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ARTICLE

Comparison of Volatile Profiles of Nine Litchi (*Litchi chinensis* Sonn.) Cultivars from Southern China

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Volatile components of nine litchi cultivars (10 samples) with high commercial value from Southern China were investigated by means of gas chromatography−mass spectrometry combined with headspace solid phase microextraction. A total of 96 volatiles were detected, of which 43 were identified. Seventeen common volatiles in all the samples included linalool, *cis*-rose oxide, α-terpineol, β-citronellol, geraniol, *p*-cymene, ethanol, 3-methyl-3-buten-1-ol, 3-methyl-2-buten-1-ol, 1-hexanol, (*E*)-2-hexen-1-ol, 2-ethyl-1-hexanol, 1-octen-3-ol, 1-octanol, ethyl acetate, *p*,α-dimethylstyrene and 3-*tert*-butyl-4-hydroxyanisole. Although the volatile composition and concentration varied between these cultivars, the components with the highest OAVs in most cultivars were still *cis*-rose oxide, *trans*-rose oxide, 1-octen-3-ol, and geraniol. Two Huaizhi samples from two producing areas exhibited similar volatile profiles, and significantly different from other cultivars according to cluster analysis performed on amounts of major volatile components.

KEYWORDS: Litchi cultivars; volatile compounds; odor activity values; cluster analysis

INTRODUCTION

Litchi (*Litchi chinensis* Sonn.), also known as lychee, is a tropical and subtropical fruit of the *Sapindaceae* family with commercial significance native to Southern China (*1*, *2*). A ripe litchi pulp usually consists of a white, slightly transparent, juicy aril, surrounding a large brown seed and covered with a reddish leathery skin. Owing to its bright color and exotic aroma, litchi has established great popularity in the international market. However, this popular fruit will rapidly lose its excellent flavor, with its attractive red appearance turning into dull brown, within 2 or 3 days after harvesting (*3*−*6*).

The distinctive flavor of litchi fruit is usually described as honey, rose-floral and citrus-fruity (*7*−*9*). Several previous studies have explored the volatile composition of fresh litchi fruit, as well as frozen litchi (*10*, *11*), dried litchi (*12*), and canned litchi (*13*). However, some of these researches did not cover the cultivar of litchi (*7*, *14*, *15*), and some investigated only a few cultivars (*8*, *9*, *16*, *17*) or only performed qualitative identification of volatiles (*18*). It remains unclear which volatile components are similar and which are unique, and what the volatile profile characters are among different commercially cultivated litchi cultivars.

In recent years, more and more litchi products have come into the daily life of people, such as litchi juice, litchi wine, canned litchi and dried litchi. Therefore, it is important to evaluate volatile profiles of litchi cultivars in order to utilize these cultivars to obtain litchi products with distinct flavor. There are more than 20 representative commercial litchi cultivars broad cultivated in South China (*1*, *19*). Here, a comparative study of volatile compounds of nine important commercial litchi cultivars widely grown in the Guangdong and Guangxi areas of Southern China was carried out through gas chromatography−mass spectrometry (GC−MS) combined with headspace solid phase microextraction (SPME) to better understand the similarities and differences of volatile profiles between these cultivars. This will provide basic data for further study on deep processing of litchi products, like litchi wine, with unique and favorite flavor.

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MATERIALS AND METHODS

Materials. The collected litchi cultivars included Xiangli (XL), Guiwei (GW), Heiye (HY), Jizuili (JZL), Nuomici (NMC), and Huaizhi 1 (HZ1) from the Guangxi area and Zhengfeng (ZF), Baila (BL), Feizixiao (FZX), and Huaizhi 2 (HZ2) from the Guangdong area, China. To obtain a sample representing a variety population, a set of 60 litchi fruits per six trees of each cultivar were collected during the stage of commercial maturity in the year of 2007, and these samples were immediately covered with ice blocks and transported to Beijing by air. They were evaluated within 36 h of being harvested.

Chemicals. Standards of ethanol (HPLC quality), 1-butanol (99.1%), 2,3-butanediol (99.0%), isoamyl alcohol (99.0%), 1-hexanol (99.0%), (*E*)-2-hexen-1-ol (96.0%), (*E*)-3-hexen-1-ol (98.0%), (*Z*)-3-hexen-1-ol (98.0%), 2-heptanol (99.0%), 1-heptanol (99.5%), 1-octanol (99.5%), 2-octanol (99.0%), 1-octen-3-ol (98.0%), 2-ethyl-1-hexanol (99.5%), 2-nonanol (99.0%), 1-dodecanol (98.0%), benzyl alcohol (99.9%), 2-phenylethanol (99.0%), ethyl acetate (99.9%), isoamyl acetate (99.5%), ethyl octanoate (99.5%), ethyl decanoate (99.5%), diethyl succinate (99.5%), hexanal (98.0%), (*E*)-2-hexenal (98.0%), nonanal (98.0%), benzaldehyde (99.0%), acetic acid (99.0%), limonene (99.5%), linalool (96.5%), linalool oxide (97.0%), α-terpineol (90.0%), β-citronellol (95.0%), citral (95.0%), rose oxide (99.0%), geranylacetone (containing 35% nerylacetone), geraniol (99.5%), nerol (97.0%), *p*-cymene (99.5%), terpinolene (97.0%),

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acetoin (96.0%), and butylated hydroxytoluene (99.5%) were purchased from Aldrich (Milwaukee, WI), Fluka (Buchs, Switzerland), and Sigma (St. Louis, MO).

