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Comparison of free and bound volatile profiles of immature *Litsea mollis* fruits grown in five distinct regions of China

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Abstract

Immature *Litsea mollis* fruits (ILMFs) are widely used as cold appetizers and seasonings in China. The free and bound volatile components of ILMFs from five distinct regions of China were determined by gas chromatography-mass spectrometry (GC-MS) combined with headspace solid phase microextraction (SPME) in the present work. The results reveal sixty six free-form volatiles and fifty two bound-form volatiles in ILMFs and terpene hydrocarbons and oxygenated terpenes are the dominate components. In addition, sixty-seven volatile compounds are found in *Litsea mollis* fruit for the first time. The free α-pinene, eucalyptol, linalool, α,β-citral, geraniol and citronellal, and bound β-myrcene, α-citral, geraniol, citronellal and eugenol are the major odor contributors of ILMFs. Multivariate statistical analysis indicates that the free and bound volatiles profiles of ILMFs of Fujian and Guangxi in southern China are similar and those of Yunnan and Sichuan in southwestern China are similar. The odor profiles of all of the ILMFs are floral, fruity and herbaceous.

Keywords: *Litsea mollis*; immature fruit; volatile; aroma; bound aroma compound.

Practical Application: The study provides valuable and reliable information of free and bound volatile aroma components in immature *Litsea mollis* fruits, and will help the development of natural foods for food industries.

1 Introduction

Litsea mollis Hemsl. is a member of the Lauraceae family. In China, it is mostly distributed in Guangdong, Guangxi, Fujian, Hunan, Hubei, Sichuan, Guizhou, Yunnan and eastern Tibet (Chinese Academy of Sciences, 2019, Figure S1). *Litsea mollis* Hemsl. is also found in some regions of Japan, Korea, New Zealand, North America, and South America (Huang et al., 2014). The large amounts of fatty acids and aromatic oils in its fruit make it of a great economic plant, especially for the production of drugs and lubricants (Kim et al., 2014). The volatile oil of ripe *Litsea mollis* fruits is a biopreservative of low-cost in the dairy industry (Cai et al., 2019).

Immature *Litsea mollis* fruit (ILMF) is generally pickled with soy sauce, vinegar, pepper, garlic, and ginger as a popular cold appetizer in southwestern China (Zhang, 2015). People usually only pay attention to the aroma of free volatiles in fruits, but the studies on the bound aroma compounds of other fruits show that the hydrolysis of bound volatiles can improve the overall aroma of the fruits. The bound forms, mainly referring to the glycosidically bound alcohols, terpenes, dimethyl isoprene, volatile organic acids and shikimic acid derivatives, can be released by enzymatic or acid hydrolysis (Sánchez-Palomo et al., 2017), and thus they are also considered as important aroma components (Sarry & Gunata, 2004). The glycosides in the bound volatile compounds identified in fruits are mainly O-β-D-glucosides and O-diglycosides. O-glycoside hydrolases (EC 3.2.1.x) are a widespread group of enzymes of significant biological, biomedical and industrial importance. They, as well as fungal enzymes (usually from *Aspergillus* niger), can release the bound aroma (Davis & Croteau, 2000).

Chen et al. (1984) extracted volatile oils from mature *Litsea mollis* fruits by steam distillation and found that the aroma of volatile oils mainly came from terpenoids. To the best of our knowledge, only one work has been reported on the free volatile compounds in ILMF (Yang et al., 2020), where six terpenoids are found to be responsible for the typical flavor of ILMF. Other than that, the aroma compounds of ILMF, either the free forms or bound forms, have been rarely studied.

In the present work, the volatile components in the ILMFs collected from different regions of China were extracted by headspace solid phase microextraction (SPME), and the glycosidically bound volatiles were enzymatically hydrolyzed with the pectinase from *Aspergillus* niger (Dziadas & Jelen, 2016; Yang et al., 2019). SPME is a simple, low cost, solvent free and sensitive method for aroma compound extraction without heating required, and thus can minimize the loss of aroma. The volatile components were then identified and quantitatively analyzed by GC-MS, and the volatile profiles of the ILMFs were compared, aiming to provide a scientific foundation for understanding the

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different free and bound aroma compositions of the ILMFs in different regions of China.

2 Materials and methods

2.1 Materials and chemicals

ILMF samples

Based on the main distribution areas of *Litsea mollis* published by the Chinese Academy of Sciences (Chinese Academy of Sciences, 2019), wild ILMFs with sizes of 3-4 mm were harvested in five main distribution areas in China including Fujian (FJ), Guangxi (GX), Hubei (HB), Yunnan (YN) and Sichuan (SC) provinces by a professional organization, Enshi Dongsheng Plant Development Co. Ltd. (Figure S1), and identified and validated by Professor Yong-Mei Yi at the School of Forestry and Horticulture, Hubei Minzu University. The samples were frozen immediately after collection, transported to the laboratory under frozen conditions in two days, and stored at -78 °C before processing and analysis. The detailed sampling protocol is provided in Table S1.

Chemicals

C7-C30 saturated alkanes reference standard, *Aspergillus* niger pectinase (1.06 U mg^{-1}) and the standards of citral (98.0%), α-pinene (98.0%), α-phellandrene (85.0%), (E)-2-hexen-1-ol (96.0%), linalool (97.0%), camphene (95.0%), α-terpinene (89.0%), allo-ocimene (80.0%), β-myrcene (90.0%), γ-terpinene (97.0%), p-cymenene (98.0%), citronellal (95.0%), (Z)-verbenol (95%), terpinolene (85.0%), geraniol (97.0%), nerol acetate (98.0%), caryophyllene (98.0%), 4-terpineol (95.0%), α-terpineol (95.0%), caryophyllene oxide (99.0%), methyl cinnamate (99.0%), methyl salicylate (99.0%), L-perillaldehyde (92.0%), nerolidol (98.0%), carveol (95.0%), humulene (96.0%), nerol (97.0%), eugenol (99.0%), (-)-borneol (99.0%), phenethanol (97.0%), sulcatone (97.0%), perilla alcohol (96.0%), rose oxide (99.0%), benzaldehyde (99.0%), methyl o-anisate (99.0%), eucalyptol (99.0%), geranic acid (85.0%) and L-pinocarveol (96.0%) were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All other chemicals were of analytical grade. Double-distilled water was used in all experiments.

2.2 Juice preparation and free volatiles extraction

The fruit samples were respectively thawed and juiced using a JYZ-E16 juicer (Joyoung Co., Ltd., Jinan, China). The juices were centrifuged at 4 °C at 8000 rpm for 20 min in an Avanti J −30I centrifuge (Beckman Coulter Inc., Brea, USA). Ten milliliters of the supernatant of each juice were collected, mixed with 20.0 µL 1-octanol (1.5707g L^{-1} in ethanol) internal standard and 2.0 g NaCl, transferred into a 20-mL sample bottle containing a magnetic stirrer, sealed with a PTFE-silicon septum, and extracted by the method reported by Yang et al. (2020). Briefly, the sample was equilibrated at 46 °C for 20 min, and extracted with a DVB/CAR/PDMS (Divinylbenzene/Carboxen/Polydimethylsiloxane) SPME fiber (50/30µm; Supelco, Bellefonte, PA) at 46 °C for 36 min. To ensure the accuracy of the analysis, three replicates of each juice sample were prepared and extracted.

2.3 Isolation of bound volatile compounds

The bound volatile compounds were extracted as described in our previous work (Yang et al., 2019). Briefly, 400 mL of juice supernatant was passed through a LC column (400 × 40 mm) packed with 50 g Amberlite XAD-2 resin (20–60 mesh, Sigma Aldrich, St. Louis, Mo., USA) at the flow rate of 3 mL min^{-1}. The column was washed with double-distilled water and diethyl ether-pentane (1:1, V/V, 5 mL min^{-1}) to remove sugars, acids and free aroma compounds. The bound compounds were then eluted with 600 mL methanol (≥99.8%) at 5 mL min^{-1}, dried under reduced pressure (0.08 MPa) with a rotary evaporator (RE-52AA, Shanghai Yarong biochemical instrument factory, China) at ≤35 °C, and reconstituted in 40 mL of citric acid-disodium hydrogen phosphate buffer (pH 5.5, close to the pH of the juice). Free volatile residues were further removed by the liquid-liquid extraction with 80 mL of dichloromethane-pentane (1:1, V/V) 3 times. The aqueous phase was equally divided into four aliquots, and hydrolyzed with 150 mg of *Aspergillus* niger pectinase (1.06 U mg^{-1}) at 37 °C for 48 h. Each aliquot, two grams of NaCl, a magnetic stirrer and 20 µL of 1-octanol (1.5707 g L^{-1} in ethanol) were sealed in an extractor with PTFE-silicon septum and extracted by SPME as described in Section 2.2 for GC-MS analysis.

