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Research Article

Comparative analysis of characteristic volatile compounds in five types of Yunnan dry-cured hams by HS-GC-IMS and HS-SPME-GC-MS

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Abstract: The quality characteristics of dry-cured hams from different regions of Yunnan province were studied by analyzing five types of Yunnan dry-cured hams (Xuanwei ham, Sanchuan ham, Nuodeng ham, Saba ham, and Heqing ham) using headspace-gas chromatography-ion mobility spectrometry (HS-GC-IMS) and headspace solid-phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC-MS). The analysis aimed to identify different volatile organic compounds (VOCs) in the dry-cured ham samples. Forty-one VOCs were qualitatively characterized by HS-GC-IMS from dry-cured ham samples, of which Nuodeng ham and Saba ham had similar fingerprint profiles and contained higher levels of aldehydes and alcohols. Meanwhile, a total of 12 qualitatively differential characteristic markers were screened by the PLS-DA model. Furthermore, 128 main VOCs were identified by HS-SPME-GC-MS, of which 26 differential characteristic markers were screened by the PLS-DA model. HCA analysis showed that the VOCs of Sanchuan ham were different from those of the other four dry-cured hams due to the unique processing. These results can contribute to a more comprehensive understanding of the flavor characteristics of dry-cured hams from different regions of Yunnan.

Keywords: Yunnan dry-cured ham; volatile compounds; headspace-gas chromatography-ion mobility spectrometry; headspace solid-phase microextraction-gas chromatography-mass spectrometry

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1 Introduction

Yunnan dry-cured ham is a special meat product processed by traditional technology, which is favored by consumers for its unique flavor[1]. Dry-cured ham is produced in many areas of Yunnan province, and mainly distributed in the northern region with an altitude of about 2 000 meters and low annual average temperature, including Xuanwei ham, Sanchuan ham, Nuodeng ham, Saba ham, and Heqing ham, etc.[2]. Due to the influence of raw materials, curing methods, ripening conditions and other factors, there are some differences in the quality of dry-cured ham in different regions. So far, there are few studies on the differences of characteristic flavor compounds of dry-cured ham in different regions of Yunnan province.

It is well known that flavor is one of the most important quality indexes of dry-cured ham, and the products have different flavor characteristics because of different volatile substances. The research on the flavor of dry-cured ham is of great significance to its quality evaluation. Recent years, headspace-gas chromatography-ion mobility spectrometry (HS-GC-IMS)[3–5] and headspace solid-phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC-MS)[6–7] are widely used in the analysis of volatile compounds in food. HS-GC-IMS has become increasingly popular for the combination of the high separation ability of gas chromatography and the fast response ability of ion mobility spectrometry, and it has the advantages of no sample pretreatment, fast analysis time, low detection limit and simple operation[8–9]. HS-GC-IMS can be regarded as a two-dimensional analysis method, in which the analytes are separated on the basis of GC in the chromatographic column, and then separated and eluted in the drift tube with a constant electric field at atmospheric pressure[10]. Arroyo-Manzanares et al.[11] reported that HS-GC-IMS was used to distinguish authentic and fraudulent samples of Iberian ham. However, due to the lack of a complete HS-GC-IMS database, some VOCs have not been identified[12]. Meanwhile, GC-MS can be combined with HS-SPME and is another widely effective technology for the analysis of volatile compounds in food samples. Martínez-Onandi et al.[13] analyzed the volatile compounds of Iberian dry-cured hams of different physicochemical characteristics and the effect of high-pressure processing on volatile compounds using HS-SPME-GC-MS. Compared with HS-GC-IMS, HS-SPME-GC-MS analysis provides detailed information about compounds based on a standard reference database like the NIST mass spectral library[14]. However, HS-SPME-GC-MS is not sensitive to lower levels of VOCs, and these can easily be neglected[15]. Accordingly, it is advisable for us to combine HS-GC-IMS analysis with HS-SPME-GC-MS analysis, which can not only provide an intuitive method for the identification of volatile compounds, but also obtain the comprehensive information of volatile compounds in food samples[16].

At present, there is no research to systematically compare the characteristic flavor differences of different Yunnan dry-cured hams. Therefore, in this study, the volatile compounds of five types of Yunnan dry-cured hams (Xuanwei ham, Sanchuan ham, Nuodeng ham, Saba ham and Heqing ham) were determined by HS-GC-IMS and HS-SPME-GC-MS, respectively, and the differences of volatile compounds in dry-cured ham from different regions were compared by multivariate statistical analysis so as to further determine the characteristic volatile flavor compounds of different types of dry-cured hams. The results of this study can provide an important theoretical basis for the quality control of dry-cured ham in different regions of Yunnan province.

2 Materials and methods

2.1 Samples

Five types of Yunnan dry-cured hams (X: Xuanwei ham; S: Sanchuan ham; N: Nuodeng ham; B: Saba ham; H: Heqing ham), a total of 30 hams, 6 hams from each region, were provided by Xuanwei Puji Huotui Food Co., Ltd., Lijiang Sanchuan Huotui Co., Ltd., Dali Yunlong Nuodeng Huotui Food Factory, Kunming Jianguo Saba Huotui Co., Ltd., and Heqing Yixiang Food Co., Ltd., respectively. The process of five Yunnan dry-cured hams usually involved five stages, including fresh legs selection, natural cooling and trimming, salting, hanging air drying and ripening. In particular, after drying, Sanchuan ham needed to be placed in a pool containing plant ash for further ripening and completely covered with plant ash. Nuodeng ham also needed to be sprayed with a local Nuodeng homebrew (over 50% alcohol content) after trimming. All the experimental hams were processed from November 2019 to November 2021. The *Biceps femoris* (BF) muscle of each ham was obtained as a sample. After removing the subcutaneous fat and connective tissue, the samples were crushed, vacuum-packed and stored at −20 ℃ until analysis.

2.2 HS-GC-IMS analysis

According to our previous procedure[17], the VOCs of ham samples were identified and analyzed by IMS commercial instrument (FlavourSpec®) from Gesellschaft für Analytische Sensorysteme mbH (Dortmund, Germany). The instrument was equipped with an autosampler (CTC Analytics AG, Zwingen, Switzerland), headspace sampling unit and 1 mL gas-tight syringe (Gerstel GmbH, Mühlheim, Germany). The GC was equipped with a FS-SE-54-CB capillary column (15 m × 0.53 mm). One gram of minced ham sample was transferred into a 20 mL headspace vial, and then incubated at 60 ℃ for 20 min. Then, 500 μL headspace was injected by a heated syringe (65 ℃) into the heated injector in splitless mode. Nitrogen of 99.99% purity was used as a carrier gas at a programmed flow as follows: 2 mL/min for 2 min, 20 mL/min for 8 min, and 100 mL/min for 10 min. The analytes were eluted and separated at 45 ℃, and ionized in the IMS ionization chamber by a 3H ionization source (300 MBq activity) in a positive ion mode. Drift tube (9.8 cm) was operated at a constant voltage (5 kV) at 45 ℃ with a nitrogen flow of 150 mL/min. Each spectrum was reported as the average of 12 scans. The identification of VOCs was based on comparing retention index (RI) and the drift time with the GC-IMS library. The final results were the averages of six replicates.