Sample Preparation. Volatiles of litchi fruit were gathered and concentrated using the headspace SPME method. Fifteen fresh fruits of each cultivar were peeled and pitted, and then the flesh was wringed and blended. After being macerated for 40 min, the flesh was immediately centrifuged at 2000*g* and 4 °C for 10 min, and clear litchi juice was obtained. It should be noted that sodium chloride was added into the litchi juice, rather than the fresh or the macerated flesh, because it was considered as an inhibitor of enzyme reactions through which garlic odors were released (*9*), and we allowed the possibility of these enzyme actions since this was the accustomed way under which litchi was consumed or processed. Five milliliters of the juice and 1.00 g of NaCl were blended in a 15 mL sample vial tightly capped with a PTFE-silicon septum and containing a magnetic stirrer. Afterward the vial containing the sample was equilibrated at 40 °C for 30 min on a heating platform agitation. The pretreated (conditioned at 270 °C for 1 h) SPME fiber (50/30 μm DVB/Carboxen/PDMS, Supelco, Bellefonte, PA) was then inserted in to the headspace, extracting for 30 min with continued heating and agitation (*20*). The fiber was instantly desorbed in the GC injector for 25 min. Three independent extractions were done for each litchi sample.

GC−MS Analysis. An Agilent 6890 GC equipped with an Agilent 5975 MS and fitted with a 60 m × 0.25 mm id HP-INNOWAX capillary column with 0.25 μm film thickness (J&W Scientific, Folsom, CA) was employed to separate and identify the aromatic volatiles. Helium was used as the carrier gas at 1 mL/min, and the GC inlet was set in the splitless mode. The temperature program was from 50 °C (1 min hold) to 220 at 3 °C/min and held at 220 °C for 5 min. Mass spectra in the electron impact mode at a voltage of 70 eV ionization energy was set to scan from m/z 20 to 450 and operated in the selective ion mode under autotune conditions (*20*). Each independent extraction was carried out in duplicate. Retention indices were calculated after analyzing C8−C24 *n*-alkane series (Supelco, Bellefonte, PA) under the same chromatographic conditions. Identifications were based on mass spectra matching in the standard NIST 05 library and retention indices of reference standards in authors' laboratories. When reference standards were not available, tentative identifications were based on the standard NIST05 library and a comparison of retention indices reported in the literature.

Quantification. The quantification procedure was carried out based on prior studies (*21*, *22*) with modifications. According to the average concentration of sugar and acids in litchi juice (*23*), a synthetic matrix was prepared in distilled water containing 160 g/L glucose and 1.34 g/L malic acid, and the pH was adjusted to 4.7 with 5 M NaOH solution. Due to a great difference in concentration of various volatile components, the standard stock solution prepared could be divided into three groups on the basis of concentration: high, medium and low. Each standard was dissolved with ethanol (HPLC quality). All the standard stock solutions were then combined together, and this mixed standard solution was diluted into seven levels in succession with the synthetic matrix. Aroma standards of each level were extracted and analyzed under the same condition as litchi samples. For quantification, calibration curves were obtained with their regression coefficients all above 98% (**Table 1**). In addition, volatile compounds without calibration curves were estimated with those standards that had the same functional group and/or similar numbers of C atoms. Roughly, the calibration curve of isoamyl acetate was used to estimate the concentrations of esters and acetic acid, calibration curves of 1-hexanol and 1-octanol were used to estimate *n*-alkanol and *n*-alkanal, the calibration curve of (*E*)-2-hexen-1-ol was used to estimate enols and olefinic aldehydes, the calibration curve of 2-heptanol was used to estimate secondary alcohols and ketones, and calibration curves of nerol, α-terpineol and 2-phenylethanol were used to estimated acyclic terpenoids, cyclic terpenoids, and aromatic compounds, respectively. Odor activity values (OAVs) were calculated by dividing the concentration by its odor thresholds from the literature.

Statistical Procedures. All statistical procedures were performed through SPSS version 16.0 statistical package for windows (SPSS Inc., USA). A one-way analysis of variance (ANOVA) was used to gauge differences between means of volatiles amounts employing Duncan's multiple range tests at a level of $p < 0.05$, and the results were presented as mean ± SD of triplicate. Hierarchical cluster analysis was carried out on the basis of the contents of principal volatile components employing Ward's method.

Table 1. Regression Equations for Some Major Volatile Compounds in Litchi Fruits

standards	calibration curves[a]	R^b	linear range (μg/L)
isoamyl acetate	$y = 128446761.9x + 7326285.6$	0.997	1.96−979.20
limonene	$y = 87143649.4x + 1387938.1$	0.999	1.00−998.40
p-cymene	$y = 539884336.4x - 211071.3$	0.981	0.11−108.80
2-heptanol	$y = 776697432.4x + 486391.3$	0.999	0.14−14.46
1-hexanol	$y = 337147824.8x + 1224974.5$	0.998	2.02−202.24
(*E*)-2-hexen-1-ol	$y = 94271537.9x + 532679.3$	0.998	1.00−200.96
linalool oxide	$y = 179217470x - 324970.4$	0.994	5.18−103.68
1-octen-3-ol	$y = 736669467.2x + 905924.4$	0.997	0.20−51.20
1-heptanol	$y = 223549273.9x + 742903.7$	0.995	0.11−56.96
2-nonanol	$y = 414132255.2x + 321172.8$	0.999	0.02−39.04
benzaldehyde	$y = 75515613.0x + 404127.0$	0.986	0.22−11.14
linalool	$y = 720939052.6x + 130464.3$	0.999	0.22−5.57
1-octanol	$y = 344560435.0x + 463284.7$	0.999	0.02−81.92
α-terpineol	$y = 345090731.1x - 180423.9$	0.986	0.68−13.70
β-citronellol	$y = 451215286.7x + 1587397.9$	0.999	1.02−1024
nerol	$y = 321222013.3x - 1512419.3$	0.989	2.36−117.76
geraniol	$y = 168811529.5x + 906513.5$	0.999	4.53−2265.60
benzyl alcohol	$y = 10253820.9x + 777121.3$	0.999	112.64−11264
2-phenylethanol	$y = 17888092.9x + 257874.8$	0.999	15.10−755.20

[a] *y*, peak area of a compound; *x*, concentration in μg/L. [b] Regression coefficient.