2.4 Measurements of total soluble solids and pHs of ILMF juices

The total soluble solids of ILMF juices were determined with a WYT-4 digital handheld refractometer (Beijing Yangtech Scientific Instruments Co., Ltd., Beijing, China) and their pHs were measured with a PB-21 pH meter (Sartorius AG Inc., Beijing, China) for the preparation of model juice. The results are listed in Supplementary material information Table S4.

2.5 GC–MS analysis

GC-MS analysis was conducted as described in our previous report (Yang et al., 2020) using a DB-5MS capillary column (30 m × 0.25 mm i.d × 0.25 µm, Agilent, Santa Clara, CA) for GC separation.

2.6 Quantification and odor activity values (OAVs) calculation

To accurately quantify the volatile compounds, working standard solutions were prepared using a model juice containing 30.0 g sucrose and 30.0 g glucose per 1000 mL double-distilled water prepared based on the average total soluble solid and pH of ILMF juices as described in our previous report (Yang et al., 2019). The pH of the model juice was adjusted with citric acid solution to 5.34 (Table S4) for the preparation of free volatile standards solutions. The bound volatile standards solutions were prepared in citric acid–disodium hydrogen phosphate buffer (pH 5.50). Each working standard was extracted with the same procedure as that for ILMF juice. The calibration curves with regression coefficients greater than 0.975 were established by SIM quantitative analysis (Table S2 & Table S3). The volatile compounds with no standards available were quantified with the standard curves of the volatiles of similar functional groups and/or similar numbers of C. The results are reported as μg L^{-1} juice. OAVs were calculated as described in our previous work (Yang et al., 2019).

2.7 Statistical analyses

All data are reported as mean value ± standard deviation of three tests. Principle component analysis (PCA) and one-way ANOVA were conducted using the IBM SPSS Statistics 22 software (IBM, Armonk, NY, USA). Hierarchical clustering and heatmap visualization and plot were conducted with the TB tools (GitHub, 2021).

3 Results and discussion

3.1 Free and bound volatile compounds identified in ILMFs

Eighty two compounds including 66 free-forms and 52 bound-forms were identified in the ILMF samples by GC-MS (Table 1 and 2). Among them, 36 compounds are present in both free and bound forms. The number of compounds in ILMFs of the five regions displayed a high degree of similarity (Figure 1). Forty three free compounds (Figure 1A) and 34 bound compounds (Figure 1B) are found in the ILMFs of all five regions. Fourteen of them including α-pinene, camphene, β-pinene, β-myrcene, p-cymenene, sulcatone, linalool, citronellal, 4-terpineol, borneol, α-terpineol, β-citral, geraniol and α-citral were also detected in the volatile oil of mature *Litsea mollis* fruit (Chen et al., 1984). Eucalyptol, linalool, α-terpineol, β-citral, geraniol, and α-citral were found in ILMFs by SPME in our previous work (Yang et al., 2020). Other 67 volatile compounds are detected in *Litsea mollis* fruit for the first time. These identified compounds are grouped as terpenoids, alcohols, esters and others for further discussion.

Terpenoids

Terpenoids including both terpene hydrocarbons and oxygenated terpenes are the major volatile compounds in ILMF, accounting for 84.8% and 78.8% of the total numbers of the free and bound volatiles, respectively. Terpenoids are secreted *via* the isoprenoid pathway in specific plant tissues (Wang et al., 2019a; Xiao et al., 2019). They are the most important aromatic compounds affecting the characteristics of fruits. There are abundant terpenoids in Lauraceae family (Niogret et al., 2013; Wang et al., 2019b). The nonvolatile precursors, especially the glycosidically bound ones, of terpenoids can be effectively hydrolyzed with enzyme to release the terpenoids (Swiegers & Pretorius, 2005).

A total number of 28 free terpene hydrocarbons are detected, and the contents of α-pinene, β-pinene, α-terpinene, α-terpinolene and 4-M-3-(1-M)-C are the highest (Table 1). The total free terpene hydrocarbons content of the ILMF harvested in FJ is the highest with the value of 89488.51 μg L^{-1}, followed by that of HB (80063.67 μg L^{-1}). The lowest is found in the ILMF of SC (32036.66 μg L^{-1}). Camphene and α,β-pinene, the terpenes widely found in plants, are the most important skeletons of naturally occurring bicyclic monoterpenes (Qiu et al., 2017). The ILMFs of GX contain the highest amount of sylvestrene with the value of 38045.93 μg L^{-1}. Caryophyllene is a sesquiterpene hydrocarbon mainly found in pepper and some spices (Goas et al., 1978). Other free sesquiterpene hydrocarbons are rarely reported and their contributions to the aroma of *Litsea mollis* is negligible, which may explain their absence in the ripe fruit of *Litsea mollis* (Chen et al., 1984).

Fourteen bound terpene hydrocarbons are identified in the ILMFs (Table 2), and 11 of them are shared by all ILMFs of the five regions. β-Myrcene, sylvestrene, γ-terpinene and α-terpinolene are the predominant bound terpene hydrocarbons. The bound terpene hydrocarbon content in the fruit of SC is the highest with the value of 22525.22 μg L^{-1}. Some sesquiterpenes are found in free form only, possibly because they are converted into other terpenoids during the hydrolysis. Further investigation on the formation and transformation mechanism of terpenoids in ILMFs is undergoing in our lab.

Oxygenated terpenes, mainly composed of alcohols, aldehydes and ketones, provide fruits many unique flavors (Stuart et al., 2001).

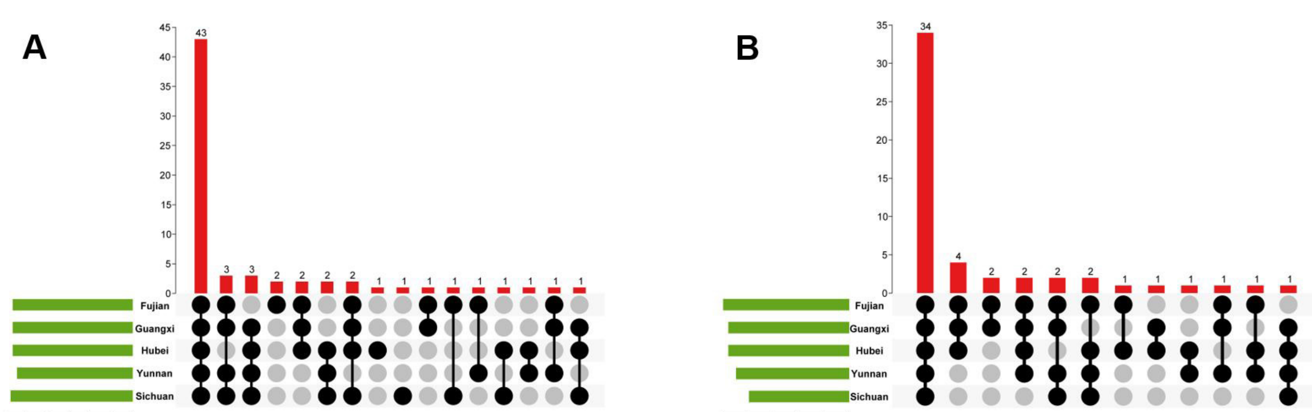

Figure 1. Common compounds in the ILMFs of five regions. (A) Upset plots of the free forms of odorants in the ILMFs of five regions; (B) Upset plot of the bound forms of odorants in the ILMFs of five regions. Green bars represent the number of volatile compounds identified in the ILMFs. Gray dots represent absence, while black dots represent the presence of the unique compounds. If more than one method contain the same unique compound, they are connected with lines. The number of the unique compounds presented individually or jointly is represented by red bars.

Table 1. Free volatile compounds detected in the ILMFs of five distinct regions of China (μg L^{-1}).