2.3 HS-SPME-GC-MS analysis

According to our previous study[1], with minor modifications. The SPME fiber (Supelco, Bellefonte, PA, USA) coated with divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, 50/30 μm) was employed to extract VOCs in Yunnan dry-cured ham. About 4 g fine ground sample was weighed into a 20 mL headspace vial, and 200 ng of 2-methyl-3-heptanone was added as the internal standard. Then the headspace vial was tightly closed with a Teflon/silicone septum and equilibrated in a water bath at 50 ℃ for 15 min. After that, the SPME fiber was inserted into the headspace of the vial for extraction of VOCs at 50 ℃ for 30 min. After extraction, the VOCs were identified and quantified by a 7890B-5977B GC-MS instrument (Agilent Technologies Inc., Santa Clara, CA, USA). The VOCs were desorbed for 5 min at 250 ℃ in the GC injector and separated using an Agilent DB-wax capillary column (30 m × 0.25 mm , 0.25 μm). The oven temperature program was: initial temperature 40 ℃ for 5 min, then increased to 250 ℃ at 5 ℃/min, and held for 5 min. Pure helium (99.999%) was used as carrier gas, and the flow rate was 1.0 mL/min. The mass spectra were conducted in an electron impact mode at 70 eV, mass spectra were scanned from *m/z* 20 to 500, quadrupole temperature 150 ℃, ion source temperature 230 ℃, transmission line temperature 260 ℃. The VOCs were tentatively identified by comparing the mass spectra and RI in the standard NIST 2011 library, and the quantitative analysis of the VOCs was performed by comparing the peak areas with internal standards used for quantitative analysis. The final results were the averages of three replicates.

2.4 Statistical analysis

One-Way analysis of variance (ANOVA) was performed to assess differences in the samples at a level of $P < 0.05$ using the SPSS 25.0 software (SPSS Inc., Chicago, USA), and SIMCA 14.1 was used to perform multivariate statistical analysis of the data. The hierarchical cluster analysis (HCA) was used to characterize the similarities and differences of VOCs between different dry-cured ham samples, and the partial least squares-discriminant analysis (PLS-DA) was used to evaluate the key VOCs.

3 Results and discussion

3.1 HS-GC-IMS topographic plots of five types of Yunnan dry-cured hams

The HS-GC-IMS was applied to obtain global IMS information on VOCs from five types of dry-cured ham samples. The 3D-topographical visualization of VOCs in five types of Yunnan dry-cured ham samples are shown in Figure 1A. We could intuitively compare the differences of VOCs in dry-cured ham from different regions in Yunnan Province. The 2D-topographic plot of HS-GC-IMS specctra of different ham samples are depicted in Figure 1B. The red line parallel to the *Y*-axis at the scale of 1.0 on the *X*-axis represented the reactive ion peak (RIP, normalized). Each point on the right of RIP represented a volatile compound extracted from the samples, and the signal intensity of the substance was indicated by the color. The white color corresponded to a relatively low intensity of volatile compounds, while red corresponded to a higher intensity, and the darker the color, the

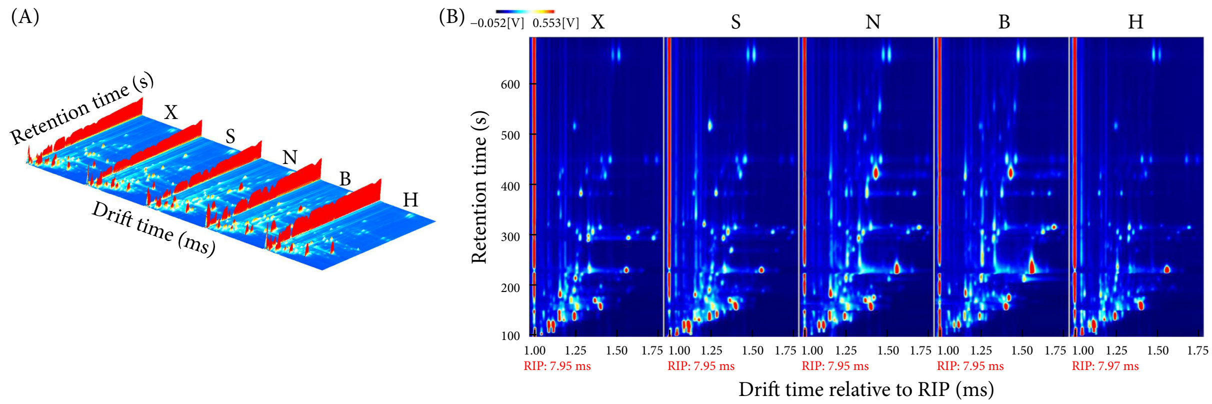

Figure 1 The (A) 3D-topographic plot and (B) 2D-topographic plot of volatile compounds in five types of Yunnan dry-cured hams. The *Y*-axis represents the retention time of the gas chromatograph, and the *X*-axis represents the ion drift time for identification. RIP, reactive ion peak.

higher the intensity[18]. It can be seen that the VOCs of dry-cured ham from different areas in Yunnan province could be well separated by HS-GC-IMS, and the differences of VOCs in different hams could be visually recognized. Among the five types of Yunnan dry-cured hams, the signal intensity of VOCs in Heqing ham was lower, while that in Nuodeng ham and Saba ham was higher.

3.2 Qualitative analysis of VOCs by HS-GC-IMS

The qualitative analysis results of the VOCs in five types of Yunnan dry-cured hams are shown in Figure 2A and Table 1. The spots in Figure 2A are labeled with corresponding numbers, which is consistent with the VOCs in Table 1. A total of 41 VOCs were identified qualitatively from five dry-cured ham samples, including 18 aldehydes, 10 alcohols, 7 ketones, 4 esters, 1 acid and 1 furan. Li et al.[19] used HS-GC-IMS to analyze VOCs in six types of Chinese dry-cured hams, and 45 VOCs were identified, the largest number of which were aldehydes and alcohols, similar to the results of this study.

3.3 The fingerprint analysis of five types of Yunnan dry-cured hams by HS-GC-IMS

In order to make a more comprehensive comparison of VOCs in dry-cured ham from different areas, it is necessary to compare their fingerprints. As shown in Figure 2B, in five Yunnan dry-cured hams, a total of 64 VOCs were detected, of which 41 were qualitative. Most of the VOCs correspond to different signal intensities in the five types of dry-cured hams, indicating that hams from different regions had their own characteristics. To provide a more clear view of the differences in VOCs among different samples, we discussed the fingerprint spectrum in terms of categories.

