ^b	470-82-6	81,581.26 $\pm$ 1,136.85	230 [31]	355	$y = 0.0613x + 0.0025$	0.9987
11	2-(3-Methyl-2-butenyl)-3-methylfuran ^c	15186-51-3	23,794.84 $\pm$ 4,610.74	100 [31]	238	$y = 0.0384x + 0.0058$	0.9979
21	Anethole ^b	104-46-1	7,133.33 $\pm$ 551.68	50 [31]	143	$y = 0.062x + 0.0085$	0.9993
23	Eugenol ^b	97-53-0	10,249.40 $\pm$ 824.78	90 [31]	114	$y = 0.0988x - 0.0039$	0.9882
18	D-Carvone ^c	2244-16-8	6,998.14 $\pm$ 682.58	160 [31]	44	$y = 0.1175x + 0.0262$	0.9936
25	Methyleugenol ^b	93-15-2	2,529.33 $\pm$ 467.25	68 [31]	37	$y = 0.0696x + 0.011$	0.9977
9	<i>p</i> -Cresol ^b	106-44-5	3,229.82 $\pm$ 41.03	100 [32]	32	$y = 0.0429x + 0.0248$	0.9928
22	Safrole ^b	94-59-7	2,328.99 $\pm$ 390.14	160 [31]	15	$y = 0.0797x + 0.0141$	0.9978
16	4-(1-methylethyl)-benzaldehyde ^b	122-03-2	2,500.23 $\pm$ 157.00	177 [31]	14	$y = 0.0612x + 0.0183$	0.9961
15	(3Z)-3,7-Dimethyl-2,6-octadien-1-ol ^a	106-25-2	3,747.99 $\pm$ 643.73	300 [30]	12	$y = 0.1214x - 0.006$	0.9983
31	Ethyl <i>p</i> -methoxycinnamate ^b	24393-56-4	5,422.92 $\pm$ 244.75	500 [34]	11	$y = 0.1576x + 0.0407$	0.9956
30	Caryophyllene oxide ^b	1139-30-6	3,829.05 $\pm$ 630.81	400 [31]	10	$y = 0.0412x + 0.0156$	0.9837
27	(+)-2-Bornanone ^d	464-49-3	3,802.95 $\pm$ 770.73	1360 [31]	3	$y = 0.0482x - 0.007$	0.9962
1	3-Methyl-butyricacid ^a	503-74-2	156.40 $\pm$ 3.15	100 [31]	1.5	$y = 0.1128x - 0.0033$	0.9982
10	Fenchone ^c	1195-79-5	97.44 $\pm$ 11.68	440 [31]	<1	$y = 0.0482x - 0.007$	0.9962
14	Carveol ^c	99-48-9	665.79 $\pm$ 637.26	4000 [35]	<1	$y = 0.4792x - 3E - 19$	0.9999
24	Decanoic acid ^a	334-48-5	2,043.84 $\pm$ 670.91	2200 [32]	<1	$y = 0.084x + 0.0068$	0.9943
29	(R)-2-Methyl-5-((S)-6-methylhept-5-en-2-yl)-cyclohexa-1,3-diene ^c	495-60-3	9,925.30 $\pm$ 535.49	—	—	$y = 0.0875x - 0.004$	0.9964
19	Phenylacetic acid ^c	103-82-2	—	—	—	—	—
8	(E)-1-methyl-4-(1-methylvinyl)cyclohex-2-en-1-ol ^c	7212-40-0	10,040.07 $\pm$ 468.85	—	—	$y = 0.0765x - 1E - 04$	0.9991

The alphabet a, b, c, and d represent compounds calibration by internal standard compounds of 2-octanol, 1,2-dichlorobenzene, and 4'-methylacetophenone, respectively; "—" represents the aroma compounds were lower than the quantitative limitation.

2.8. Calculation of the Odor Activity Value (OAV). The OAVs of aroma-active compounds were measured by dividing their concentration detected in the LPH's fruit sample by their odor threshold detected in water. Each threshold value was referenced from the corresponding literature studies and book, which labeled in Table 1 [30–33, 36, 37]. The aroma-active compounds with OAV ≥ 1 are considered to be the potent key odorants of LPH fruit.

2.9. Addition Experiment. The addition tests were conducted to validate the potent odorants and elucidate their specifically contributions with high OAV to LPH's fruit sample by adding the aroma compounds to the LPH's fruit sample (5.00 g) based on the detected concentration. Two original LPH's fruit samples (5.00 g) and one aroma added sample were subjected to the panelists. Panelists were requested to evaluate the difference by triangle tests and quantitative descriptive analysis (Section 2.3) [38] as shown in Table 2.