Nos.[a]	RI[b]	Compounds	ID[c]	Fujian	Guangxi	Hubei	Yunnan	Sichuan	Sig[d]
A1	856	3-Hexen-1-ol	B	54.39 ± 3.97	138.19 ± 22.45	134.40 ± 6.72	531.79 ± 20.27	220.40 ± 5.54	***
A2	908	2,6-D-3,5-H-2-O [e]	C	ND [f]	5125.13 ± 109.44	3832.37 ± 234.93	5572.82 ± 62.24	4150.30 ± 100.25	***
Alcohols				**54.39**	**5263.32**	**3966.76**	**6104.61**	**4370.70**	–
H4	927	α-Thujene	B	ND	ND	779.27 ± 3.12	ND	780.68 ± 5.35	***
H5	932	α-Pinene	A	15081.05 ± 782.44	7525.61 ± 207.03	8738.81 ± 358.68	8608.65 ± 356.54	6163.02 ± 287.53	*
H6	947	Camphene	A	6431.92 ± 164.73	4265.03 ± 102.68	4975.29 ± 805.53	1849.39 ± 108.70	3028.03 ± 135.04	**
H7	971	Sabinene	B	11963.48 ± 526.36	ND	ND	1919.15 ± 89.75	ND	***
H9	990	β-Pinene	B	9367.42 ± 766.38	6160.68 ± 141.22	22227.87 ± 1969.73	13730.93 ± 460.05	4984.88 ± 277.91	***
H11	1006	α-Phellandrene	A	ND	ND	14203.05 ± 1381.48	623.66 ± 20.20	ND	***
H12	1016	α-Terpinene	A	4965.40 ± 463.85	2709.60 ± 19.57	7319.30 ± 760.61	3890.80 ± 26.06	3155.98 ± 108.81	***
H13	1028	Sylvestrene	B	19998.38 ± 1047.06	38045.93 ± 1284.32	ND	11583.14 ± 694.23	ND	***
H14	1037	(E)-β-Ocimene	B	2622.37 ± 174.93	2812.69 ± 207.72	2950.91 ± 225.28	5311.72 ± 162.57	4279.45 ± 224.12	***
H16	1058	γ-Terpinene	A	3463.10 ± 572.84	1086.54 ± 1.79	2120.14 ± 179.55	1941.59 ± 21.96	1375.43 ± 27.46	***
H17	1085	α-Terpinolene	A	4285.81 ± 500.98	1860.98 ± 19.99	6496.17 ± 589.14	5597.02 ± 137.28	3039.00 ± 264.45	***
H18	1112	4-M-3-(1-M) C [g]	C	7670.04 ± 317.43	4425.41 ± 666.64	4048.38 ± 574.16	7658.84 ± 613.53	2118.97 ± 270.78	***
H20	1131	3-M-1,5,5-T [h]	C	1996.99 ± 108.36	1733.43 ± 47.20	4383.56 ± 327.73	2334.38 ± 115.84	1524.09 ± 108.90	***
H21	1390	β-Elemene	B	ND	ND	274.17 ± 5.89	352.03 ± 21.14	120.34 ± 2.74	***
H22	1422	Caryophyllene	A	209.27 ± 22.99	170.57 ± 25.64	268.32 ± 20.61	200.99 ± 19.59	147.06 ± 19.03	**
H23	1452	(Z)-β-Farnesene	B	196.79 ± 20.74	232.81 ± 34.46	116.39 ± 1.24	116.71 ± 1.83	107.57 ± 0.51	***
H24	1459	Humulene	A	137.12 ± 7.55	112.66 ± 2.64	114.95 ± 0.80	123.57 ± 3.42	112.50 ± 1.33	***
H25	1474	γ-Selinene	B	111.62 ± 1.09	117.00 ± 0.65	ND	109.76 ± 0.53	113.75 ± 0.95	***
H26	1482	Germacrene D	B	112.93 ± 1.88	111.50 ± 0.98	123.05 ± 0.39	123.93 ± 4.51	109.79 ± 1.06	***
H27	1491	β-Selinene	B	117.60 ± 2.79	118.70 ± 0.23	113.99 ± 1.33	119.82 ± 0.77	112.18 ± 1.04	**
H28	1495	Zingiberene	B	110.59 ± 1.22	117.59 ± 1.62	114.33 ± 1.38	111.14 ± 1.82	108.30 ± 0.42	***
H29	1497	α-Selinene	B	ND	108.40 ± 0.72	118.10 ± 1.44	113.74 ± 1.51	110.35 ± 0.30	***
H30	1504	α-Farnesene	A	122.37 ± 3.78	120.32 ± 0.69	108.36 ± 0.27	ND	107.38 ± 0.76	***
H31	1508	β-Bisabolene	B	138.00 ± 8.09	122.48 ± 6.90	114.44 ± 1.11	112.57 ± 1.18	107.69 ± 0.40	***
H32	1514	γ-Cadinene	B	113.18 ± 0.80	107.71 ± 0.24	115.27 ± 0.50	113.74 ± 2.15	107.21 ± 0.22	***
H33	1519	β-Cadinene	B	145.84 ± 10.76	114.04 ± 3.40	117.38 ± 0.95	116.67 ± 2.50	110.34 ± 0.39	***
H34	1522	α-Panasinsanene	B	ND	123.06 ± 8.06	122.16 ± 0.43	122.37 ± 3.91	112.68 ± 1.88	***
H35	1524	β-Sesquiphellandrene	B	127.25 ± 4.17	116.22 ± 3.69	ND	ND	ND	***
Terpene hydracarbons				**89488.51**	**72418.94**	**80063.67**	**66886.30**	**32036.66**	–
O36	1028	Eucalyptol	A	ND	ND	155474.32 ± 19356.96	16608.86 ± 2919.15	10197.25 ± 1278.60	***
O37	1099	Linalool	A	30241.82 ± 913.90	17769.48 ± 2652.07	18053.45 ± 1154.29	33305.34 ± 1177.75	26162.66 ± 1869.56	*
O39	1122	Chrysanthenone	B	2199.59 ± 73.20	1561.94 ± 18.28	1756.51 ± 21.31	1941.05 ± 59.29	1578.31 ± 10.02	***
O41	1152	Citronellal	A	4069.40 ± 376.95	2228.00 ± 83.50	5129.30 ± 221.68	3169.32 ± 327.60	5572.62 ± 161.93	***
O42	1154	(Z)-Verbenol	A	9545.83 ± 122.40	7549.77 ± 847.28	16554.63 ± 1474.87	13801.63 ± 1044.52	7693.93 ± 896.93	***
O44	1173	Borneol	A	13270.97 ± 381.32	4936.13 ± 676.29	4452.83 ± 584.95	13333.24 ± 1278.24	1247.48 ± 74.88	***
O46	1181	1,3,4-T-3-C-1-C [i]	B	15292.56 ± 2123.34	10988.88 ± 781.45	18229.39 ± 1429.26	19070.96 ± 1981.57	12818.69 ± 1014.47	**
O48	1198	α-Terpineol	A	31107.81 ± 2818.67	8726.71 ± 971.16	15359.84 ± 573.09	65267.71 ± 5096.07	33382.97 ± 5788.27	***
O49	1206	(E)-Carveol	A	515.31 ± 113.71	186.67 ± 30.26	149.42 ± 9.09	1241.72 ± 183.58	312.02 ± 42.24	***
O50	1215	Verbenone	B	1887.00 ± 74.58	1531.62 ± 105.34	1530.26 ± 22.58	1950.31 ± 206.53	1738.62 ± 197.49	*
O52	1228	(Z)-Carveol	A	614.96 ± 104.33	1995.59 ± 263.77	288.32 ± 17.74	1350.52 ± 194.75	148.43 ± 4.24	***
O53	1233	Nerol	A	27412.90 ± 2469.07	27740.57 ± 764.43	18428.53 ± 1985.58	11832.71 ± 1217.56	46894.77 ± 4555.18	***
O55	1244	β-Citral	A	220756.57 ± 14414.10	191590.32 ± 7604.93	268892.06 ± 17592.10	250397.49 ± 14811.45	135043.10 ± 8479.75	***
O56	1268	Geraniol	A	80037.17 ± 5258.47	41901.49 ± 2862.81	234708.52 ± 21092.55	198710.28 ± 16931.30	140769.68 ± 5359.06	***
O57	1277	α-Citral	A	692011.89 ± 57541.98	404244.26 ± 63537.69	575005.80 ± 43352.25	483946.06 ± 21300.77	302333.20 ± 4609.95	***
O59	1288	Lavandulal	C	1749.14 ± 76.12	1341.55 ± 20.75	3350.70 ± 226.00	1865.15 ± 41.07	1440.79 ± 22.65	***
O60	1296	p-M-1(7),8(10)-D-9-O [j]	C	499.77 ± 21.53	553.82 ± 19.51	ND	353.64 ± 19.07	326.59 ± 35.23	***
O61	1299	Perilla alcohol	A	382.10 ± 17.75	525.00 ± 115.44	1155.24 ± 152.95	684.46 ± 9.09	860.85 ± 43.68	***
O62	1318	L-Perillaldehyde	A	330.02 ± 15.58	171.01 ± 5.26	108.53 ± 10.07	71.50 ± 10.36	59.70 ± 14.09	***
O63	1321	Methyl geranate	B	ND	ND	186.19 ± 23.43	ND	ND	***
O65	1347	α-Terpinyl acetate	B	399.75 ± 56.78	ND	ND	ND	ND	***
O66	1354	Geranic acid	A	ND	ND	ND	ND	744.31 ± 59.22	***
O67	1367	Nerol acetate	A	225.20 ± 53.88	ND	ND	ND	ND	***
O68	1392	2-E-6-M-3,5-H [k]	B	2767.67 ± 223.66	30.61 ± 0.44	174.65 ± 24.67	24.91 ± 2.12	154.39 ± 20.43	*
O69	1560	Nerolidol	A	63.48 ± 8.41	69.38 ± 15.59	68.28 ± 6.44	21.02 ± 2.62	14.86 ± 0.61	**
O70	1580	Caryophyllene oxide	A	16.90 ± 1.62	28.09 ± 4.06	76.67 ± 5.29	ND	ND	***

Table 1. Continued...