The majority of aldehydes are produced by the oxidation of unsaturated fatty acids, with a very tiny amount coming through the Maillard reaction[20]. These substances are the key compounds of ham flavor, have low thresholds for perception, and have a typically fruity taste[21]. In Nuodeng ham, Saba ham and Heqing ham, the signal intensities of heptanal-monomer (M), hexanal, 2-methylbutanal and 3-methylbutanal were higher. 2-Methylbutanal and 3-methylbutanal belong to the methyl-branched aldehydes with strong volatility, and they may be degraded by fat and carbohydrate metabolism or produced by protein decomposition in meat[22–23]. Meanwhile, Nuodeng ham and Saba ham contained more abundant aldehydes, such as nonanal, octanal, (*E*)-2-octenal, (*E*)-2-heptanal, heptanal-dimer (D), pentanal-D and methylpropanal. Nonanal has a sweet and fruity aroma, while octanal has an oily and spicy taste[24].The signal intensities of butanal, phenylacetaldehyde and furfurol were higher in Sanchuan ham, while Xuanwei ham contained

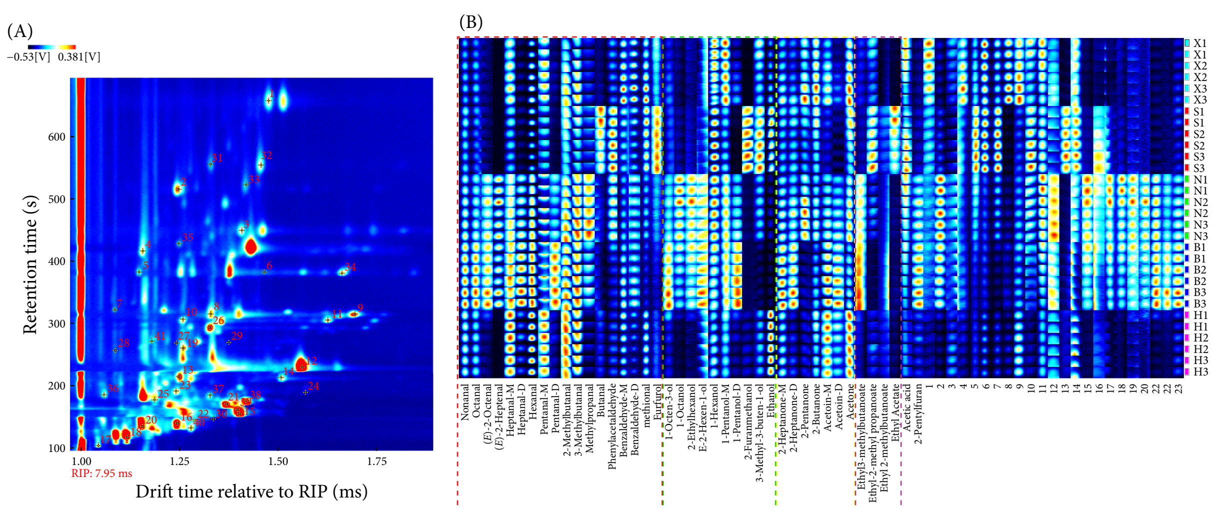

Figure 2 The GC-IMS spectra from five types of Yunnan dry-cured ham samples. (A) The 2D-topographic plot, each number represents a volatile compound corresponding to Table 1. (B) The gallery plot, it includes all signals that can be detected in the instrument, in which the *Y*-axis represents the sample and the *X*-axis represents the compound. Known compounds are labeled with existing names and unknown compounds are labeled with numbers.

Table 1 GC-IMS integration parameters of volatile compounds in five types of Yunnan dry-cured hams.

No.	Compound	CAS	Formula	Relative molecular mass	RI	Retention time (s)	Drift time relative to RIP (ms)	Comment
1	Nonanal	C124196	$C_9H_{18}O$	142.2	1 104.0	657.241	1.478 0	
2	Phenylacetaldehyde	C122781	C_8H_8O	120.2	1 039.7	514.665	1.249 7	
3	Octanal	C124130	$C_8H_{16}O$	128.2	1 005.8	448.984	1.410 6	
4	1-Octen-3-ol	C3391864	$C_8H_{16}O$	128.2	985.8	415.558	1.159 2	
5	Benzaldehyde	C100527	C_7H_6O	106.1	962.4	381.906	1.149 5	Monomer
6	Benzaldehyde	C100527	C_7H_6O	106.1	962.4	381.906	1.467 5	Dimer
7	Methional	C3268493	C_4H_8OS	104.2	908.4	320.630	1.087 0	
8	Heptanal	C111717	$C_7H_{14}O$	114.2	901.6	314.084	1.331 3	Monomer
9	Heptanal	C111717	$C_7H_{14}O$	114.2	901.3	313.755	1.698 4	Dimer
10	2-Heptanone	C110430	$C_7H_{14}O$	114.2	892.8	305.871	1.260 8	Monomer
11	2-Heptanone	C110430	$C_7H_{14}O$	114.2	891.0	304.229	1.629 1	Dimer
12	Hexanal	C66251	$C_6H_{12}O$	100.2	792.5	228.346	1.563 3	
13	1-Pentanol	C71410	$C_5H_{12}O$	88.1	769.5	213.172	1.250 5	Monomer
14	1-Pentanol	C71410	$C_5H_{12}O$	88.1	767.4	211.828	1.510 2	Dimer
15	3-Methylbutanal	C590863	$C_5H_{10}O$	86.1	647.9	153.353	1.405 1	
16	2-Butanone	C78933	C_4H_8O	72.1	599.4	138.566	1.246 6	
17	Ethanol	C64175	C_2H_6O	46.1	472.0	102.047	1.046 3	
18	Acetone	C67641	C_3H_6O	58.1	521.2	116.162	1.119 2	
19	Ethyl-3-methylbutanoate	C108645	$C_7H_{14}O_2$	130.2	836.3	259.460	1.261 2	
20	Acetic acid	C64197	$C_2H_4O_2$	60.1	581.6	133.470	1.157 2	
21	2-Pentanone	C107879	$C_5H_{10}O$	86.1	689.8	169.260	1.370 8	
22	Butanal	C123728	C_4H_8O	72.1	607.3	140.844	1.291 1	
23	3-Methyl-3-buten-1-ol	C763326	$C_5H_{10}O$	86.1	731.4	190.114	1.243 3	
24	Ethyl-2-methylpropanoate	C97621	$C_6H_{12}O_2$	116.2	727.0	187.650	1.572 0	
25	Pentanal	C110623	$C_5H_{10}O$	86.1	704.6	176.036	1.188 7	
26	1-Hexanol	C111273	$C_6H_{14}O$	102.2	877.0	291.977	1.328 8	
27	Ethyl-2-methylbutanoate	C7452791	$C_7H_{14}O_2$	130.2	847.0	267.640	1.244 7	
28	Furfurol	C98011	$C_5H_4O_2$	96.1	830.5	255.161	1.088 1	
29	2-Furanmethanol	C98000	$C_5H_6O_2$	98.1	848.0	268.372	1.377 9	
30	Ethyl acetate	C141786	$C_4H_8O_2$	88.1	620.8	144.814	1.338 1	
31	(*E*)-2-Octenal	C2548870	$C_8H_{14}O$	126.2	1058.5	554.996	1.330 5	
32	1-Octanol	C111875	$C_8H_{18}O$	130.2	1058.1	554.124	1.459 1	
33	2-Ethylhexanol	C104767	$C_8H_{18}O$	130.2	1043.1	521.851	1.421 8	
34	(*E*)-2-Heptenal	C18829555	$C_7H_{12}O$	112.2	960.7	379.629	1.665 2	
35	2-Pentylfuran	C3777693	$C_9H_{14}O$	138.2	993.5	427.892	1.250 4	
36	Acetoin	C513860	$C_4H_8O_2$	88.1	721.1	184.479	1.060 0	Monomer
37	Acetoin	C513860	$C_4H_8O_2$	88.1	718.5	183.080	1.329 5	Dimer
38	Pentanal	C110623	$C_5H_{10}O$	86.1	701.9	174.767	1.423 6	
39	2-Methylbutanal	C96173	$C_5H_{10}O$	86.1	666.7	159.957	1.400 4	
40	Methylpropanal	C78842	C_4H_8O	72.1	571.9	130.690	1.281 4	
41	(*E*)-2-Hexen-1-ol	C928950	$C_6H_{12}O$	100.2	851.0	270.747	1.181 5	