3. Results and Discussion

3.1. Sensory Evaluation. The QDA result data of LPH's fruit were plotted on a spider diagram shown in Figure 2, suggesting that lemon note was the strongest, followed by floral and sweet, mint and fresh, eucalyptus-like, and green characteristics. The sour note of LPH's fruit had the lowest intensity. This result also elucidated the LPH's fruit had a potent flavor enhancer or improving ability.

3.2. Aroma Compounds in LPH's Fruit. In this work, a total of 159 volatile compounds including 55 olefins, 34 alcohols, 10 aldehydes, 8 acids, 18 ketones, 14 esters, 7 phenolics, 4 furans, 3 alkanes, 3 ethers, and 3 sulfur compounds were detected by SAFE-GC-MS analysis (Table S1). Among the 55 olefins, terpenoids were the most prevalent substances, including the monoterpenes (C10), sesquiterpenes (C15), and diterpenes (C20). These plant-derived compounds were biosynthesized from the active isoprene C5 units

TABLE 2: Results of the addition tests.

No.	Omitted compounds	Correct numbers	Significance ^a	Contribution aroma
1	D-Limonene	9/12	*	Lemon
2	3,7-Dimethyl-2,6-octadienal	12/12	**	Lemon
3	(Z)-3,7-Dimethylocta-2,6-dienal	12/12	**	Lemon
4	β -Myrcene	10/12	**	Green
5	(E)-3-Phenyl-2-propenoic acid ethyl	5/12		
6	β -Caryophyllene	5/12		
7	Citronellal	12/12	**	Mint and fresh
8	Linalool	11/12	*	Floral and sweet
9	6-Methyl-5-hepten-2-one	8/12		
10	Methional	7/12		
11	Estragole	5/12		
12	Eucalyptol	11/12	**	Eucalyptus-like
13	2-(3-Methyl-2-butenyl)-3-methylfuran	8/12		
14	Anethole	8/12		
15	Eugenol	6/12		

^a “**” and “*” represent the significance at $P < 0.001$ and $P < 0.05$, respectively.

FIGURE 2: Aroma profiles of *Litsea pungens* Hemsl. fruit by quantitative descriptive analysis.

dimethylallyl diphosphate and isopentenyl diphosphate, which belong to the most diverse family of natural products [39, 40]. As the results showed, α -pinene, camphene, β -myrcene, α -phellandrene, 3-carene, D-limonene, (Z)-3,7-dimethyl-1,3,6-octatriene, γ -terpinene, *p*-mentha-1,4(8)-diene, β -ocimene, and *o*-cymene were monoterpenes (C10); (Z)- α -bergamotene, humulene, copaene, caryophyllene, guaia-1(10),11-diene, (E,E)-1-methyl-5-methylene-8-(1-methylethyl)-1,6-cyclodecadiene, (E)- β -farnesene, α -cubebene, α -guaiene, and β -bisabolene were sesquiterpenes (C15). On the contrary, only one diterpene (2,6,11,15-tetramethyl-hexadeca-2,6,8,10,14-pentaene) was detected in LPH's fruit. These results elucidated that the most of the volatile terpenoids were mono and sesquiterpenes with only a few diterpenes [40, 41]. Applying isotope-labeled pathway-specific precursors and green fluorescent protein-labeled terpene synthases have shown that monoterpenes' biosynthesis was located in plastids and fueled by the methylerythritol phosphate pathway. In contrast, the biosynthesis of sesquiterpenes was located in the cytosol and fueled by the

cytosolic/peroxisomal mevalonic acid pathway and, under certain conditions, the methylerythritol phosphate pathway via the abovementioned metabolic crosstalk [42]. Acyclic (geraniol, linalool, and myrcene), monocyclic (D-limonene and α -terpineol), and bicyclic (car-3-ene and α -pinene) monoterpenes were biosynthesis from geranyl diphosphate by terpene synthases that shared a coupled isomerization-cyclization reaction sequence [42]. These compounds were usually found in plant-derived monoterpenes. Carvone could be generated by a series of enzyme-catalyzed synthesis, including geranyl diphosphate synthase, (–)-limonene synthase, (–)-limonene 6-hydroxylase, and (–)-*trans*-carveol dehydrogenase [43]. β -Caryophyllene was derived from farnesyl diphosphate by sesquiterpene cyclization reactions leading to volatile sesquiterpene hydrocarbons [44].