RESULTS AND DISCUSSION

Volatile Composition. The contents of volatile compounds detected by GC−MS from the 10 litchi samples are listed in **Table 2**. A total of 43 volatiles were identified, and another 53 were tentatively identified in these litchi samples: 35 terpenoids, 27 alcohols, 10 aromatic compounds, 9 aldehydes, 8 esters, 4 ketones, 2 sulfurs, and 1 organic acid. Among these 96 volatiles, seventeen common constituents in all these samples were linalool, *cis*-rose oxide, α-terpineol, β-citronellol, geraniol, *p*-cymene, ethanol, 3-methyl-3-buten-1-ol, 3-methyl-2-buten-1-ol, 1-hexanol, (*E*)-2-hexen-1-ol, 2-ethyl-1-hexanol, 1-octen-3-ol, 1-octanol, ethyl acetate, *p*,α-dimethylstyrene and 3-*tert*-butyl-4-hydroxyanisole. In these cultivars, Guangxi Huaizhi contained at most 67 volatiles and Jizuili contained at least 36 volatiles. Alcohols were the predominant volatile components in various cultivars, representing 35.1% (Guangdong Huaizhi) to 81.6% (Jizuili) of the main fraction. Heiye, Guangxi Huaizhi and Guangdong Huaizhi contained a large amount of terpenes, accounting for 42.0%, 18.5%, and 24.5% respectively. Contrastively, Xiangli, Guiwei and Jizuili had the least terpenes, 3.9%, 4.9%, 4.3% respectively. Besides, aromatic compounds accounted for a relatively great percentage in Nuomici, Guangxi Huaizhi and Guangdong Huaizhi: 38.9%, 26.5%, and 32.9% respectively. Two prior studies (*10*, *15*) also revealed that alcohols and terpenoids were the major components of litchi volatiles, while another study (*17*) employing a purge and trap method reported that nearly all the volatiles from two South African litchi cultivars were terpenoids or derivatives, and presumably this different result might be due mainly to diverse isolation methods.

Ethanol was found to be the most abundant alcohol in each litchi sample, and showed obviously higher levels in Nuomici and Huaizhi (Guangdong and Guangxi) than those in the other cultivars. An investigation (*27*) confirmed that litchi fruits usually contained increasing amounts of ethanol during maturation. In addition to ethanol, 3-methyl-3-buten-1-ol, 3-methyl-2-buten-1-ol, 1-hexanol, (*E*)-2-hexen-1-ol, 2-ethyl-1-hexanol, 1-octen-3-ol, and 1-octanol were common alcohols in all analyzed samples, while 1-butanol, isoamyl alcohol, (*E*)-3-hexen-1-ol, (*Z*)-3-hexen-1-ol,

Table 2. Mean and Standard Deviation (n = 3) of Amounts of Volatile Components in 10 Fresh Litchi Samples (μg/L)[a]