Note: RI represents retention index calculated in the experiment.

slightly higher benzaldehyde. For alcohols, the signal intensities of 1-octen-3-ol, 1-octanol, 2-ethylhexanol, (*E*)-2-hexen-1-ol and 1-pentanol-D were higher in Nuodeng ham and Saba ham. The signal intensities of 2-furanmethanol and 3-methyl-3-butene-1-ol were higher in Sanchuan ham, while the Heqing ham contained more abundant ethanol. Alcohols have a relatively high threshold, which has minimal effect on the flavor of dry-cured ham, but individual unsaturated alcohols, such 1-octen-3-ol, have a very low threshold, which significantly affects the flavor of dry-cured ham[25]. For ketones, the signal intensities of 2-heptanone and acetoin were higher in Saba ham, 2-pentanone was higher in Nuodeng ham, and acetone was higher in Heqing ham. They are usually associated with creamy and fruity flavors, especially 2-ketones, which play an important role in the flavor of meat products[26–27]. Acetoin has also been reported to have a buttery note[19]. As for esters, they are mainly derived from the esterification of carboxylic acids and alcohols, which gives the dry-cured ham a fruity and sweet flavor[1]. Nuodeng ham and Saba ham contained more ethyl 3-methylbutanoate, while ethyl-2-methyl propanoate and ethyl acetate were higher in Sanchuan ham. Finally, higher concentrations of acetic acid were found in Xuanwei ham and Sanchuan ham, and 2-pentylfuran was higher in Saba ham. Thus, compared to the other three dry-cured hams, the relative content of aldehydes and alcohols was higher in Nuodeng ham and Saba ham, and the fingerprint profiles were similar between them.

3.4 Analysis of VOCs in five types of Yunnan dry-cured hams by HS-SPME-GC-MS

The results of the HS-SPME-GC-MS analysis of VOCs in five types of Yunnan dry-cured hams are shown in Table 2. In total, 128

Table 2 The content of volatile compounds in five types of Yunnan dry-cured hams (ng/g).

Compounds	X	S	N	B	H
Aldehydes					
Acetaldehyde	0.21 ± 0.19^b	0.88 ± 0.08^a	0.33 ± 0.06^b	0.42 ± 0.12^b	0.81 ± 0.09^a
Propanal	0.66 ± 0.03^c	0.76 ± 0.09^c	15.40 ± 1.46^b	20.07 ± 1.48^a	1.07 ± 0.41^c
Methional	8.18 ± 0.22^a	8.56 ± 1.33^a	2.68 ± 0.36^b	0.96 ± 0.06^c	ND
Butanal	0.46 ± 0.05^c	ND	1.77 ± 0.20^b	2.34 ± 0.47^a	ND
2-Methyl-butanal	29.59 ± 1.26^c	65.74 ± 5.94^a	43.33 ± 4.50^b	9.13 ± 1.59^d	28.45 ± 3.51^c
2-Methyl-propanal	6.83 ± 0.21^c	15.86 ± 1.10^a	10.62 ± 1.33^b	2.06 ± 0.22^d	7.49 ± 0.74^c
3-Methyl-butanal	57.36 ± 4.26^b	115.78 ± 7.66^a	85.36 ± 11.22^{ab}	18.97 ± 1.81^b	61.35 ± 7.03^b
Hexanal	189.90 ± 6.00^{bc}	169.25 ± 14.94^c	1 082.95 ± 109.55^a	1 222.64 ± 120.67^a	321.89 ± 53.74^b
Heptanal	3.14 ± 0.10^c	3.26 ± 0.58^c	7.87 ± 0.78^a	8.71 ± 1.07^a	4.54 ± 0.32^b
(*Z*)-2-Heptenal	0.62 ± 0.15^c	0.52 ± 0.02^c	7.75 ± 0.69^a	6.31 ± 0.58^b	0.54 ± 0.14^c
Octanal	11.06 ± 0.50^c	10.94 ± 1.84^c	16.98 ± 0.87^b	19.98 ± 1.26^a	13.31 ± 1.52^c
(*E*)-2-Octenal	0.65 ± 0.14^b	0.74 ± 0.21^b	11.74 ± 1.41^a	10.44 ± 3.88^a	1.18 ± 0.85^b
Nonanal	6.52 ± 0.66^c	10.50 ± 1.40^b	12.41 ± 0.89^a	12.39 ± 0.63^a	13.75 ± 1.05^a
(*E,E*)-2,4-Nonadienal	0.22 ± 0.02^c	0.35 ± 0.01^c	7.24 ± 1.12^a	5.03 ± 0.84^b	0.90 ± 0.46^c
2,4-Decadienal	ND	ND	1.23 ± 0.32^a	0.88 ± 0.21^a	ND
(*E,E*)-2,4-Decadienal	0.64 ± 0.07^c	1.11 ± 0.14^c	15.77 ± 2.57^a	9.54 ± 1.34^b	1.79 ± 1.00^c
Pentadecanal	6.06 ± 1.14^a	1.88 ± 0.53^c	0.88 ± 0.20^c	1.74 ± 0.08^c	4.25 ± 0.30^b
Benzaldehyde	20.27 ± 0.38^a	17.13 ± 2.23^b	22.28 ± 2.33^a	15.35 ± 0.24^b	13.04 ± 0.24^{bc}
3-Ethyl-benzaldehyde	ND	ND	0.26 ± 0.02^a	0.30 ± 0.08^a	ND
Benzeneacetaldehyde	17.94 ± 1.18^b	41.34 ± 7.57^a	43.30 ± 5.84^a	9.7 ± 0.53^c	19.52 ± 2.05^b
Total	360.31 ± 16.46^c	464.60 ± 45.67^b	1 390.15 ± 145.72^a	1 376.96 ± 137.41^a	493.88 ± 73.83^b
Alcohols					
Ethanol	61.17 ± 3.58^c	500.33 ± 26.76^a	12.23 ± 1.76^d	4.60 ± 0.37^d	379.67 ± 28.29^b
1-Propanol	2.44 ± 0.06^b	24.43 ± 0.87^a	2.14 ± 0.18^b	2.64 ± 0.66^b	2.03 ± 0.06^b
1-Pentanol	48.27 ± 4.64^c	16.67 ± 1.04^d	79.87 ± 6.87^b	115.67 ± 10.69^a	26.07 ± 2.56^d
3-Methyl-1-butanol	1.09 ± 0.10^c	4.40 ± 0.18^a	0.63 ± 0.09^c	0.19 ± 0.02^c	3.52 ± 0.54^b
1-Hexanol	30.53 ± 1.21^b	32.00 ± 5.93^b	60.27 ± 6.90^a	55.00 ± 8.72^a	36.37 ± 1.90^b
1-Heptanol	6.56 ± 0.13^c	6.33 ± 1.13^c	12.90 ± 0.62^b	15.75 ± 0.73^a	6.04 ± 1.34^c
1-Octanol	4.81 ± 0.30^b	5.19 ± 1.19^b	10.40 ± 0.55^a	11.60 ± 1.73^a	6.60 ± 0.63^b
2,4,4-Trimethyl-1-pentanol	5.75 ± 0.51^b	11.17 ± 0.72^a	3.40 ± 0.90^b	3.45 ± 0.78^b	7.31 ± 0.98^{ab}
2-Ethyl-1-hexanol	2.58 ± 0.27^b	2.55 ± 0.03^b	1.96 ± 0.13^c	1.64 ± 0.35^c	3.81 ± 0.26^a