The chain acids, such as acetic acid, 3-methyl-butyric acid, and decanoic acid, were derived from the degradation of fatty acids. Phenylacetic acid was generated from L-phenylalanine via the shikimic acid pathway under the action of phenylalanine ammonia-lyase. Esters in the plants mainly generated from two ways: alcohol acyltransferase and carboxylesterase, belonging to the biosynthesis of amino acid-derived odor compounds [45]. Three kinds of ketone, including sesquiterpenes ketones, furan ketones, and chain ketones, were detected in the LPH's fruit. Sesquiterpenes ketones were mainly derived from the biosynthesis of the terpenoid pathway; furan ketones were carbohydrate-derived or carotenoid-derived volatile compounds, and chain ketones generated from lipids degradation [46]. The phenolic compounds were mainly derived from L-phenylalanine. Cinnamic acid was generated under the action of a phenylalanine ammonia-lyase, and then cinnamic aldehyde produced through reduction reaction [47]. Aldehydes with fresh green characteristics were mainly derived from the fatty acids (linolenic acid) by the lipoxygenase pathway [45]. Generally, the fatty acids were stored in plants as triacylglycerides and were liberated by lipases before they are acted as direct precursors for various volatiles. The sulfur compounds were derived from amino acid degradation [36]. Most of the alcoholics (monoterpenes), such as (3Z)-3,7-

TABLE 3: Qualitative analysis results of aroma-active compounds (FD = 9) in *Litsea pungens* Hemsl. fruit.

No.	Compounds	CAS	Structure	RI (TG-5MS/ TG-WAX)	Aroma descriptions	Identification
1	3-Methyl-butyricacid	503-74-2		863/859	Sour, sweaty, cheese	MS/RI/S/O
2	Methional	3268-49-3		903/906	Cooked potato	MS/RI/S/O
3	β -Myrcene	123-35-3		989/990	Spicy, peppery, green	MS/RI/S/O
4	6-Methyl-5-hepten-2-one	110-93-0		991/986	Citrus, green, musty	MS/RI/S/O
5	D-Limonene	5989-27-5		1027/1039	Citrus, orange, fresh, sweet	MS/RI/S/O
6	Eucalyptol	470-82-6		1032/1029	Eucalyptus, camphor, medicine	MS/RI/S/O
7	Linalool	78-70-6		1104/1116	Floral, sweet, woody, green	MS/RI/S/O
8	(E)-1-Methyl-4-(1-methylvinyl)cyclohex-2-en-1-ol	7212-40-0		1105/1127	Fresh, minty, woody	MS/RI/S/O
9	p-Cresol	106-44-5		1085/1076	Phenolic, leather, animal	MS/RI/S/O
10	Fenchone	1195-79-5		1090/1083	Fresh, woody	MS/RI/S/O
11	2-(3-Methyl-2-butenyl)-3-methylfuran	15186-51-3		1093/1100	Caramel, sweet, floral	MS/RI/S/O
12	Citronellal	106-23-0		1151/1156	Sweet, floral, citrus	MS/RI/S/O
13	Estragole	140-67-0		1196/1204	Spicy, green, herbal, fennel	MS/RI/S/O
14	Carveol	99-48-9		1225/1210	Spearmint, caraway	MS/RI/S/O
15	(3Z)-3,7-Dimethyl-2,6-octadien-1-ol	106-25-2		1236/1225	Sweet, floral, citrus, magnolia	MS/RI/S/O
16	4-(1-Methylethyl)-benzaldehyde	122-03-2		1239/1233	Spicy, cumin, green, herbal	MS/RI/S/O
17	(Z)-3,7-Dimethylocta-2,6-dienal	106-26-3		1242/1254	Floral, sweet, citral, lemon	MS/RI/S/O
18	D-Carvone	2244-16-8		1246/1255	Spicy, mint, green	MS/RI/S/O
19	Phenylacetic acid	103-82-2		1262/1274	Sweet, honey, floral	MS/RI/S/O
20	3,7-Dimethyl-2,6-Octadienal	5392-40-5		1276/1290	Lemon, sweet, citura	MS/RI/S/O
21	Anethole	104-46-1		1279/1298	Anise, licorice, medicinal	MS/RI/S/O
22	Safrole	94-59-7		1287/1264	Spicy, woody, floral, anise	MS/RI/S/O
23	Eugenol	97-53-0		1348/1361	Clove, sweet, spicy, woody	MS/RI/S/O
24	Decanoic acid	334-48-5		1373/1423	Rancid sour, fatty, waxy	MS/RI/S/O
25	Methyleugenol	93-15-2		1404/1411	Sweet, fresh, spicy, clove, cinnamon	MS/RI/S/O
26	β -Caryophyllene	87-44-5		1420/1424	Clove, sweet, woody, spicy	MS/RI/S/O

TABLE 3: Continued.