compound	RI[b]	ID[c]	XL	GW	HY	JZL	NMC	HZ1	HZ2	ZF	BL	FZX
ethyl acetate	890	A	171.8 ± 4.4 b	342.0 ± 0.7 d	358.9 ± 6.8 d	59.0 ± 0.3 a	170.4 ± 3.6 b	268.4 ± 6.6 c	38.3 ± 7.5 a	364.4 ± 37.1 d	359.9 ± 2.4 d	197.7 ± 17.7 b
ethanol	933	A	755.4 ± 9.5 c	569.7 ± 6.1 a	705.9 ± 26.1 bc	677.3 ± 4.5 bc	1454.8 ± 12.4 d	2680.3 ± 76.8 f	1810.0 ± 99.7 e	571.8 ± 6.9 a	621.7 ± 42.8 ab	643.8 ± 4.9 ab
hexanal	1086	A	1.0 ± 0.2 a	–[d]	–	–	–	19.1 ± 4.7 b	44.4 ± 2.2 d	31.2 ± 1.7 c	4.9 ± 0.7 a	3.6 ± 0.6 a
isoamyl acetate	1122	A	–	nq	2.8 ± 1.5	–	–	–	–	nq	–	–
4-methyl-2-hexanone[e]	1123	C	4.249 ± 0.003 b	–	–	2.7 ± 0.2 a	–	–	–	–	3.6 ± 0.4 b	nq
1-butanol	1140	A	–	–	–	–	–	–	11.0 ± 0.7	–	–	–
β-myrcene	1164	B	–	–	109.9 ± 12.8 b	–	65.3 ± 1.3 a	236.8 ± 13.7 c	–	–	–	–
2,6-dimethyl-4-heptanone	1176	C	1.49 ± 0.05 a	1.02 ± 0.09 a	0.88 ± 0.08 a	1.10 ± 0.15 a	–	–	8.3 ± 1.3 b	–	nq	nq
4-penten-1-yl acetate	1199	C	–	nq	–	–	–	–	–	–	–	–
limonene	1202	A	–	–	47.0 ± 1.6	–	nq	nq	–	–	2.5 ± 1.1	–
3-methyl-2-butenal	1203	C	–	24.10 ± 0.14 a	–	–	155.15 ± 0.04 c	–	211.2 ± 15.6 d	–	–	69.2 ± 2.2 b
isoamyl alcohol	1206	A	–	–	–	2.11 ± 0.08	–	–	–	–	–	–
2-hexenol	1220	B	0.06 ± 0.02 a	0.078 ± 0.008 a	–	0.082 ± 0.001 a	–	–	–	–	–	–
(*E*)-2-hexenal	1223	A	8.4 ± 0.3 a	11.20 ± 0.02 a	8.1 ± 1.2 a	–	7.6 ± 1.2 a	74.6 ± 1.7 b	154.9 ± 3.3 d	203.4 ± 1.0 e	103.9 ± 7.2 c	10.9 ± 1.4 a
3-heptanol	1230	B	nq	nq	nq	nq	nq	–	–	–	–	–
(*Z*)-β-ocimene	1240	B	–	–	7.65 ± 0.14	–	–	17.1 ± 0.7	nq	–	–	–
1,3,5-trimethylbenzene	1249	B	–	–	–	–	–	–	20.7 ± 3.2	–	–	–
3-methyl-3-buten-1-ol	1251	C	19.6 ± 0.5 d	18.8 ± 0.3 d	8.47 ± 0.14 b	nq	58.0 ± 0.2 f	77.4 ± 1.0 h	38.7 ± 1.8 e	14.3 ± 2.5 c	2.4 ± 0.5 a	64.0 ± 3.0 g
3-methyl-2-buten-1-yl acetate	1255	C	nq	26.0 ± 0.2	–	–	nq	–	–	nq	–	–
(*E*)-β-ocimene	1257	B	–	–	18.3 ± 0.6 b	–	–	26.9 ± 1.1 c	24.5 ± 1.2 c	–	–	9.9 ± 1.7 a
p-cymene	1275	A	11.10 ± 0.13 c	6.27 ± 0.03 ab	13.2 ± 0.5 d	5.42 ± 0.14 a	5.66 ± 0.05 ab	7.2 ± 0.6 ab	10.7 ± 0.6 c	10.9 ± 2.3 c	7.5 ± 0.6 b	6.8 ± 0.6 ab
acetoin	1284	A	34.7 ± 0.6 e	12.17 ± 0.12 c	9.5 ± 0.2 b	35.6 ± 0.2 e	73.0 ± 3.4 f	0.87 ± 0.07 a	–	–	8.98 ± 0.11 b	15.6 ± 0.2 d
terpinolene	1290	A	–	–	–	–	nq	8.4 ± 0.3	11.9 ± 6.6	–	–	–
2,4-dithiapentane	1299	B	–	–	–	–	–	nq	–	–	–	–
2-heptanol	1314	A	–	–	nq	–	–	6.4 ± 0.0	4.6 ± 0.0	–	–	–
3-methyl-2-buten-1-ol	1316	B	19.0 ± 0.4 d	42.90 ± 0.05 e	16.28 ± 0.02 cd	2.1 ± 0.2 a	170.4 ± 0.2 h	162.5 ± 1.0 g	106.6 ± 2.4 f	15.1 ± 0.6 c	5.5 ± 0.2 b	103.6 ± 3.0 f