Nos.[a]	RI[b]	Compounds	ID[c]	Fujian	Guangxi	Hubei	Yunnan	Sichuan	Sig[d]
O71	1595	Cedrol	B	19.73 ± 0.90	19.79 ± 2.72	18.41 ± 0.61	ND	ND	***
O72	1659	Juniper camphor	B	59.68 ± 9.73	58.42 ± 9.10	129.15 ± 10.66	ND	34.64 ± 5.25	***
Oxygenated monoterpene				**1135477.25**	**725749.10**	**1339280.99**	**1118947.88**	**729529.84**	–
E73	1192	Methyl salicylate	A	6866.53 ± 163.72	2000.39 ± 319.13	3180.77 ± 68.43	9948.81 ± 493.58	3401.67 ± 605.50	***
E74	1333	Methyl o-anisate	A	478.79 ± 61.34	104.75 ± 3.67	195.51 ± 32.32	341.58 ± 65.59	119.33 ± 25.15	***
E75	1385	Methyl cinnamate	A	252.59 ± 65.05	331.98 ± 17.95	ND	2629.33 ± 315.69	90.89 ± 14.62	***
Esters				**7597.90**	**2437.11**	**3376.28**	**12919.72**	**3611.89**	–
T76	<800	2,5-Dihydrotoluene	C	1323.69 ± 40.33	893.73 ± 52.52	929.71 ± 26.99	897.08 ± 84.74	907.11 ± 26.36	***
T77	886	2,6-D-1,5-H [l]	B	2308.02 ± 196.65	5194.48 ± 1159.29	5418.70 ± 570.53	5795.73 ± 41.64	1814.03 ± 54.39	***
T79	985	Sulcatone	A	ND	1718.74 ± 130.19	14293.04 ± 1038.42	ND	1858.66 ± 68.49	***
T80	1090	p-Cymenene	A	3625.01 ± 484.14	2364.51 ± 338.63	10991.11 ± 613.30	3209.60 ± 498.54	1668.70 ± 296.17	***
T82	1446	(E)-Isoeugenol	A	114.97 ± 20.21	ND	ND	ND	52.71 ± 3.31	***
Others				**7371.71**	**10171.45**	**31632.56**	**9902.40**	**6301.21**	–

[a]A-Alcohols, H-Terpenehydracarbons, O-Oxygenated terpenes, E-Esters, T-Others; [b]Retention indices; cReliability of the identification proposal: A, identified, mass spectrum, LRI agreed with literature data (National Institute of Standards and Technology, 2018) and LRI agreed with standards; [B], tentatively identified, mass spectrum agreed with the mass spectral database and LRI agreed with literature data (National Institute of Standards and Technology, 2018); [C], tentatively identified, mass spectrum agreed with the mass spectral database; [d]Sig: Statistical significance; *Significant at $p<0.05$; **Significant at $p<0.01$; ***Significant at $p<0.001$, ns: no significant ($p>0.05$); [e]2,6-D-3,5-H-2-O represents the compound of 2,6-dimethyl-3,5-heptadien-2-ol; [f]Not detected; [g]4-M-3-(1-M) C represents the compound of 4-methyl-3-(1-methylethylidene)cyclohexene; [h]3-M-1,5,5-T represents the compound of 3-methylene-1,5,5-trimethylcyclohexene; [i]1,3,4-T-3-C-1-C represents the compound of 1,3,4-trimethyl-3-cyclohexen-1-carboxaldehyde; [j]p-M-1(7),8(10)-D-9-O represents the compound of p-mentha-1(7),8(10)-dien-9-ol; [k]2-E-6-M-3,5-H represents the compound of 2-ethylidene-6-methyl-3,5-heptadienal; [l]2,6-D-1,5-H represents the compound of 2,6-dimethyl-1,5-heptadiene.

Table 2. Bound volatile compounds found in the ILMFs of five distinct regions of China (μg L^{-1}).

Nos.[a]	RI [b]	Compounds	ID[c]	Fujian	Guangxi	Hubei	Yunnan	Sichuan	Sig[d]
A1	856	3-Hexen-1-ol	B	23.22 ± 1.41	32.42 ± 2.56	51.92 ± 1.98	42.80 ± 3.94	ND [e]	***
A3	1111	Phenethanol	A	3376.61 ± 589.00	1704.99 ± 93.87	2157.13 ± 164.54	2561.34 ± 197.11	2149.66 ± 210.25	**
Alcohols				**3399.82**	**1737.41**	**2209.05**	**2604.14**	**2149.66**	–
H6	947	Camphene	A	50.47 ± 7.65	84.36 ± 6.04	11.42 ± 1.29	34.06 ± 4.44	99.01 ± 5.16	***
H8	986	α-Myrcene	B	40.06 ± 5.96	44.74 ± 3.26	165.38 ± 13.94	167.48 ± 20.84	304.54 ± 53.22	***
H10	991	β-Myrcene	A	2079.55 ± 243.35	2547.70 ± 181.60	3418.26 ± 327.19	3636.85 ± 411.73	5841.88 ± 660.54	***
H11	1006	α-Phellandrene	A	336.82 ± 30.72	471.07 ± 24.30	445.74 ± 22.25	529.05 ± 50.03	664.70 ± 63.59	***
H12	1016	α-Terpinene	A	633.19 ± 33.98	845.26 ± 58.20	993.24 ± 103.08	1076.81 ± 125.09	1549.18 ± 176.84	***
H13	1028	Sylvestrene	B	796.38 ± 99.09	2087.43 ± 87.46	2054.42 ± 154.27	4532.16 ± 484.39	5733.13 ± 558.04	***
H14	1037	(E)-β-Ocimene	B	414.42 ± 45.45	841.87 ± 42.79	536.46 ± 52.68	1272.24 ± 153.30	1610.46 ± 167.60	***
H15	1047	(Z)-β-Ocimene	B	690.31 ± 74.63	1375.89 ± 121.03	1130.98 ± 65.37	1305.19 ± 153.69	3344.49 ± 360.19	***
H16	1058	γ-Terpinene	A	1376.87 ± 103.89	1022.65 ± 39.48	1732.48 ± 279.44	1096.33 ± 125.50	994.57 ± 107.61	**
H17	1085	α-Terpinolene	A	714.10 ± 69.94	1295.32 ± 66.48	1058.56 ± 88.80	1676.15 ± 186.17	2033.15 ± 190.86	***
H19	1128	allo-Ocimene	A	155.29 ± 28.65	131.29 ± 12.44	215.21 ± 19.43	215.90 ± 28.41	338.24 ± 45.09	***
H22	1422	Caryophyllene	A	8.07 ± 0.93	ND	14.94 ± 1.46	23.96 ± 6.62	11.85 ± 2.04	***
H23	1452	(Z)-β-Farnesene	B	36.16 ± 6.04	17.11 ± 3.28	3.29 ± 0.53	ND	ND	***
H24	1459	Humulene	A	ND	ND	15.45 ± 1.62	29.48 ± 7.59	ND	***
Terpene hydracarbons				**7331.71**	**10764.69**	**11795.83**	**15595.67**	**22525.22**	–
O37	1099	Linalool	A	85.04 ± 5.02	78.46 ± 2.36	112.68 ± 6.44	75.98 ± 6.02	129.11 ± 9.73	***
O38	1120	(Z)-p-2,8-Menthadien-1-ol	B	9.44 ± 0.71	15.06 ± 0.20	8.03 ± 0.29	11.18 ± 0.88	14.86 ± 0.70	***
O40	1151	Nerol oxide	B	121.35 ± 6.08	117.18 ± 9.33	745.36 ± 91.09	ND	ND	***
O41	1152	Citronellal	A	229.83 ± 17.08	218.12 ± 26.20	1982.50 ± 352.45	314.36 ± 41.53	313.86 ± 29.90	***
O42	1154	(Z)-Verbenol	A	147.57 ± 24.41	15.60 ± 2.02	ND	80.63 ± 11.74	38.57 ± 3.93	***
O43	1162	Pinocarvone	B	320.65 ± 62.67	38.42 ± 0.85	75.32 ± 8.62	ND	ND	***
O44	1173	Borneol	A	2304.02 ± 197.03	2354.00 ± 278.98	1932.86 ± 217.31	1607.55 ± 146.89	1833.93 ± 110.94	*
O45	1180	4-Terpineol	A	613.49 ± 75.32	328.35 ± 13.93	292.40 ± 23.01	254.10 ± 8.93	246.44 ± 4.90	***
O47	1186	3,9-Epoxy-1-p-menthene	B	109.00 ± 6.13	57.08 ± 2.35	43.55 ± 0.21	68.77 ± 2.09	ND	***
O48	1198	α-Terpineol	A	490.72 ± 43.54	513.80 ± 59.08	749.72 ± 102.77	296.59 ± 28.35	474.35 ± 36.65	***
O49	1206	(E)-Carveol	A	82.43 ± 11.88	170.31 ± 8.66	ND	ND	ND	***
O51	1219	p-Menth-1-en-9-al	B	1185.42 ± 77.94	498.02 ± 46.03	1924.39 ± 262.25	288.27 ± 18.06	339.91 ± 59.71	***
O52	1228	(Z)-Carveol	A	46.04 ± 0.94	44.11 ± 6.03	ND	ND	ND	***
O53	1233	Nerol	A	1188.39 ± 52.83	612.35 ± 5.57	825.21 ± 99.94	842.40 ± 69.77	3737.01 ± 231.89	***
O54	1237	2-Hydroxycineol	B	43.72 ± 5.40	ND	21.30 ± 0.78	47.32 ± 1.92	ND	***
O55	1244	β-Citral	A	1436.22 ± 159.82	144.82 ± 2.55	193.62 ± 14.69	875.16 ± 1140.35	1436.57 ± 158.83	***
O56	1268	Geraniol	A	2886.74 ± 95.24	3962.00 ± 543.32	5033.78 ± 43.12	5244.25 ± 761.23	19398.16 ± 2427.27	***

Table 2. Continued...