No.	Compounds	CAS	Structure	RI (TG-5MS/ TG-WAX)	Aroma descriptions	Identification
27	(+)-2-Bornanone	464-49-3		1443/1143	Camphor, minty, herbal, woody	MS/RI/S/O
28	(E)-3-Phenyl-2-propenoic acid ethyl	4192-77-2		1474/1463	Floral, honey, balsamic, wine	MS/RI/S/O
29	(R)-2-Methyl-5-((S)-6-methylhept-5-en-2-yl)-cyclohexa-1,3iene	495-60-3		1500/1494	Spicy, fresh, sharp woody	MS/RI/S/O
30	Caryophyllene oxide	1139-30-6		1561/1543	Sweet, fresh, woody, spicy	MS/RI/S/O
31	Ethyl p-methoxycinnamate	24393-56-4		1773/1748	Cinnamon, sweet, wine	MS/RI/S/O

"MS," compounds were identified by mass spectra (NIST 14); "RI," compounds were identified on TG-WAX and TG-5MS columns; "S," compounds were identified by standards; "O," compounds were identified by GC-O.

dimethyl-2,6-octadiene-1-ol, (*E*)-linalool oxide, (*E*)-4-thujanol, and (*E*)-p-mentha-2,8-dien-1-ol were derived from the precursor of granyl diphosphate, whereas the sesquiterpenes alcohols including nerolidol and decahydro-1,4-dimethyl-7-(1-methylvinyl)azulen-4-ol were derived from the precursor of (*E,E*)-farnesyl diphosphate [47].

These volatile compounds with the properties of sweet, floral, citrus, green, herb, fresh, eucalyptus, phenolic, spicy, and sour characteristics also had an antimicrobial activity or other biological functional activities. They were detected in LPH's fruits and existed in the roots, stems, and leaves, which played an important role in plant-animal interactions, including the attraction of pollinators and seed disseminators, protecting the plants from pathogen attack, and repellence of herbivores [36].

3.3. Quantitation and Calculation of the Odor Active Values (OAVs). A total of 31 aroma-active compounds (FD=9) were detected by gas chromatography-olfactory. Among them, terpenoids (16) were the most prevalent. β -Myrcene, linalool, and citronellal were acyclic monoterpenes. D-limonene, carveol, eucalyptol, and D-carvone were monocyclic monoterpenes; (+)-2-bornanone and fenchone were bicyclic monoterpenoids; (3Z)-3,7-dimethyl-2,6-octadien-1-ol (nerolidol) was acyclic sesquiterpenes; β -caryophyllene, caryophyllene, and (*R*)-2-methyl-5-((*S*)-6-methylhept-5-en-2-yl)-cyclohexa-1,3-diene oxide were bicyclic sesquiterpenes. These compounds with spicy, floral, green, fresh, woody, citrus, and herb spearmint characteristics consist of the main aroma profiles of LPH's fruits. The other compounds, such as acids, phenolics, and esters, also played an important role in the overall aroma profiles.

To confirm the potent aroma compounds in the overall aroma profiles of LPH's fruit, the aroma-active compounds were quantified, and their odor activity values (OAVs, the ratio of concentration to odor threshold) were also calculated. The standard curves of each aroma-active compound are shown in Table 1. All the calibration curves obtained had

good linearity ($R^2 > 0.99$). Quantification results showed (Table 3) that linalool had the highest concentration (360,841.19 $\mu\text{g/kg}$), followed by D-limonene (333,321.19 $\mu\text{g/kg}$), 3,7-dimethyl-2,6-octadienal (251,978.13 $\mu\text{g/kg}$), (*Z*)-3,7-dimethylocta-2,6-dienal (189,366.87 $\mu\text{g/kg}$), β -myrcene (179,800.17 $\mu\text{g/kg}$), eucalyptol (81,581.26 $\mu\text{g/kg}$), and β -caryophyllene (76,771.87 $\mu\text{g/kg}$). Fenchone (97.44 $\mu\text{g/kg}$) and 3-methyl-butyric acid (156.40 $\mu\text{g/kg}$) had the lowest concentrations.