(*E*)-*2-heptenal*	1327	B	–	–	–	–	–	–	17.8 ± 1.2	–	–	–
6-methyl-5-hepten-2-one	1339	B	nq	0.34 ± 0.02 a	–	0.42 ± 0.04 a	1.2 ± 0.4 b	1.214 ± 0.001 b	8.1 ± 0.5 c	0.54 ± 0.01 a	0.42 ± 0.08 a	0.80 ± 0.07 ab
2,6-dimethyl-4-heptanol	1345	C	4.26 ± 0.11 e	3.08 ± 0.06 cd	3.45 ± 0.11 de	3.5 ± 0.3 de	2.51 ± 0.03 bcd	–	10.7 ± 1.3 f	2.09 ± 0.04 abc	1.1 ± 0.2 a	1.8 ± 0.4 ab
1-hexanol	1348	A	13.60 ± 0.11 d	10.27 ± 0.09 bc	8.2 ± 0.4 b	0.4 ± 0.2 a	7.6 ± 0.6 b	69.5 ± 4.5 g	55.4 ± 0.9 f	37.85 ± 0.03 e	56.2 ± 3.0 f	36.6 ± 0.5 e
cis-rose oxide	1356	A	4.83 ± 0.07 a	8.7 ± 0.2 b	15.2 ± 0.8 c	12.8 ± 2.4 c	5.1 ± 0.2 a	22.3 ± 1.5 d	42.8 ± 4.1 e	4.2 ± 0.4 a	2.70 ± 0.07 a	5.9 ± 0.6 ab
(*E*)-3-hexen-1-ol	1358	A	–	–	–	–	–	–	–	–	nq	–
trans-rose oxide	1370	A	2.43 ± 0.03 a	4.18 ± 0.05 b	6.3 ± 0.3 c	6.1 ± 1.1 c	2.29 ± 0.07 a	9.1 ± 0.6 d	23.6 ± 1.6 e	1.9 ± 0.2 a	–	2.5 ± 0.3 a
(*Z*)-*alloocimene*	1378	B	–	–	6.55 ± 0.12 a	–	–	11.7 ± 0.4 c	8.7 ± 0.2 b	–	–	–
(*Z*)-3-hexen-1-ol	1385	A	–	–	–	–	–	nq	–		–	–
3-octanol	1388	B	–	–	0.19 ± 0.04 a		nq	8.4 ± 0.2 b	12.5 ± 0.9 c		nq	nq
nonanal	1393	A	nq	nq	–	nq	–	–	nq	nq	–	–
methyl benzyl ether	1395	B	–	–	–	nq	–	–	–	–	–	76.5 ± 4.3
(*E*)-*alloocimene*	1396	C	–	–	8.1 ± 0.3	–	–	9.2 ± 0.4	nq	–	–	–
(*E*)-2-hexen-1-ol	1401	A	32.3 ± 0.4 c	56.92 ± 0.12 d	21.5 ± 1.0 b	11.2 ± 0.8 a	11.0 ± 0.3 a	50.6 ± 0.4 d	26.7 ± 0.2 bc	76.3 ± 1.0 e	151.3 ± 10.7 g	88.9 ± 2.9 f
2-octanol	1417	A	–	–	–	–	–	0.23 ± 0.01	tr	–	–	–
(*E*)-*2-octenal*	1433	B	–	–	–	–	–	–	nq	–	–	–
ethyl octanoate	1437	A	–	–	–	–	–	–	nq	–	–	–
p,a-dimethylstyrene	1441	B	2.30 ± 0.05 c	1.38 ± 0.01 a	2.8 ± 0.2 d	1.46 ± 0.05 a	1.42 ± 0.03 a	6.1 ± 0.3 e	9.01 ± 0.03 f	2.6 ± 0.4 cd	1.88 ± 0.16 b	1.45 ± 0.12 a
1-octen-3-ol	1446	A	nq	0.68 ± 0.04 a	19.6 ± 1.0 d	nq	nq	58.8 ± 1.5 e	57.1 ± 0.7 e	3.3 ± 0.3 b	1.7 ± 0.4 ab	11.5 ± 0.8 c
1-heptanol	1448	A	–	–	–	–	nq	1.5 ± 0.2	3.69 ± 0.08	–	–	nq
acetic acid	1449	A	2.9 ± 0.4 a	nq	11.8 ± 1.8 c	8.2 ± 1.2 b	–	–	–	14.8 ± 0.9 d	9.8 ± 0.2 bc	–
6-methyl-5-hepten-2-ol	1458	C	–	–	nq	–	nq	1.4 ± 0.1	nq	nq	nq	nq
trans-furan linalool oxide	1468	A	4.42 ± 0.01 a	16.04 ± 0.07 cd	7.3 ± 0.3 ab	10.7 ± 0.2 bc	17.6 ± 0.6 d	55.5 ± 0.9 e	63.2 ± 6.6 f	7.9 ± 2.4 ab	6.3 ± 0.5 ab	–
nerol oxide	1471	B	1.53 ± 0.02 a	2.27 ± 0.01 a	1.62 ± 0.07 a	1.6 ± 0.2 a	2.98 ± 0.11 a	14.6 ± 1.1 b	35.4 ± 3.7 c	1.80 ± 0.01 a	–	–