Table 2 (*Continued*)

Compounds	X	S	N	B	H
2-Octanol	0.43 ± 0.08[c]	1.02 ± 0.08[b]	ND	ND	1.41 ± 0.28[a]
(*R*)-2-Octanol	3.68 ± 0.72[ab]	4.72 ± 0.53[a]	2.50 ± 0.84[b]	4.28 ± 1.31[ab]	4.98 ± 0.63[a]
3-Methyl-3-buten-1-ol	1.09 ± 0.14[b]	0.89 ± 0.14[b]	ND	ND	2.29 ± 0.50[a]
1-Penten-3-ol	2.63 ± 0.08[a]	3.30 ± 0.35[a]	2.60 ± 0.72[a]	2.06 ± 0.36[a]	ND
(*Z*)-2-Penten-1-ol	2.06 ± 0.36[b]	2.35 ± 0.31[b]	6.48 ± 0.89[a]	8.02 ± 1.46[a]	2.54 ± 0.74[b]
1-Octen-3-ol	108.00 ± 1.00[b]	96.53 ± 12.08[b]	352.67 ± 23.97[a]	354.67 ± 20.11[a]	77.40 ± 11.87[bc]
(*E*)-2-Octen-1-ol	1.16 ± 0.03[c]	0.64 ± 0.09[d]	3.11 ± 0.13[b]	3.63 ± 0.33[a]	0.73 ± 0.14[d]
2,2-Dichloro-ethanol	59.67 ± 1.51[a]	ND	9.58 ± 0.89[b]	ND	10.60 ± 1.25[b]
Methanethiol	5.38 ± 0.18[a]	3.73 ± 0.08[b]	1.50 ± 0.16[c]	1.15 ± 0.18[c]	3.68 ± 0.68[b]
Phenylethyl alcohol	ND	4.43 ± 0.77[a]	1.09 ± 0.19[c]	1.24 ± 0.17[c]	2.50 ± 0.43[b]
Total	347.3 ± 15.14[c]	720.68 ± 55.06[a]	563.33 ± 45.79[b]	585.59 ± 47.72[b]	577.55 ± 53.08[b]
Ketones					
1-Hydroxy-2-butanone	2.21 ± 0.15[b]	2.29 ± 0.18[b]	2.52 ± 0.20[ab]	2.96 ± 0.83[ab]	3.42 ± 0.93[a]
2,3-Pentanedione	ND	ND	14.66 ± 2.64[b]	25.22 ± 1.41[a]	1.50 ± 0.54[c]
Acetone	41.67 ± 3.62[a]	42.43 ± 6.13[a]	19.24 ± 1.81[b]	12.72 ± 3.83[b]	45.75 ± 3.04[a]
2-Heptanone	43.88 ± 6.75[b]	74.59 ± 7.55[a]	45.13 ± 3.78[b]	43.45 ± 8.60[b]	28.68 ± 2.74[c]
2-Octanone	3.45 ± 0.10[b]	15.10 ± 3.97[a]	4.75 ± 0.28[b]	2.71 ± 0.35[b]	3.75 ± 1.02[b]
2-Pentanone	15.62 ± 2.61[a]	12.95 ± 1.04[b]	8.13 ± 0.99[c]	ND	ND
3-Octen-2-one	ND	ND	27.36 ± 2.77[a]	17.02 ± 2.78[b]	0.99 ± 0.30[c]
6-Methyl-5-hepten-2-one	6.11 ± 0.35[a]	1.86 ± 0.29[b]	0.93 ± 0.06[b]	2.51 ± 2.49[b]	1.52 ± 0.12[b]
(*E*,*E*)-3,5-Octadien-2-one	ND	ND	5.15 ± 0.76[a]	2.93 ± 0.61[b]	0.48 ± 0.16[c]
Acetoin	31.57 ± 0.81[b]	15.77 ± 0.20[c]	24.41 ± 2.96[b]	26.98 ± 7.93[b]	46.97 ± 6.14[a]
2-Butanone	21.93 ± 0.50[a]	19.93 ± 1.75[b]	7.57 ± 0.50[d]	2.72 ± 0.67[e]	15.06 ± 0.42[c]
Total	166.44 ± 15.03[b]	184.92 ± 21.15[a]	159.85 ± 16.84[b]	139.22 ± 29.93[c]	148.12 ± 15.60[c]
Acids					
Acetic acid	71.32 ± 1.12[c]	259.26 ± 1.21[a]	56.76 ± 2.26[d]	14.85 ± 0.98[e]	97.14 ± 2.12[b]
Propanoic acid	3.52 ± 0.24[b]	11.20 ± 0.33[a]	2.18 ± 0.19[c]	1.45 ± 0.27[d]	3.51 ± 0.04[b]
Butanoic acid	255.91 ± 0.36[a]	98.71 ± 2.04[c]	50.78 ± 1.44[d]	36.29 ± 1.58[e]	163.15 ± 2.37[b]
Hexanoic acid	211.08 ± 2.26[c]	126.78 ± 10.87[e]	335.53 ± 9.15[a]	247.42 ± 5.92[b]	150.54 ± 9.27[d]
Heptanoic acid	4.54 ± 0.37[b]	4.54 ± 0.93[b]	8.08 ± 1.19[a]	3.90 ± 0.29[b]	3.59 ± 0.42[b]
Octanoic acid	10.42 ± 0.70[c]	20.43 ± 1.35[a]	21.41 ± 1.12[a]	8.24 ± 1.46[d]	15.56 ± 0.60[b]
Nonanoic acid	1.75 ± 0.09[c]	3.82 ± 0.45[a]	2.50 ± 0.15[b]	1.28 ± 0.23[c]	2.26 ± 0.25[b]
n-Decanoic acid	1.42 ± 0.38[cd]	3.11 ± 1.00[b]	4.78 ± 0.56[a]	0.91 ± 0.18[d]	2.42 ± 0.45[bc]
Pentanoic acid	27.29 ± 0.66[b]	16.15 ± 0.91[c]	30.63 ± 1.80[a]	16.14 ± 1.13[c]	15.83 ± 1.58[c]
2-Methyl-propanoic acid	1.63 ± 0.06[c]	24.27 ± 1.03[a]	4.83 ± 0.69[b]	1.25 ± 0.04[c]	22.89 ± 2.23[a]
(*Z*)-2-Methyl-2-butenedioic acid	0.21 ± 0.01[c]	ND	0.79 ± 0.08[a]	0.64 ± 0.11[b]	ND
Methoxy-acetic acid	1.05 ± 0.07[a]	0.84 ± 0.08[b]	0.80 ± 0.11[b]	0.92 ± 0.09[ab]	1.08 ± 0.07[a]
Methylene-butanedioic acid	1.38 ± 0.04[c]	0.13 ± 0.01[d]	3.22 ± 0.06[b]	4.42 ± 0.58[a]	1.10 ± 0.04[c]
2-Methyl-butanoic acid	4.27 ± 0.20[d]	45.31 ± 2.83[a]	11.68 ± 1.21[c]	2.37 ± 0.07[d]	33.93 ± 3.87[b]
3-Methyl-butanoic acid	14.24 ± 0.82[d]	215.14 ± 5.40[a]	63.93 ± 1.13[c]	8.48 ± 0.15[d]	187.93 ± 14.78[b]
Total	610.03 ± 11.27[c]	829.69 ± 35.26[a]	597.9 ± 21.7[c]	348.56 ± 15.97[d]	700.93 ± 39.62[b]
Esters					
5-Butyldihydr-2(3*H*)-furanone	4.46 ± 0.09[a]	3.70 ± 0.60[ab]	4.40 ± 0.60[a]	3.17 ± 0.37[bc]	2.53 ± 0.11[c]
5-Ethyldihydro-2(3*H*)-furanone	9.96 ± 0.20[b]	7.35 ± 0.63[c]	16.30 ± 1.21[a]	6.93 ± 0.51[cd]	6.01 ± 0.13[d]