Nos.[a]	RI [b]	Compounds	ID[c]	Fujian	Guangxi	Hubei	Yunnan	Sichuan	Sig[d]
O57	1277	α-Citral	A	813.31 ± 106.83	1200.86 ± 104.72	358.29 ± 20.75	5187.31 ± 651.11	4059.58 ± 478.43	***
O58	1279	Phellandral	B	48.70 ± 1.19	ND	70.96 ± 15.19	37.68 ± 4.16	191.86 ± 13.52	***
O60	1296	p-M-1(7),8(10)-D-9-O [f]	C	591.06 ± 54.50	538.13 ± 48.36	186.98 ± 21.89	393.98 ± 36.80	387.02 ± 47.48	***
O61	1299	Perilla alcohol	A	58.12 ± 3.22	26.55 ± 3.87	33.72 ± 2.45	33.18 ± 1.76	47.80 ± 3.45	***
O63	1321	Methyl geranate	B	1253.60 ± 183.20	939.75 ± 115.87	473.06 ± 4.73	428.06 ± 51.85	393.47 ± 80.23	**
O64	1331	p-Mentha-1,4-dien-7-ol	B	182.23 ± 15.00	23.95 ± 0.88	29.72 ± 5.16	22.59 ± 0.81	34.92 ± 2.40	***
O65	1347	α-Terpinyl acetate	B	136.12 ± 15.77	35.90 ± 3.47	41.57 ± 6.31	ND	ND	***
O66	1354	Geranic acid	A	902.51 ± 110.65	46.94 ± 0.81	30.49 ± 1.70	36.24 ± 1.53	28.44 ± 0.57	***
O67	1367	Nerol acetate	A	112.33 ± 10.62	ND	34.16 ± 1.70	ND	ND	***
O68	1392	2-E-6-M-3,5-H [g]	B	62.58 ± 4.48	10.24 ± 0.49	5.92 ± 0.21	29.57 ± 2.38	28.87 ± 2.08	***
Oxygenated terpenes				**15460.63**	**11990.00**	**15205.59**	**16175.17**	**33134.74**	–
E73	1192	Methyl salicylate	A	2027.92 ± 408.25	2758.99 ± 145.34	1212.37 ± 307.04	2411.68 ± 302.71	2253.22 ± 91.40	**
E74	1333	Methyl o-anisate	A	171.10 ± 6.76	39.73 ± 4.42	24.72 ± 3.28	25.59 ± 2.14	23.97 ± 2.27	***
E75	1385	Methyl cinnamate	A	2125.60 ± 243.98	110.18 ± 1.38	184.89 ± 28.60	982.44 ± 155.18	410.07 ± 77.46	***
Esters				**4324.62**	**2908.90**	**1421.98**	**3419.71**	**2687.27**	–
T77	886	2,6-D-1,5-H [h]	B	127.83 ± 9.17	68.90 ± 1.73	ND	127.01 ± 6.92	65.66 ± 0.29	***
T78	964	Benzaldehyde	A	60.71 ± 12.45	4.28 ± 0.75	ND	15.45 ± 1.65	ND	***
T79	985	Sulcatone	A	ND	0.39 ± 0.00	0.39 ± 0.00	ND	ND	**
T80	1090	p-Cymenene	A	260.75 ± 44.39	244.72 ± 17.56	181.15 ± 17.35	258.48 ± 37.74	264.56 ± 20.41	ns
T81	1352	Eugenol	A	ND	105.26 ± 15.12	333.52 ± 19.12	998.14 ± 119.42	939.70 ± 219.04	***
T82	1446	(E)-Isoeugenol	A	73.58 ± 7.71	129.07 ± 23.45	153.01 ± 19.91	248.57 ± 27.11	386.90 ± 63.99	***
Others				**522.87**	**552.62**	**668.06**	**1647.65**	**1656.82**	–

[a]A-Alcohols, H-Terpenehydracarbons, O-Oxygenated terpenes, E-Esters, T-Others; [b]Retention indices; cReliability of the identification proposal: A, identified, mass spectrum, LRI agreed with literature data (National Institute of Standards and Technology, 2018) and LRI agreed with standards; [B], tentatively identified, mass spectrum agreed with the mass spectral database and LRI agreed with literature data (National Institute of Standards and Technology, 2018); [C], tentatively identified, mass spectrum agreed with the mass spectral database; [d]Sig: Statistical significance; *Significant at $p< 0.05$; **Significant at $p< 0.01$; ***Significant at $p< 0.001$, ns: no significant ($p> 0.05$); [e]Not detected; [f]p-M-1(7),8(10)-D-9-O represents the compound of p-mentha-1(7),8(10)-dien-9-ol; [g]2-E-6-M-3,5-H represents the compound of 2-ethylidene-6-methyl-3,5-heptadienal; [h]2,6-D-1,5-H represents the compound of 2,6-dimethyl-1,5-heptadiene.

In addition, they can show better antimicrobial activities than hydrocarbons (Guimarães et al., 2019). A total number of 37 free and bound oxygenated terpenes are found in the ILMFs, which include 28 free forms dominated by linalool, 1,3,4-T-3-C-1-C, α-terpineol, nerol, β-citral, geraniol and α-citral. Linalool is an important fruity and floral terpenoid that is usually produced by hydrolysis, redox reactions, and rearrangement (Davis & Croteau, 2000; Qian & Wang, 2005). Citral is the most abundant compound among these free oxygenated terpenes, accordant with the results reported in our previous study (Yang et al., 2020). The total content of α-citral and β-citral accounts for 60-82% of the total free oxygenated terpenes for all the fruits from different regions. Citral possesses excellent antibacterial activities, and has been used in preservatives, soap and cosmetics industries (Onawunmi, 2008). It is worth noting that free eucalyptol is only detected in the fruits of HB, YN and SC, with the highest content of 155474.32 μg L^{-1} in the ILMF of HB. Its absence in the ILMFs of FJ and GX may be explained with their geographic locations. HB, YN and SC are in the southwestern China, and FJ and GX are located in the southern China.

All bound oxygenated terpenes, except for borneol and geraniol, are present in the ILMFs at low levels. The highest total content of bound oxygenated terpenes is found in the ILMF of SC with the value of 33134.74 μg L^{-1}, which is mainly attributed to the high contents of nerol, geraniol and α-citral. Releasing the flavor compounds is generally positive for the overall aroma, as most odoriferous terpene alcohols, such as geraniol, nerol, citronellol and linalool, can enhance floral aroma (Dziadas & Jelen, 2016).

Eighteen oxygenated terpenes including linalool, citronellal, (Z)-verbenol and borneol are found in both free and bound forms. α-Terpineol is a monoterpene alcohol with fruity aroma, and has been extensively reported in fruits and vegetables (Sharma et al., 2010). Bornol is a reduction product of camphor, which emits a peppery and peppermint odor. Geraniol can contribute notably to the aroma of ILMF, yet only trace amount found in the volatile oil of mature *Litsea mollis* fruit (Chen et al., 1984). It has also been found in lychee (Chyau et al., 2003), kiwifruit (Garcia et al., 2011), black velvet tamarind (Lasekan & See, 2015) and other fruits.

Alcohols and esters

Three alcohols including 3-hexen-1-ol, 2,6-D-3,5-H-2-O and phenethanol are identified in the ILMFs. 3-Hexen-1-ol is an important C6 compound. It has also been found in the Spanish cultivars of pomegranate (Melgarejo et al., 2011). It is both in free and bound forms in all ILMFs, except for those of SC in the bound form. 2,6-D-3,5-H-2-O is present in all ILMFs only in the free form at very high contents ranging from 3832.37–5572.82 μg L^{-1}.