As the OAV analysis results shown above, there were 25 odorants with OAVs ≥ 1 in LPH's fruit, but 3 compounds (fenchone, carveol, and decanoic acid) had OAVs < 1 , indicating that they were not the potent odorants in the overall aroma of LPH's fruit. The odor threshold value of (*E*)-1-methyl-4-(1-methylvinyl) cyclohex-2-en-1-ol and (*R*)-2-methyl-5-((*S*)-6-methylhept-5-en-2-yl)-cyclohexa-1,3-diene was not found; therefore, their OAVs were not calculated. Moreover, the concentration of phenylacetic acid was lower than the limit of quantitation. The remained 25 odorants with OAVs ranged from 1.5 to 9803 were the potent odorants for LPH's fruit aroma. Among them, D-limonene had the highest OAV (OAV = 9803) for its significant high concentration and low threshold (34 $\mu\text{g/kg}$), followed by 3,7-dimethyl-2,6-octadienal (OAV = 8399), (*Z*)-3,7-dimethylocta-2,6-dienal (OAV = 1893), β -myrcene (OAV = 1798), (*E*)-3-phenyl-2-propenoic acid ethyl (OAV = 1603), β -caryophyllene (OAV = 1129), citronellal (OAV = 819), linalool (OAV = 722), 6-methyl-5-hepten-2-one (OAV = 582), methional (OAV = 494), estragole (OAV = 382), eucalyptol (OAV = 355), 2-(3-methyl-2-butenyl)-3-methylfuran (OAV = 238), anethole (OAV = 143), and eugenol (OAV = 114). Interestingly, most of the terpenoids exhibited higher OAVs, which are similar to other species, for example, *Toona sinensis* (A. Juss.) and pepper (*Zanthoxylum bungeanum*) [30, 37]. The results of aroma characteristics quality, OAV analysis, and the sensory evaluation elucidated that D-limonene, citronellal, 3,7-dimethyl-2,6-octadienal, (*Z*)-3,7-dimethylocta-2,6-dienal, (*E*)-3-phenyl-2-propenoic acid ethyl, β -myrcene, linalool, methional, 6-methyl-5-

hepten-2-one, 2-(3-methyl-2-butenyl)-3-methylfuran, β -caryophyllene, estragole, anethole, eucalyptol, and eugenol might be the key odorants of LPH's fruit.

3.4. Addition Experiment. Addition tests results elucidated that 3,7-dimethyl-2,6-octadienal and (Z)-3,7-dimethylocta-2,6-dienal had high significant ($P < 0.001$) contribution to the lemon attribute of LPH's fruit. Besides, D-limonene also had contribution to lemon attribute ($P < 0.05$). β -Myrcene had high significant ($P < 0.001$) contribution to the green attribute of LPH's fruit. Citronellal had high significant ($P < 0.001$) contribution to the mint and fresh attribute. Eucalyptol had high significant ($P < 0.001$) contribution to the eucalyptus-like note of LPH's fruit. In summary, 3,7-dimethyl-2,6-octadienal and (Z)-3,7-dimethylocta-2,6-dienal, D-limonene, β -myrcene, citronellal, and eucalyptol were confirmed as the key odorants of LPH's fruit. As the significant difference results elucidated, 3,7-dimethyl-2,6-octadienal and (Z)-3,7-dimethylocta-2,6-dienal were greater than that of D-limonene.

4. Conclusions

The aroma compounds in LPH's fruit were isolated by SAFE. By application of frequency combined with aroma dilution analysis, 31 aroma-active compounds were detected, and 30 of them were further quantified by the external standard method. OAVs ≥ 1 were obtained for 25 odorants among which D-limonene (OAV = 9803) and 3,7-dimethyl-2,6-octadienal (OAV = 8399) had the highest OAV value. (Z)-3,7-dimethylocta-2,6-dienal (OAV = 1893), β -myrcene (OAV = 1798), (E)-3-phenyl-2-propenoic acid ethyl (OAV = 1603), and β -caryophyllene (OAV = 1129) had OAV over 1000. These results elucidated that they played an important role to the overall aroma profiles of LPH's fruit. Based on the addition tests, 3,7-dimethyl-2,6-octadienal and (Z)-3,7-dimethylocta-2,6-dienal and D-limonene contributing to lemon attribute, β -myrcene contributing to green attribute, citronellal contributing to mint and fresh note, and eucalyptol contributing to eucalyptus-like note were confirmed as the key odorants in LPH's fruit.

Data Availability

All the data used in this work could be found in the manuscript and the supplemental materials.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Supplementary Materials

Table S1: the aroma compounds isolated by solvent-assisted evaporation extraction in *Litsea pungens* Hemsl. fruit. (Supplementary Materials)

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