Table 2 (*Continued*)

Compounds	X	S	N	B	H
Dihydro-5-methyl-2(3*H*)-furanone	1.36 ± 0.10^{a}	0.88 ± 0.07^{c}	1.50 ± 0.12^{a}	0.40 ± 0.03^{d}	1.06 ± 0.08^{b}
Dihydro-5-pentyl-2(3*H*)-furanone	2.03 ± 0.11^{c}	3.99 ± 1.24^{b}	6.39 ± 1.27^{a}	1.73 ± 0.07^{c}	2.89 ± 0.27^{bc}
Tetrahydro-6-methyl-2*H*-pyran-2-one	0.70 ± 0.02^{a}	0.31 ± 0.06^{c}	0.45 ± 0.03^{b}	0.15 ± 0.05^{d}	0.74 ± 0.09^{a}
Ethyl ester 2-methyl-butanoic acid	ND	1.49 ± 0.28^{a}	ND	ND	0.25 ± 0.06^{b}
Ethyl ester 3-methyl-butanoic acid	ND	2.37 ± 0.14^{a}	ND	ND	0.74 ± 0.04^{b}
Ethyl ester butanoic acid	1.02 ± 0.02^{c}	3.14 ± 0.17^{a}	ND	ND	1.92 ± 0.10^{b}
Butyrolactone	7.77 ± 0.10^{b}	15.93 ± 0.91^{a}	3.11 ± 0.21^{d}	2.12 ± 0.32^{e}	5.84 ± 0.39^{c}
Ethyl acetate	ND	17.21 ± 1.03^{a}	ND	ND	2.43 ± 0.19^{b}
2-Propylpentyl ester formic acid	0.54 ± 0.03^{c}	0.27 ± 0.03^{d}	1.09 ± 0.12^{a}	0.94 ± 0.01^{b}	0.33 ± 0.08^{d}
Ethyl ester hexanoic acid	0.55 ± 0.14^{c}	2.98 ± 0.27^{a}	0.38 ± 0.04^{c}	0.01 ± 0.00^{d}	1.14 ± 0.22^{b}
Ethyl ester linoleic acid	0.47 ± 0.02^{a}	0.26 ± 0.02^{b}	ND	0.10 ± 0.01^{c}	0.22 ± 0.04^{b}
n-Caproic acid vinyl ester	12.66 ± 0.60^{c}	17.75 ± 2.31^{c}	80.69 ± 10.07^{b}	134.95 ± 14.42^{a}	21.56 ± 1.31^{c}
Ethyl ester octanoic acid	ND	0.92 ± 0.17^{a}	ND	ND	0.54 ± 0.03^{b}
Monomethyl ester pentanedioic acid	0.28 ± 0.00^{b}	0.37 ± 0.08^{a}	0.15 ± 0.01^{c}	0.20 ± 0.01^{c}	ND
Total	41.8 ± 1.48^{d}	78.92 ± 8.13^{c}	114.46 ± 13.68^{b}	150.7 ± 15.84^{a}	48.2 ± 3.16^{d}
Hydrocarbons					
Pentane	7.63 ± 1.01^{c}	10.11 ± 1.14^{bc}	13.24 ± 2.64^{b}	20.21 ± 2.16^{a}	11.01 ± 2.97^{bc}
n-Hexane	2.62 ± 0.09^{c}	3.53 ± 0.11^{a}	1.24 ± 0.26^{d}	1.35 ± 0.27^{d}	3.11 ± 0.04^{b}
Heptane	6.05 ± 0.60^{c}	12.35 ± 0.83^{a}	2.83 ± 0.47^{e}	4.73 ± 0.41^{d}	7.31 ± 0.95^{b}
Octane	62.62 ± 8.27^{b}	111.48 ± 2.05^{a}	16.60 ± 1.78^{c}	16.36 ± 1.74^{c}	67.63 ± 4.54^{b}
Decane	49.44 ± 6.77^{c}	69.20 ± 5.47^{b}	39.78 ± 9.22^{cd}	29.58 ± 1.24^{d}	84.75 ± 9.66^{a}
Dodecane	5.02 ± 0.70^{a}	5.98 ± 0.53^{a}	3.74 ± 0.37^{b}	3.31 ± 1.03^{b}	6.28 ± 0.62^{a}
Tridecane	0.11 ± 0.01^{a}	0.10 ± 0.01^{a}	0.06 ± 0.01^{b}	0.05 ± 0.01^{b}	0.11 ± 0.01^{a}
Hexadecane	4.98 ± 0.46^{bc}	5.88 ± 0.73^{bc}	4.90 ± 0.28^{c}	6.97 ± 2.07^{ab}	8.72 ± 0.63^{a}
Heptadecane	1.33 ± 0.09^{b}	1.24 ± 0.15^{b}	1.37 ± 0.43^{b}	2.94 ± 0.29^{a}	3.17 ± 0.19^{a}
Octyl-cyclopropane	0.16 ± 0.01^{c}	0.11 ± 0.03^{c}	0.33 ± 0.03^{b}	0.45 ± 0.10^{a}	0.38 ± 0.06^{ab}
Cyclopentane	ND	ND	0.52 ± 0.05^{b}	0.77 ± 0.15^{a}	ND
1-Nitro-hexane	ND	ND	1.49 ± 0.10^{b}	2.13 ± 0.50^{a}	2.07 ± 0.33^{a}
2,2,4,6,6-Pentamethyl-heptane	360.51 ± 59.72^{c}	571.23 ± 61.68^{b}	312.65 ± 90.78^{c}	240.64 ± 16.18^{c}	716.23 ± 116.17^{a}
2,6-Dimethyl-octane	2.05 ± 0.03^{c}	7.05 ± 0.70^{a}	2.21 ± 0.42^{c}	3.20 ± 0.49^{b}	3.20 ± 0.26^{b}
2,2,4,4-Tetramethyloctane	75.69 ± 11.18^{b}	108.48 ± 10.46^{a}	57.40 ± 17.24^{bc}	39.16 ± 2.74^{c}	130.42 ± 20.67^{a}
2,3,6-Trimethyl-decane	1.63 ± 0.36^{b}	1.70 ± 0.16^{b}	1.31 ± 0.27^{b}	1.22 ± 0.54^{b}	2.39 ± 0.33^{a}
4-Ethyl-octane	1.63 ± 0.24^{c}	3.09 ± 0.27^{b}	1.40 ± 0.38^{c}	1.85 ± 0.28^{c}	4.38 ± 0.62^{a}
1-Chloro-octane	0.62 ± 0.15^{c}	0.67 ± 0.08^{c}	0.89 ± 0.01^{b}	0.72 ± 0.07^{c}	1.16 ± 0.05^{a}
2,3-Dimethyl-nonane	1.08 ± 0.05^{a}	1.41 ± 0.38^{a}	0.64 ± 0.15^{b}	0.46 ± 0.27^{b}	1.44 ± 0.19^{a}
3-Methylene-nonane	4.29 ± 0.92^{c}	8.05 ± 1.44^{b}	4.42 ± 1.33^{c}	3.26 ± 0.36^{c}	10.34 ± 1.22^{a}
5-Ethyldecane	4.67 ± 0.85^{b}	5.02 ± 0.41^{b}	3.40 ± 0.74^{c}	2.74 ± 0.36^{c}	6.34 ± 0.85^{a}
3-Methyl-undecane	14.40 ± 2.12^{b}	14.23 ± 1.57^{b}	11.27 ± 1.84^{b}	12.72 ± 2.29^{b}	18.88 ± 2.71^{a}
4-Methyl-dodecane	2.26 ± 0.26^{a}	1.11 ± 0.06^{b}	1.08 ± 0.22^{b}	2.36 ± 0.75^{a}	2.57 ± 0.32^{a}
Limonene	4.61 ± 0.34^{cd}	5.14 ± 0.33^{c}	6.19 ± 0.29^{b}	3.98 ± 0.85^{d}	8.37 ± 0.40^{a}
1-Heptene	0.28 ± 0.05^{c}	0.27 ± 0.03^{c}	0.59 ± 0.06^{ab}	0.67 ± 0.14^{a}	0.44 ± 0.07^{b}
1-Octene	0.55 ± 0.09^{c}	2.01 ± 0.12^{b}	0.82 ± 0.09^{c}	0.59 ± 0.13^{c}	3.59 ± 0.42^{a}
2-Octene	1.41 ± 0.23^{a}	ND	ND	ND	1.36 ± 0.33^{a}
Total	615.64 ± 105.54^{b}	949.44 ± 96.63^{a}	490.37 ± 138.83^{b}	402.42 ± 39.81^{c}	1 105.65 ± 177.43^{a}