Table 2. Continued

compound	RI[b]	ID[c]	XL	GW	HY	JZL	NMC	HZ1	HZ2	ZF	BL	FZX
2-ethyl-1-hexanol	1484	A	0.8 ± 0.2 ab	1.80 ± 0.03 c	0.6 ± 0.2 ab	0.30 ± 0.01 a	1.44 ± 0.11 bc	4.2 ± 0.4 d	6.3 ± 1.0 e	2.253 ± 0.002 c	1.8 ± 0.2 c	1.8 ± 0.3 c
methyl phenethyl ether	1491	B	–	–	2.5 ± 1.7 a	4.9 ± 1.6 a	1498.0 ± 47.5 b	1913.0 ± 41.2 c	2219.9 ± 122.1 d	–	–	68.3 ± 13.0 a
α-copaene	1494	B	–	–	–	–	nq	–	–	–	–	–
(E,E)-2,4-heptadienal	1499	B	–	–	–	–	–	nq	–	–	–	–
(E)-2-hepten-1-ol	1510	C	–	–	–	–	–	4.9 ± 0.3	14.40 ± 0.06	–	–	–
benzaldehyde	1529	A	–	–	–	–	–	nq	–	–	–	3.25 ± 0.01
linalool	1547	A	3.44 ± 0.02 e	1.52 ± 0.01 c	1.20 ± 0.06 bc	0.26 ± 0.06 a	4.5 ± 0.2 f	10.9 ± 0.5 g	19.4 ± 0.3 h	2.5 ± 0.2 d	0.92 ± 0.07 b	2.58 ± 0.07 d
1-octanol	1551	A	nq	nq	nq	nq	0.5 ± 0.2 a	5.5 ± 0.3 d	6.5 ± 0.2 e	2.5 ± 0.3 c	1.93 ± 0.04 b	0.22 ± 0.11 a
2,3-butanediol	1568	A	nq	–	–	–	–	–	–	–	–	–
4-terpinenol	1607	B	5.1 ± 0.2 c	8.80 ± 0.03 e	nq	–	3.5 ± 0.2 b	7.5 ± 0.4 d	11.6 ± 0.2 f	1.51 ± 0.12 a	nq	nq
(E)-2-octen-1-ol	1614	B	–	–	–	–	–	1.1 ± 0.1	0.03 ± 0.01	–	–	–
(E)-p-mentha-2,8-dienol	1626	C	–	–	–	–	–	–	–	nq	nq	–
ethyl decanoate	1641	A	–	–	–	–	–	nq	nq	–	–	–
menthol	1644	B	–	–	–	–	–	nq	–	–	–	–
γ-elemene	1645	B	–	–	–	–	8.1 ± 1.0	nq	5.4 ± 3.4	–	–	–
1-nonanol	1656	B	nq	nq	nq	–	nq	nq	nq	nq	0.30 ± 0.14	nq
1,2-dimethoxy-1-phenylethane	1675	C	–	–	–	–	–	–	–	–	–	10.0 ± 3.2
2,3,5-trithiahexane	1676	C	–	–	–	–	–	nq	–	–	–	–
diethyl succinate	1682	A	–	–	–	–	–	nq	–	–	–	–
neral	1689	A	–	–	10.8 ± 0.6 c	–	5.85 ± 0.11 a	11.11 ± 0.11 c	14.7 ± 0.3 d	5.2 ± 0.4 a	5.91 ± 0.13 a	7.3 ± 0.4 b
γ-muurolene	1697	B	–	–	–	–	6.1 ± 0.7	4.8 ± 0.1	nq	–	–	–
α-terpineol	1703	A	6.27 ± 0.06 c	2.74 ± 0.04 ab	1.41 ± 0.04 a	nq	8.8 ± 0.3 d	16.6 ± 1.0 e	33.2 ± 2.3 f	5.6 ± 0.7 c	2.44 ± 0.06 ab	4.4 ± 0.4 bc
borneol	1709	B	–	–	–	–	–	nq	–	–	–	–
1,2-dimethoxybenzene	1729	C	–	–	–	–	–	–	–	–	nq	–
α- muurolene	1731	B	–	–	–	–	13.2 ± 2.1 ab	5.6 ± 0.3 a	34.9 ± 13.8 b	–	–	–
(Z)-myrtanol	1737	C	–		–	–	–	3.82 ± 0.15	–	–	–	–
cis-pyran linalool oxide	1739	B	–	nq	–	–	–	–	–	–	–	–
geranial	1742	A	–	nq	53.6 ± 5.0 d	–	2.1 ± 0.6 a	38.9 ± 1.3 c	93.8 ± 9.4 e	4.9 ± 1.5 a	9.1 ± 3.2 a	23.1 ± 3.7 b
β-citronellol	1771	A	nq	nq	156.8 ± 7.6 e	nq	4.3 ± 0.5 a	26.5 ± 1.4 b	52.3 ± 3.7 c	11.5 ± 1.7 a	10.9 ± 1.5 a	72.4 ± 6.6 d
γ-geraniol[f]	1790	C	–	–	0.5 ± 0.2 a	–	nq	2.6 ± 0.4 b	6.1 ± 0.6 c	nq	nq	nq
ethyl phenylacetate	1796	B	–	–	–	–	–	3.6 ± 1.6	–	–	–	–
nerol	1808	A	–	nq	12.9 ± 0.5 b	–	9.4 ± 0.3 ab	35.2 ± 1.8 d	47.3 ± 2.5 e	6.5 ± 0.3 a	7.4 ± 0.3 a	21.8 ± 2.2 c
cis-isogeraniol	1816	B	–	–	6.54 ± 0.10 a	–	–	7.3 ± 0.2 ab	9.4 ± 0.4 c	–	–	7.8 ± 0.5 b
trans-isogeraniol	1819	B	–	5.481 ± 0.004 a	21.9 ± 0.9 d	nq	9.4 ± 0.3 b	26.4 ± 1.4 e	37.0 ± 2.7 f	5.8 ± 0.2 a	5.82 ± 0.01 a	14.5 ± 1.4 c
p-cymen-8-ol	1853	B	4.2 ± 0.5 a	nq	–	–	–	87.4 ± 37.0 b	120.1 ± 50.9 c	nq	–	–
geraniol	1857	A	nq	2.67 ± 0.02 a	349.8 ± 19.0 c	nq	13.3 ± 1.2 a	648.2 ± 38.6 d	964.1 ± 39.6 e	47.9 ± 11.5 a	38.5 ± 5.8 a	125.5 ± 19.4 b
geranylacetone	1864	A	–	–	–	nq	5.4 ± 0.4 a	–	12.1 ± 0.2 d	nq	6.1 ± 0.2 b	6.9 ± 0.2 c
cis-carveol	1871	B	–	–	–	–	–	–	nq	–	–	–
benzyl alcohol	1884	A	–	–	–	–	–	98.7 ± 5.0	nq	–	–	nq
2-phenylethanol	1917	A	–	7.7 ± 0.2 a	–	–	62.2 ± 0.2 b	418.3 ± 22.4 d	247.6 ± 10.0 c	–	nq	273.4 ± 35.5 c
butylated hydroxytoluene	1920	A	nq	nq	nq	nq	nq	–	nq	nq	–	–
1-dodecanol	1969	A	–	–	–	–	–	nq	2.1 ± 0.3	–	–	nq
eugenol methyl ether	2027	B	–	–	–	–	–	7.0 ± 1.8	nq	–	–	–
3-tert-butyl-4-hydroxyanisole	2103	C	8.4 ± 0.5 cd	6.5 ± 0.8 abc	6.70 ± 0.14 bc	6.96 ± 0.11 bc	6.85 ± 0.03 bc	10.2 ± 2.6 de	11.4 ± 2.7 e	5.0 ± 0.3 ab	3.79 ± 0.03 a	4.3 ± 0.4 ab
2,4-ditert-butyl phenol	2317	B	nq	nq	nq	nq	–	nq	–	nq	nq	nq
dibutylhydroxybenzaldehyde	>2400	C	–	–	–	–	–	–	38.0 ± 23.2	–	–	–

[a] Values (mean ± SD, $n = 3$) of the same compounds followed by different letters are significantly different ($P < 0.05$). nq: Not quantified (detection limit < concentration < quantification limit). [b] Retention indices on the poly(ethylene glycol) (PEG) column. [c] Reliability of the identification proposal: A, identified, mass spectrum and RI agreed with standards; B, tentatively identified, mass spectrum agreed with the mass spectral database and RI agreed with literature data (refs *8*, *9*, *13*, *15*, *24*−*26*); C, tentatively identified, mass spectrum agreed with the mass spectral database. [d] Not detected (concentration < detection limit). [e] Compounds in italics were newly reported as litchi fruit volatiles. [f] γ-Geraniol: 7-methyl-3-methylene-6-octen-1-ol.

and 2,3-butanediol existed only in Guangdong Huaizhi, Jizuili, Baila, Guangxi Huaizhi, and Xiangli respectively. Nuomici, Huaizhi (Guangdong and Guangxi) and Feizixiao had relatively higher concentrations of 3-methyl-2-buten-1-ol, 3-methyl-3-buten-1-ol and 2-phenylethanol. 1-Hexanol was highest in all of the litchi samples from Guangdong as well as Guangxi Huaizhi, while 1-octanol, 1-octen-3-ol, and 2-ethyl-1-hexanol were with the highest levels only in two Huaizhi samples. In particular, Guangxi Huaizhi contained the absolutely high concentration of benzyl alcohol.