Esters are the major contributors of the fruity and sweet odor notes of fruits and vegetables (Wu et al., 2016). They can be produced by the enzymatic reactions of alcohols and acyl CoA′s derived from the fatty acid and amino acid metabolism (Wyllie & Fellman, 2000). Three esters including methyl salicylate, methyl o-anisate and methyl cinnamate are detected in the ILMFs, which are not reported in the volatile oil of mature *Litsea mollis* fruit (Chen et al., 1984). The content of free ester methyl salicylate varies with the geographic origin of the fruit, with the highest value of 9948.81 μg L^{-1} (YN). The bound forms of the esters are also found in all the ILMFs with the highest total content in the ILMFs of FJ (4324.62 μg L^{-1}) and the lowest in the ILMFs harvested in HB (1421.98 μg L^{-1}).

Others

Seven compounds including three norisoprenoids, two phenols, one aldehyde and one aromatic hydrocarbon are classified as other compounds. They are detected in *Litsea mollis* fruit for first time (Chen et al., 1984). The content of 2,6-D-1,5-H ranges from 1814.03 μg L^{-1} to 5795.73 μg L^{-1} for the free form and the highest content is only 127.83 μg L^{-1} for the bound form. Sulcatone is an oxidation byproduct or degradation product of lycopene, α-farnesene, citral, or conjugated trienols (Liu et al., 2018). It has also been detected in apple (Whitaker & Saftner, 2000). High contents of 2,5-dihydrotoluene are present in all ILMFs, but in the free form only. High contents of bound eugenol are found in the ILMFs of YN (998.14 μg L^{-1}) and SC (939.70 μg L^{-1}). The contents of free form of (E)-isoeugenol are low, but high amounts of bound form are found in the ILMFs of SC, suggesting that glucoside bound eugenol and isoeugenol can be hydrolyzed into their free forms with pectinase, and the natural ILMF lacks hydrolysis enzymes of the bound eugenol and isoeugenol. Benzaldehyde is in bound forms in the ILMFs. However, these volatiles account for a very small proportion of the total volatile.

3.2 Odor profiles of ILMFs of different regions

OAV is an important parameter evaluating the contribution of a volatile compound to food odor. Table 3 lists the OAVs, odor descriptions and aromatic series of the volatile compounds found in the ILMFs. The overall odors are classified into six categories including fruity, floral, sweet, herbaceous, woody and chemical. Thirty-three volatiles found in the ILMFs exhibit OAVs greater than 1, and thus are considered as the odor contributors. The bound aroma compounds can contribute to the odor of ILMFs only after they are released by enzymes or acids, thus indirectly.

Terpene hydrocarbons and oxygenated terpenes, such as α-pinene, eucalyptol, linalool, α,β-citral, geraniol and citronellal, are the major free volatiles contributing to the odor of ILMF. The contributions of the alcohols are negligible due to the high odor threshold of 3-hexen-1-ol. α,β-Citral and citronellal enhance the fruity aroma, and the floral scent is mainly attributed to geraniol. Linalool, commonly found in citrus fruit, flowers and spice plants, is responsible for the pleasant fruity and floral odor notes of ILMF due to its low odor threshold in water (Buettner & Schieberle, 2001; Elmaci & Altug, 2005). The herbaceous odor mainly originates from eucalyptol and α-pinene, especially in the ILMFs of HB where eucalyptol displays the highest OAV of 2429.29. The odors completely opposite to the odor of ILMF, such as muddy and oily odors, are grouped as "chemical" odor, which also significantly affect fruit odor. Although there are abundant γ-terpinene and α-terpineol in all ILMFs samples, they make no significant contributes to the fruit odor because of their high odor thresholds. The bound aroma compounds that contribute to the odor of ILMF mainly include β-myrcene (OAV, 57.77–162.27), α-citral (OAV, 11.20–126.86), geraniol (OAV, 72.17–484.95), citronellal (OAV, 36.35–330.42) and eugenol (OAV, 17.54–166.36).

The odor vectors of the free and bound aroma compounds are plotted based on their aromatic series and the sum of their OAVs in natural logarithmic scale (Figure 2). It is found that the overall odor profiles of the free aroma compounds detected in the ILMFs of the five regions are similar, predominated by floral and fruity odors that are usually derived from the free volatiles rather than the bound volatiles, followed by herbaceous odor (Figure 2A). The effects of sweet and woody odors on the ILMF aroma are also similar. Most aroma characteristics of the ILMFs of HB are more pronounced than those of other regions. The bound volatiles also exhibit floral, fruity and herbaceous odor profiles, but with stronger sweet and woody odors than those of free forms (Figure 2B). It explains that the sweet and woody odor of ILMF juice becomes stronger after the enzymatic hydrolysis. All odors of the bound volatiles, except for the fruity odor, of the ILMFs collected in SC are notably stronger than those of other regions, which is closely related to the highest altitude, less sunshine and low temperature in SC (Table S1). The herbaceous, sweet, woody and floral odors are more evident in the ILMFs of YN and SC. It is worth noting that the OAVs of the bound aroma compounds with chemical odor is very low or even negligible, and thus enzyme or acid hydrolysis does not increase chemical odor.

3.3 Multivariate statistical analysis of free and bound volatile compounds

PCA

To better understand the similarity of the ILMFs from five distinct regions of China, PCA analysis was conducted using 66 free and 52 bound volatile compounds found in the ILMFs as the variables for dimensionality reduction without losing much information (Shin et al., 2010. Figure 3).

Figure 3A shows the first two principal components (PC1 and PC2) of the free volatile compounds respectively with 63.82% and 35.18% of data variances. The score distributions in PC1 and PC2 divide the ILMFs of the five regions into two groups. The five regions are all positioned at the right side of the loading plot, indicating that their high scores in PC1. FJ and GX are positively correlated to PC2, suggesting that the similarity of the ILMFs in these two regions is high. The free volatile compounds of the samples harvested in SC, YN and HB are close to each other and are negatively correlated to PC2, suggesting that their free volatile profiles are similar.

The bound volatile compounds are also positively correlated to PC1 (Figure 3B), and those of YN and SC are still on the PC2 negative axis. However, unlike the free volatile compounds, the

Table 3. Odor description and odor activity values (OAVs) of most potent volatiles in the ILMFs of five distinct regions of China.