Table 2 (*Continued*)

Compounds	X	S	N	B	H
Others					
Allomatrine	0.84 ± 0.05[c]	ND	2.60 ± 0.21[a]	1.26 ± 0.25[b]	ND
Benzene	0.93 ± 0.11[b]	1.04 ± 0.16[b]	1.17 ± 0.06[b]	1.22 ± 0.31[b]	1.63 ± 0.18[a]
Toluene	11.55 ± 0.99[a]	11.59 ± 0.98[a]	4.96 ± 0.34[c]	7.63 ± 2.50[b]	11.50 ± 0.70[a]
1,3-Dimethyl-benzene	2.08 ± 0.15[c]	ND	1.48 ± 0.17[c]	5.14 ± 1.06[a]	3.06 ± 0.22[b]
Ethyl ether	6.21 ± 1.34[a]	8.14 ± 1.91[a]	2.11 ± 0.18[b]	2.55 ± 0.67[b]	7.99 ± 1.11[a]
Tetrahydro-2-methyl-furan	ND	ND	3.63 ± 0.21[b]	9.46 ± 0.82[a]	1.36 ± 0.17[c]
2-Ethyl-furan	0.69 ± 0.15[c]	0.63 ± 0.06[c]	2.39 ± 0.14[b]	4.84 ± 0.69[a]	0.85 ± 0.15[c]
2-Pentyl-furan	6.85 ± 1.69[c]	11.20 ± 3.65[bc]	66.73 ± 11.51[a]	65.57 ± 4.87[a]	21.47 ± 3.15[b]
Heptanonitrile	2.90 ± 0.20[bc]	1.86 ± 0.30[c]	3.31 ± 0.26[b]	3.42 ± 1.22[b]	5.17 ± 0.40[a]
Hexanenitrile	50.50 ± 2.75[a]	8.93 ± 1.31[b]	6.12 ± 0.47[c]	3.02 ± 0.70[d]	11.59 ± 1.31[b]
3-Methyl-butanenitrile	4.98 ± 0.72[b]	6.42 ± 0.14[ab]	2.26 ± 0.34[c]	ND	7.52 ± 1.70[a]
Tetradecylamine	1.04 ± 0.23[a]	0.32 ± 0.06[c]	0.19 ± 0.01[c]	0.57 ± 0.12[b]	0.35 ± 0.04[c]
Formamide	0.70 ± 0.08[ab]	0.65 ± 0.10[ab]	0.58 ± 0.03[b]	ND	0.81 ± 0.21[a]
N-Benzylaniline	3.05 ± 0.38[c]	2.23 ± 0.3[c]	0.84 ± 0.16[d]	7.12 ± 1.11[a]	4.40 ± 0.77[b]
8-Chlorotheophylline	1.31 ± 0.05[b]	4.47 ± 0.27[a]	0.64 ± 0.02[c]	0.62 ± 0.11[c]	1.08 ± 0.09[b]
2,6-Dimethyl-pyrazine	2.55 ± 0.17[c]	14.94 ± 1.27[a]	2.08 ± 0.15[cd]	1.17 ± 0.03[d]	3.73 ± 0.36[b]
Trimethyl-pyrazine	3.52 ± 0.26[b]	22.32 ± 2.03[a]	3.65 ± 0.31[b]	1.54 ± 0.13[c]	2.80 ± 0.39[bc]
Pyridine	5.84 ± 0.68[b]	4.37 ± 0.68[b]	9.79 ± 1.31[b]	7.99 ± 0.53[b]	16.91 ± 6.32[a]
1-Methoxy-2-propanol	40.42 ± 1.71[a]	18.72 ± 1.33[b]	19.35 ± 1.93[b]	4.73 ± 0.72[c]	44.45 ± 4.93[a]
Anhydride 2-methyl-pentanoic acid	15.57 ± 1.16[c]	1.81 ± 0.25[d]	41.35 ± 2.36[b]	56.92 ± 5.12[a]	10.42 ± 1.66[cd]
Total	161.53 ± 12.87[b]	119.64 ± 14.82[c]	175.23 ± 20.17[a]	184.77 ± 20.96[a]	157.09 ± 23.86[b]

Note: Different lower case letters (a–d) in the same row indicate significant differences ($P < 0.05$). ND indicates not detected.