Terpenoids played a very important role in the unique flavor of fresh litchi fruits. **Table 2** suggested these litchi cultivars held different terpenoid profiles. Both Xiangli and Jizuili contained only 11 terpenoids, while Guangxi Huaizhi and Guangdong Huaizhi held 30 and 27 terpenoids, respectively. Linalool, *cis*-rose oxide, α-terpineol, *β*-citronellol, geraniol, and *p*-cymene existed in all 10 litchi samples, while α-copaene and *cis*-pyran linalool oxide were found only in Nuomici and Guiwei respectively, *cis*-carveol only in Guangdong Huaizhi, as well as menthol, borneol, and (*Z*)-myrtanol only in Guangxi Huaizhi. Two Huaizhi samples held very high levels of *trans*-furan linalool oxide, nerol oxide, linalool, α-terpineol, *trans*-isogeraniol, geraniol, *γ*-geraniol (7-methyl-3-methylene-6-octen-1-ol) and *p*-cymen-8-ol, and *β*-citronellol and limonene in Heiye, α-muurolene in Guangdong Huaizhi, *β*-myrcene in Guangxi Huaizhi also showed high concentrations.

Sulfur compounds usually have low contents to detect by MS but are responsible for garlic character of litchi flavor (*9*) for their extremely low odor thresholds. Excitingly two sulfur volatiles, 2,4-dithiapentane and 2,3,5-trithiahexane, were observed using GC−MS and noticeably they were only detected in Guangxi Huaizhi, none in Guangdong Huaizhi or other cultivars. One of the sulfides, 2,4-dithiapentane, was reported to present cabbage notes(*9*), while the other one, 2,3,5-trithiahexane, has not been noted in previous reports about litchi volatiles.

The types of esters and aldehydes were not as many as alcohols or terpenoids in litchi fruits. Ethyl octanoate and 4-penten-1-yl acetate were detected only in Guangdong Huaizhi and Guiwei respectively, and diethyl succinate and ethyl phenylacetate existed only in Guangxi Huaizhi. Among nine aldehydes found in this study, (*E*)-2-heptenal, (*E*)-2-octenal, and dibutylhydroxybenzaldehyde appeared only in Guangdong Huaizhi, and (*E,E*)-2,4-heptadienal only in Guangxi Huaizhi.

Aromatic compounds in fresh litchi fruits also varied with varieties. All of the litchi samples contained two common benzene compounds, *p*,α-dimethylstyrene and 3-*tert*-butyl-4-hydroxyanisole, while 1,3,5-trimethylbenzene, 1,2-dimethoxybenzene and 1,2-dimethoxy-1-phenylethane were unique in Guangxi Huaizhi, Baila and Feizixiao respectively. In addition, methyl benzyl ether was identified only in Feizixiao and Jizuili, and eugenyl methyl ether only in two Huaizhi samples. Methyl phenethyl ether showed very high concentrations in Huaizhi (Guangdong and Guangxi) and Nuomici.

To our knowledge, the present investigation identified or tentatively identified some new compounds (marked in italics in **Table 2**) that were not reported in previous studies concerning litchi fruits volatile constituents. These newly identified compounds (confirmed by reference standards) included 1-butanol, 2,3-butanediol, 2-heptanol, 2-octanol, 1-dodecanol, geranylacetone, ethyl octanoate, ethyl decanoate, diethyl succinate, butylated hydroxytoluene, and tentatively identified compounds (confirmed by MS and/or retention indices) included 2-hexanol, 3-heptanol, 1-nonanol, (*E*)-2-hepten-1-ol, (*E*)-2-octen-1-ol, 2,6-dimethyl-4-heptanol, (*E*)-alloocimene, (*Z*)-alloocimene, nerol oxide, (*E*)-*p*-mentha-2,8-dienol, menthol, *γ*-muurolene, borneol, (*Z*)-myrtanol, *γ*-geraniol, *cis*-carveol, 4-penten-1-yl acetate, ethyl phenylacetate, (*E*)-2-heptenal, (*E*)-2-octenal, (*E,E*)-2,4-heptadienal, dibutylhydroxybenzaldehyde, 6-methyl-5-hepten-2-one, 2,6-dimethyl-4-heptanone, 4-methyl-2-hexanone, 1,3,5-trimethyl benzene, *p*,α-dimethylstyrene, methyl benzyl ether, eugenyl methyl ether, 3-*tert*-butyl-4-hydroxyanisole, 2,4-di-*tert*-butyl phenol, and 2,3,5-trithiahexane. Most of these newly detected volatiles came from litchi cultivars (Huaizhi, Xiangli, Jizuili and Zhengfeng) that were not previously investigated.

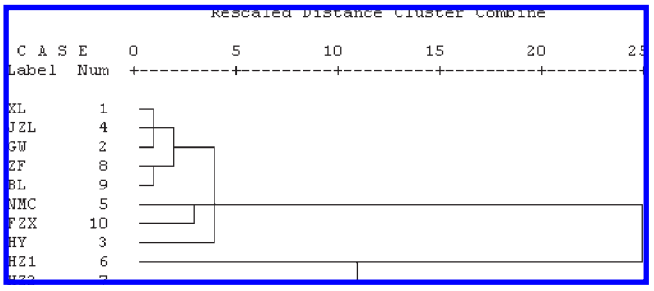

Figure 1. Dendrogram obtained from cluster analysis (Ward's method) based on amounts of major volatiles.