Compounds	Odor description[a]	Aromatic series [a]	OT (μg L^{-1}) [a]	OAVs of free-form [b]					OAVs of bound-form[b]				
				Fujian	Guangxi	Hubei	Yunnan	Sichuan	Fujian	Guangxi	Hubei	Yunnan	Sichuan
3-Hexen-1-ol	Green, leafy	Herbaceous	1630	0.03	0.08	0.08	0.33	0.14	0.01	0.02	0.03	0.03	– [c]
Phenethanol	Rose-like, honey	Floral	1100	–	–	–	–	–	3.07	1.55	1.96	2.33	1.95
Alcohols				**0.03**	**0.08**	**0.08**	**0.33**	**0.14**	**3.08**	**1.57**	**1.99**	**2.35**	**1.95**
α-Phellandrene	Sweet, rose-like	Sweet, floral	40	–	–	355.08	15.59	–	8.42	11.78	11.14	13.23	16.62
α-Terpinene	Woody, citrus, lemon	Fruity, woody	80	62.07	33.87	91.49	48.64	39.45	7.91	10.57	12.42	13.46	19.36
β-Myrcene	Green	Herbaceous	36	–	–	–	–	–	57.77	70.77	94.95	101.02	162.27
γ-Terpinene	Citrus	Fruity	1000	3.46	1.09	2.12	1.94	1.38	1.38	1.02	1.73	1.10	0.99
Terpinolene	Woody, lemon, lime-like	Fruity, woody	200	21.43	9.30	32.48	27.99	15.19	3.57	6.48	5.29	8.38	10.17
α-Pinene	Camphor, pine	Herbaceous	6	2513.51	1254.27	1456.47	1434.78	1027.17	–	–	–	–	–
Sabinen	Woody, spicy, camphoreous	Chemical	980	12.21	–	–	1.96	–	–	–	–	–	–
β-Pinene	Green, woody	Herbaceous, woody	140	66.91	44.00	158.77	98.08	35.61	–	–	–	–	–
Caryophyllene	Sweet, woody	Sweet, woody	64	3.27	2.67	4.19	3.14	2.30	0.13	–	0.23	0.37	0.19
Humulene	Woody	Woody	160	0.86	0.70	0.72	0.77	0.70	–	–	0.10	0.18	–
Terpene hydracarbons				**2683.72**	**1345.90**	**2101.32**	**1632.89**	**1121.80**	**79.18**	**100.62**	**125.86**	**137.74**	**209.60**
Eucalyptol	Eucalyptus, herbal	Herbaceous	64	–	–	2429.29	259.51	159.33	–	–	–	–	–
(E)-Ocimene	Sweet, herbal	Sweet, herbaceous	34	77.13	82.73	86.79	156.23	125.87	12.19	24.76	15.78	37.42	47.37
(Z)-Ocimene	Floral	Floral	55	–	–	–	–	–	12.55	25.02	20.56	23.73	60.81
Linalool	Citrus, floral, grape-like	Floral, fruity	6	5040.30	2961.58	3008.91	5550.89	4360.44	14.17	13.08	18.78	12.66	21.52
Nerol oxide	Oil, flower	Floral	3000	–	–	–	–	–	0.04	0.04	0.25	–	–
β-Citral	Lemon	Fruity	53	4165.22	3614.91	5073.44	4724.48	2547.98	27.10	2.73	3.65	16.51	27.11
α-Citral	Citrus, lemon	Fruity	32	21625.37	12632.63	17968.93	15123.31	9447.91	25.42	37.53	11.20	162.10	126.86
α-Terpineol	Oil, anise, mint	Chemical	330	94.27	26.44	46.54	197.78	101.16	1.49	1.56	2.27	0.90	1.44
(E)-Carveol	Carawa, spearmint	Herbaceous, woody	250	2.06	0.75	0.60	4.97	1.25	0.33	0.68	–	–	–
(Z)-Carveol	Caraway	Woody	250	2.46	7.98	1.15	5.40	0.59	0.18	0.18	–	–	–
Nerol	Grass, floral	Floral, herbaceous	300	91.38	92.47	61.43	39.44	156.32	3.96	2.04	2.75	2.81	12.46
Geraniol	Rose-like, citrus	Floral	40	2000.93	1047.54	5867.71	4967.76	3519.24	72.17	99.05	125.84	131.11	484.95
Perilla alcohol	Green, fatty	Herbaceous,chemical	7000	0.05	0.08	0.17	0.10	0.12	0.01	–	–	–	0.01
Citronellal	Rose, citrus, lemon	Fruity	6	678.23	371.33	854.88	528.22	928.77	38.31	36.35	330.42	52.39	52.31
Borneol	Camphor	Herbaceous	140	94.79	35.26	31.81	95.24	8.91	16.46	16.81	13.81	11.48	13.10
4-Terpineol	Pepper, woody, musty	Chemical, woody	130	–	–	–	–	–	4.72	2.53	2.25	1.95	1.90
Caryophyllene oxide	Woody, sweet	Woody, sweet	410	0.04	0.07	0.19	–	–	–	–	–	–	–
Nerolidol	Green, floral	Herbaceous, floral	15	4.23	4.63	4.55	1.40	0.99	–	–	–	–	–
Cedrol	Cool, camphor	Herbaceous	0.5	39.46	39.58	36.81	–	–	–	–	–	–	–
L-Perillaldehyde	Oil, mint, cherry	Chemical, fruity	30	11.00	5.70	3.62	2.38	1.99	–	–	–	–	–
α-Terpinyl acetate	Citrus, spicy, floral	Floral, chemical	2500	0.16	–	–	–	–	0.05	0.01	0.02	–	–
Geranic acid	Green	Herbaceous	40	–	–	–	–	18.61	22.56	1.17	0.76	0.91	0.71
Nerol acetate	Rose, citrus, pear	Fruity	2000	0.11	–	–	–	–	0.06	–	0.02	–	–
Oxygenated terpenes				**33927.19**	**20923.68**	**35476.82**	**31657.11**	**21379.49**	**251.76**	**263.54**	**548.35**	**453.98**	**850.53**
Methyl salicylate	Green, pine	Herbaceous	40	171.66	50.01	79.52	248.72	85.04	50.70	68.97	30.31	60.29	56.33

Table 3. Continued...

Compounds	Odor description[a]	Aromatic series [a]	OT ($\mu g\ L^{-1}$) [a]	OAVs of free-form [b]					OAVs of bound-form[b]				
				Fujian	Guangxi	Hubei	Yunnan	Sichuan	Fujian	Guangxi	Hubei	Yunnan	Sichuan
Esters				**171.66**	**50.01**	**79.52**	**248.72**	**85.04**	**50.70**	**68.97**	**30.34**	**60.29**	**56.33**
Benzaldehyde	Sweet, oily, almond	Sweet, chemical	350	–	–	–	–	–	0.17	0.01	–	0.04	–
Sulcatone	Citrus, green, apple	Fruity, herbaceous	50	–	34.37	285.86	–	37.17	–	0.01	0.01	–	–
p-Cymenene	Spicy, musty, guaiacol-like	Chemical	11.4	317.98	207.41	964.13	281.54	146.38	22.87	21.47	15.89	22.67	23.21
Eugenol	Sweet, woody	Sweet, woody	6	–	–	–	–	–	–	17.54	55.59	166.36	156.62
(E)-Isoeugenol	Floral	Floral	6	19.16	–	–	–	8.78	12.26	21.51	25.50	41.43	64.48
Others				**337.15**	**241.79**	**1249.99**	**281.54**	**192.33**	**35.31**	**60.54**	**96.99**	**230.50**	**244.31**

[a]Odor description, aromatic series and OT (odor threshold in water) were obtained from literature data (Fenoll et al., 2009; Javed et al., 2018; Leffingwell & Associates, 2020; Pino & Mesa, 2006; Van Gemert, 2011; Wu et al., 2016); [b]OAVs, ratio of the concentration of a molecule to its odor threshold; [c]Not detected.

hydrolyzed bound volatiles in the ILMFs of HB are positively correlated with PC2, similar to the bound volatile profile of the ILMFs in GX. The bound volatile compounds found in the ILMF of FJ exhibit high scores in positive PC2. Phenethanol (A3), (Z)-verbenol (O42), p-M-1(7),8(10)-D-9-O (O60), p-mentha-1,4-dien-7-ol (O64), geranic acid (O66), methyl o-anisate (E74), methyl cinnamate (E75) and benzaldehyde (T78) are positively related to FJ, indicating that these compounds are abundant in the ILMFs of FJ. Unlike the free volatile profile, the bound aroma profile is mainly correlated to oxygenated terpenes (Figure 3B).

Cluster analysis

To further distinguish the free and bound volatile compounds of the ILMFs from different geological regions, all the identified volatile compounds were subjected to hierarchical clustering.

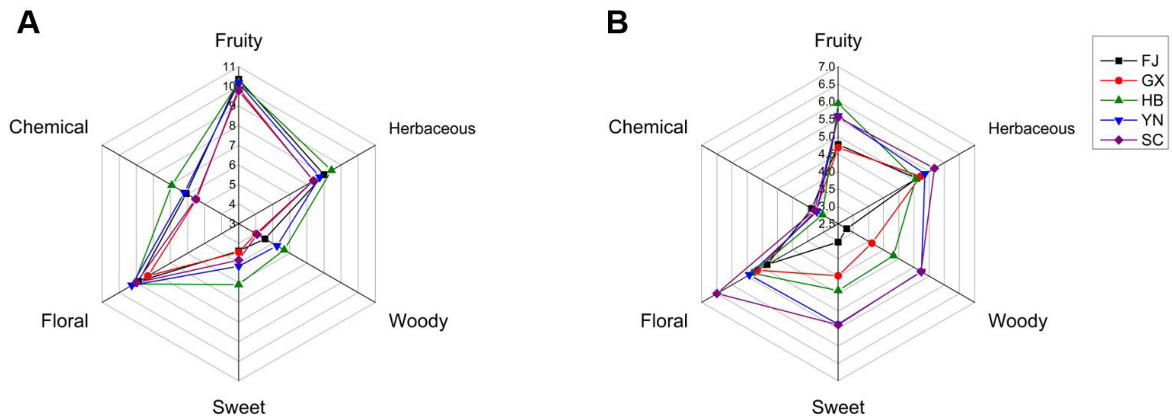

Figure 2. Aromatic plots of free (A) and bound (B) aroma compounds according to its odor activity values (in natural logarithmic scale). The colors of lines represent different odor profiles of the ILMFs in the five regions.

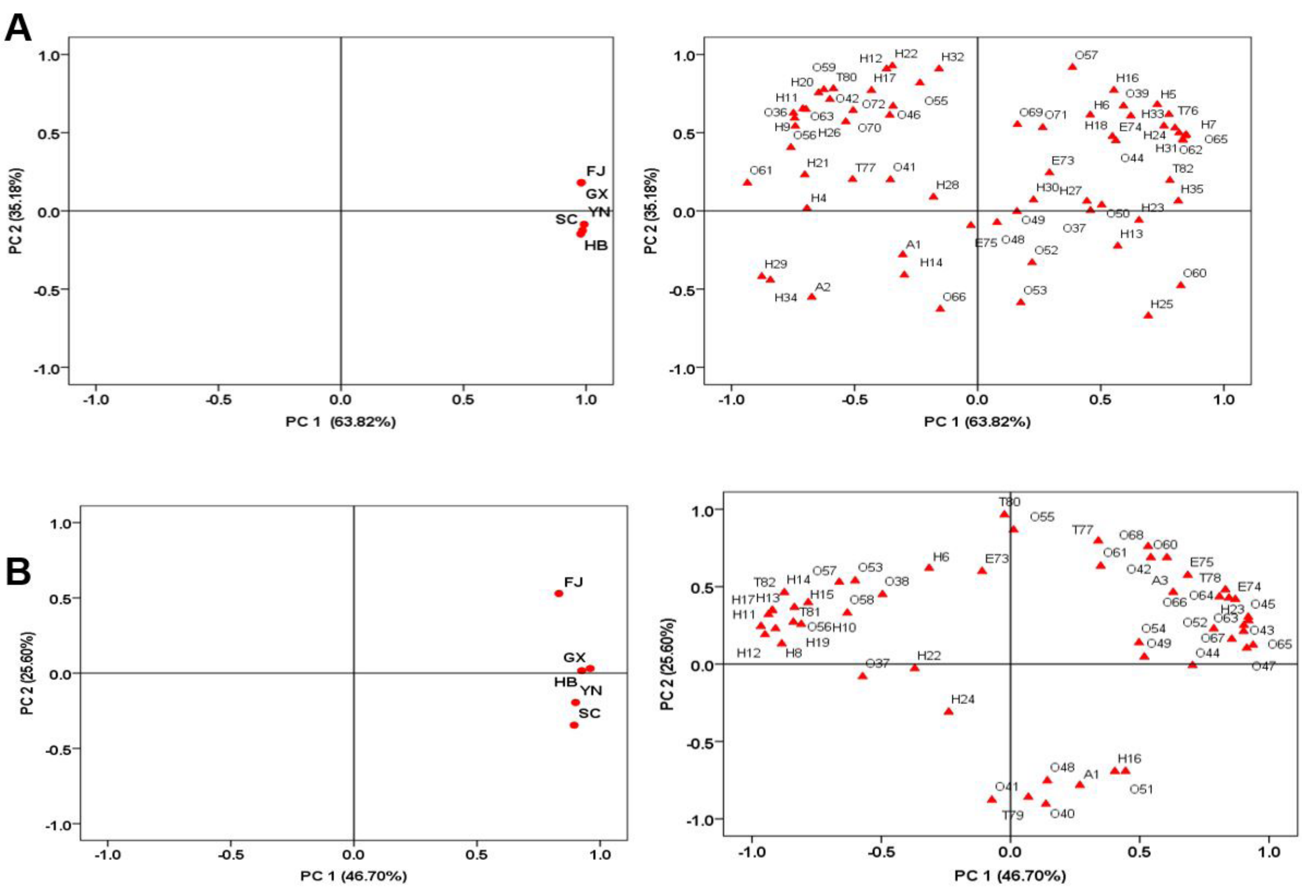

Figure 3. PCA of free (A) and bound (B) volatile compounds found in ILMFs. Compound nos. correspond to Table 1 and Table 2.

The clustering of the free volatile compounds divides the ILMFs of the five regions into two groups (Figure 4). GX and FJ are in the same group for their similar contents of free volatiles. α-Thujene, β-pinene, α-phellandrene, β-elemene, eucalyptol, methyl geranate and geranic acid are absent in the ILMFs of these two regions, and their contents of (Z)-β-farnesene, β-citral and juniper camphor are similar. The ILMFs of SC are assembled with those of HB and YN because of the high resemblance of their free volatile profiles, especially their similar eucalyptol concentrations.

The ILMFs of the different regions are also divided into two groups by the clustering of their bound volatile compositions, with FJ and GX as one group for their similar contents of α-myrcene, β-myrcene, allo-ocimene, α-citral and p-M-1(7),8(10)-D-9-O. The other group of HB, GX and FJ shows similar profiles of other compounds, especially for the ILMFs of YN and SC that contain similar amounts of sylvestrene, (E)-β-ocimene, nerol oxide, pinocarvone, α-terpinyl acetate and eugenol.

The PCA and clustering results infer that the divergences of the ILMFs in the five regions are due to their different geographic location and environments. YN and SC are located in southwestern China with high altitudes and relatively cold weather. Both FJ and GX are in southern China where the altitude is low, and the weather is relatively warm (Table S1). Therefore, the free and bound volatile profiles of the ILMFs grown in YN and SC are similar and those of FJ and GX are similar. HB is a province in central China. Its ILMF shows similar free volatiles profile to those of YN and SC and shares bound volatiles profile with those of FJ and GX.

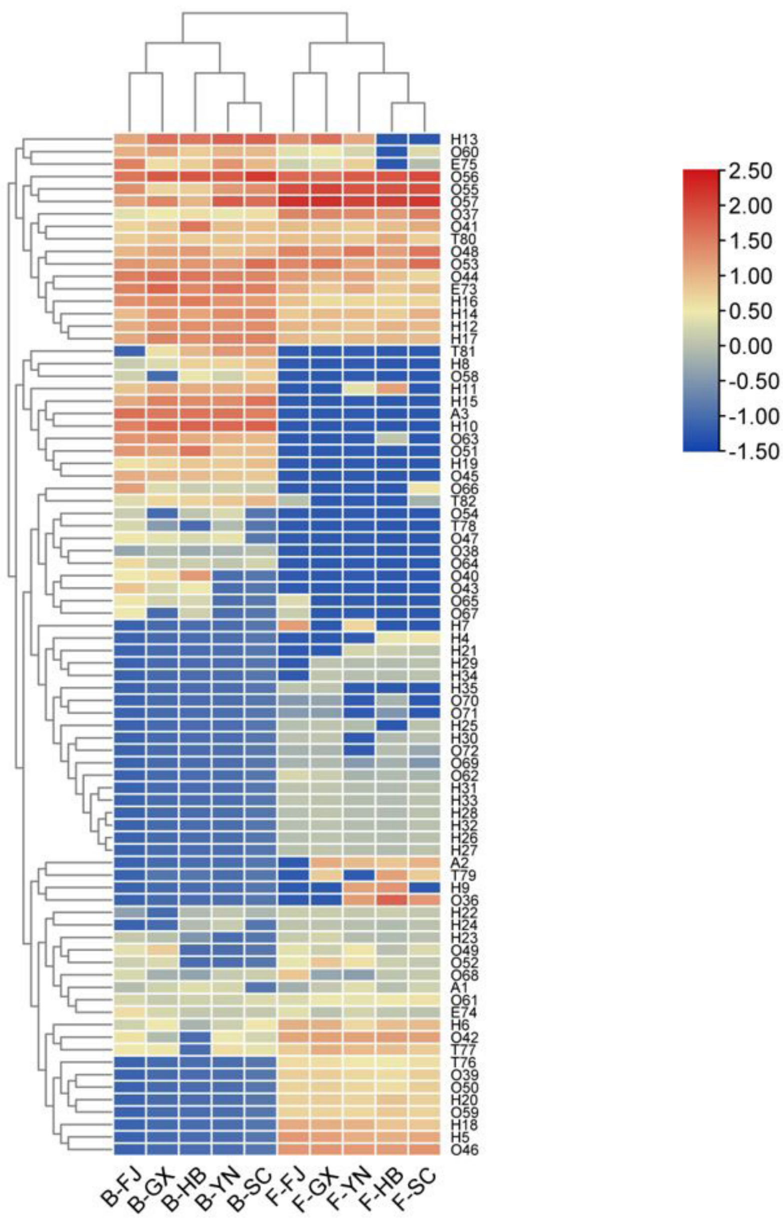

Figure 4. Hierarchical clustering and heatmap visualization of free and bound volatile compounds in ILMFs. The compound numbers are as listed in Table 1 and Table 2. F represensts free volatile compounds and B represents bound volatile compounds. The concentrations of aroma compounds are normalized.

4 Conclusions

In summary, total 82 volatiles with 66 free and 52 bound forms are identified and quantified in the ILMFs harvested in FJ, GX, HB, YN and SC by headspace SPME–GC–MS. Thirty six volatiles are found in both free and bound forms and 67 volatile compounds are detected in *Litsea mollis* fruit for the first time. Terpene hydrocarbons and oxygenated terpenes are the major volatile compounds in ILMFs. The free aroma compounds are dominated by α-pinene, eucalyptol, linalool, α,β-citral, geraniol and citronellal. β-Myrcene, α-citral, geraniol, citronellal and eugenol are the characteristic bound aroma compounds of ILMFs. The free volatiles of the ILMFs from the five regions share the floral, fruity and herbaceous odor profile. The bound volatiles exhibit similar characteristic odor as the free forms, but with stronger sweet and woody odor, suggesting that the enzymatic hydrolysis can make ILMF sweeter and woodier. The statistical analyses reveal that the volatiles of ILMF is correlated to geographic location. The ILMFs of FJ and GX in southern China show similar free and bound volatiles profiles, and those of YN and SC in southwestern China are similar. The ILMF of HB displays similar free volatiles profile to that of the ILMF in southwestern China, and shares bound volatiles profile with the ILMF of southern China.

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Supplementary material

Supplementary material accompanies this paper.

Legend S1. COMPARISON OF FREE AND BOUND VOLATILE PROFILES OF IMMATURE LITSEA MOLLIS FRUITS GROWN IN FIVE DISTINCT REGIONS OF CHINA.

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