Cluster Analysis. Hierarchical cluster analysis was carried out following Ward's method on the basis of contents of 75 quantified volatiles in **Table 2**. The result showed clearly that these litchi samples were appropriately divided into two clusters (**Figure 1**): two Huaizhi samples were grouped as one cluster, and the other nine cultivars, including Guiwei, Jizuili, Xiangli, Nuomici, Zhengfeng, Heiye, Feizixiao, Baila, linked together as the other one. Namely, two Huaizhi samples from different areas showed similar volatile profiles, but differed greatly from the others. **Table 2** showed that most volatiles in Huaizhi cultivar (Guangdong and Guangxi) had higher contents than others, and this indicated that the flavor of this cultivar could be much more complex than those of the other nine cultivars. It was expected that more potent odorants, especially terpenoids, in Huaizhi could survive during litchi further processing than in other cultivars, while there still were small differences of volatile profile between the two Huaizhi samples: the Guangdong sample contained much more aldehydes and ketones, and the Guangxi sample held much more esters. In addition, two sulfur volatiles were only detected in Guangxi Huaizhi, but not in Guangdong Huaizhi. These could answer for the relatively big distance between the two Huaizhi samples in **Figure 1**.

Odor Profiles. Though dozens of volatiles were detected in each litchi sample, not all of the components have great impact on the overall aroma character of this fruit. To evaluate the contribution of various volatile compounds to olfactory impression of this fruit, OAVs of the most potent odorants, especially those having been considered to contribute much to litchi flavor (*7*−*9*), were calculated on the basis of their odor thresholds (OT), and only those with OAVs above 1 were listed in **Table 3**.

Table 3 indicated that the odor profiles of litchi samples differed greatly across cultivars. Xiangli, Guiwei and Jizuili had the fewest odorants with OAVs above 1, and in contrast, Huaizhi (Guangdong and Guangxi), Zhengfeng and Heiye contained the most odor-active volatiles, suggesting that these three cultivars presented more complex flavor than the others. Two Huaizhi cultivars were characterized by their relatively high OAVs of 1-octen-3-ol, *cis*-rose oxide, *trans*-rose oxide, linalool, geranial and geraniol, indicating that more rose-floral and citrus notes might be sensed in this cultivar. Isoamyl acetate and *β*-citronellol in Heiye endowed this cultivar with more tropical fruit, citrus

Table 3. Odor Activity Values (OAVs) of Most Potent Odorants in 10 Litchi Samples

odorants	OT[a] (ppb in water)	XL	GW	HY	JZL	NMC	HZ1	HZ2	ZF	BL	FZX
hexanal	4.5[b]	0.2	–[c]	–	–	–	4.3	9.9	6.9	1.1	0.8
isoamyl acetate	2[b]	–	<0.1	1.4	–	–	–	–	<0.1	–	–
β-myrcene	15[b]	–	–	7.3	–	4.4	15.8	–	–	–	–
(*E*)-2-hexenal	17[b]	0.5	0.7	0.5	–	0.4	4.4	9.1	12.0	6.1	0.6
(*E*)-2-heptenal	13[b]	–	–	–	–	–	–	1.4	–	–	–
1-octen-3-ol	1[b]	<0.1	0.7	19.6	<0.1	<0.1	58.8	57.1	3.3	1.7	11.5
p-cymene	11.4[b]	1.0	0.6	1.2	0.5	0.5	0.6	1.0	1.0	0.7	0.6
cis-rose oxide	0.5[d]	9.7	17.5	30.4	25.5	10.2	44.6	85.6	8.4	5.4	11.8
trans-rose oxide	0.5[d]	4.9	8.4	12.6	12.2	4.6	18.2	47.2	3.8	–	5.0
linalool	6[b]	0.6	0.3	0.2	<0.1	0.8	1.8	3.6	0.4	0.2	0.4
geranial	32[b]	–	<0.1	1.7	–	0.1	1.2	2.9	0.2	0.3	0.7
β-citronellol	40[d]	<0.1	<0.1	3.9	<0.1	0.1	0.7	1.3	0.3	0.3	1.8
geraniol	40[d]	<0.1	<0.1	8.7	<0.1	0.3	16.2	24.1	1.2	1.0	3.1

[a] Odor threshold. [b] Reference *28*. [d] Reference *29*. [c] Not detected (concentration < detection limit).

flavor note because of their higher OAVs (*9*). Zhengfeng, Baila and Huaizhi (Guangdong and Guangxi) were observed with higher OAVs than other cultivars of hexanal and (*E*)-2-hexenal, which were responsible for green/woody odor (*9*, *21*).

As shown in **Table 3**, terpenoids accounted for the largest part of odor-active volatiles. In most litchi cultivars, *cis*-rose oxide, *trans*-rose oxide, 1-octen-3-ol, and geraniol showed relatively high OAVs, suggesting their great contributions to litchi fruit flavor. Ong et al. (*13*) confirmed that *cis*-rose oxide, geraniol, linalool, β-citronellol, and eight other common odorants were responsible for the litchi flavor in Gewürztraminer wine. In detail, geraniol, β-citronellol, limonene, geranial, and neral were found to be responsible for the citrus-fruity notes, nerol responsible for honey odor attribute, and 2-phenylethanol, *cis*-rose oxide, linalool and *cis*-furan linalool oxide responsible for the rose-floral odor by means of GC−O and GC−MS research (*7*−*9*). However, limonene, 2-phenylethanol, nerol, and neral showed low OAVs in all cultivars containing them in this study, which might result from their high odor thresholds (limonene and 2-phenylenthanol, 1000 and 1100 ppb in water, respectively (*28*); nerol and neral, 300 and 32 ppb in water, respectively (*29*)).

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