VOCs were isolated and identified, and they could be divided into seven categories, including 20 aldehydes, 19 alcohols, 11 ketones, 15 acids, 16 esters, 27 hydrocarbons and 20 other compounds.

3.4.1 Aldehydes

A total of 20 aldehydes were detected in the five types of Yunnan dry-cured hams, of which the total content of aldehyde compounds was higher in Nuodeng ham and Saba ham, at 1 390.15 and 1 376.96 ng/g, respectively. The content of hexanal was the highest in the five types of ham samples. Previous research showed that hexanal could be detected in most dry-cured hams, such as Istrain ham, Mianning ham, Sanchuan ham and Nuodeng ham[19,28]. Meanwhile, the content of hexanal was higher in Saba ham (1 222.64 ng/g) and Nuodeng ham (1 082.95 ng/g), which was significantly higher than that in Xuanwei ham, Sanchuan ham and Heqing ham ($P < 0.05$). Hexanal has been found to be the main odour-active compound in Iberian ham, which helps to increase the sweetness and aroma of grass[29]. As a result, the content of hexanal in Saba ham and Nuodeng ham may bring strong grassy aromas to the two types of dry-cured hams. Furthermore, Nuodeng ham and Saba ham also had greater contents of heptanal, (*Z*)-2-heptenal, octanal, (*E*)-2-octenal, (*E,E*)-2,4-nonadienal and (*E,E*)-2,4-decadienal; Sanchuan ham had greater contents of methional, 2-methyl-butanal, 2-methyl-propanal and 3-methyl-butanal, and Xuanwei ham had greater contents of pentadecanal. 3-Methyl-butanal is a branched Strecker aldehyde with a malty and toasted flavor[23]. This is related to the unique process of covering Sanchuan ham with plant ash. In this study, the contents of hexanal, 2-methyl-butanal and 3-methyl-butanal were relatively rich in these dry-cured hams from five regions.

3.4.2 Alcohols

The formation of alcohol compounds is closely related to the oxidation of fats and the reduction of ketones[30]. As shown in Table 2, Sanchuan ham had a significantly higher total alcohol content, and ethanol was more abundant than that in the other four types of dry-cured hams ($P < 0.05$). Liu et al.[31] also found abundant ethanol in Jinhua ham. Additionally, high concentration of 1-pentanol was found in Saba ham, while 1-hexanol was found in high concentrations in Nuodeng ham. 1-Octen-3-ol was the most abundant alcohol in Xuanwei ham (108.00 ng/g), Nuodeng ham (352.67 ng/g) and Saba ham (354.67 ng/g). Due to 1-octen-3-ol has a relatively low odor threshold, which may impart a more intense mushroom flavor to these three dry-cured hams[32]. However, the relatively low levels of 1-octen-3-ol in Sanchuan ham and Heqing ham might be due to the higher levels of ethanol in these two dry-cured hams.

3.4.3 Ketones

Sanchuan ham had the highest 2-heptanone level of any of the five types of dry-cured hams in our investigation. 2-Heptanone is a characteristic flavor compound in burnt meat[33], which may make a unique contribution to the flavor of Sanchuan ham. Furthermore, the content of 2,3-pentanedione was higher in Nuodeng ham and

Saba ham, acetoin was higher in Heqing ham, and 2-pentanone and 2-butanone were higher in Xuanwei ham.

3.4.4 Acids

The acid compounds mainly come from aldehyde oxidation and enzymatic lipolysis during dry-cured ham ripening, which has a high threshold[34]. In this study, many straight-chain aliphatic acids were detected, which could be derived from the degradation of phospholipids and triglycerides or lipid oxidation. The highest content of acetic acid was found in Sanchuan ham, which may be related to the growth and reproduction of microorganisms. Meanwhile, the highest content of hexanoic acid was found in Nuodeng ham. As a short-chain acid, hexanoic acid made an important contribution to the aroma of dry-cured ham[35].

3.4.5 Esters

The most esters were found in Sanchuan ham, and a total of 16 esters were detected. The *n*-caproic acid vinyl ester was the most abundant in the five types of dry-cured hams, with the highest content found in Saba ham. Ethyl ester hexanoic acid and ethyl ester octanoic acid, with sweet and fruity flavors, were mainly found in Sanchuan ham and Heqing ham but were rarely or not found in the other three dry-cured hams. Meanwhile, ethyl acetate was detected in both Sanchuan ham and Heqing ham, and its content was highest in Sanchuan ham. This may be related to the high content of ethanol and acetic acid in Sanchuan ham. Ethyl acetate has a fresh fruit aroma and is an important compound of the aroma of fermented foods[36].

3.4.6 Hydrocarbons

Most hydrocarbons are produced by the decomposition of lipids, which usually have a minor effect on the flavor of dry-cured ham due to their high odor thresholds[1]. In this study, 9 kinds of normal alkanes, 12 kinds of branched chain alkanes, 4 kinds of olefins and 2 kinds of naphthenics were detected in five types of dry-cured hams. Among them, 2,2,4,6,6-pentamethyl-heptane was the most abundant hydrocarbon in the five types of dry-cured hams. The *N*-alkanes may derive from the auto-oxidation of fat, branched chain alkanes may come from the auto-oxidation of branched chain fatty acids, and some unsaturated hydrocarbons may also come from the oxidation of unsaturated fatty acids[22]. Additionally, olefins can be used as flavor precursors for aldehydes and ketones, which may contribute to the flavor of dry-cured ham to some extent.

3.4.7 Others

The contents of furans were significantly higher in Nuodeng ham and Saba ham ($P < 0.05$). Furan compounds in food generally have the flavor of caramel and nuts[37]. The content of 2-pentyl-furan in Nuodeng ham and Saba ham was approximately 3–10 times higher than that in the other three dry-cured hams. 2-pentyl-furan, which has a fruity and buttery flavor, is probably derived from the oxidation of linoleic acid[38]. Several nitrogenous compounds, mainly derived from the catabolism of proteins, free amino acids and nucleic acids[39], were also found, such as 2,6-dimethyl-pyrazine, trimethyl-pyrazine and 8-chlorotheophylline. Interestingly, the levels of pyrazines in Sanchuan ham were significantly higher than those in the other four dry-cured hams ($P < 0.05$). The presence of pyrazines indicated that the Sanchuan ham had been subjected to a more severe heat treatment[1].

3.5 Multivariate statistical analysis of VOCs in five Yunnan dry-cured hams

The HCA results are shown in Figures 3A and B. The results of the HS-GC-IMS analysis and the HS-SPME-GC-MS analysis were consistent. The samples of Nuodeng ham and Saba ham were first clustered into one category, indicating a more similar composition

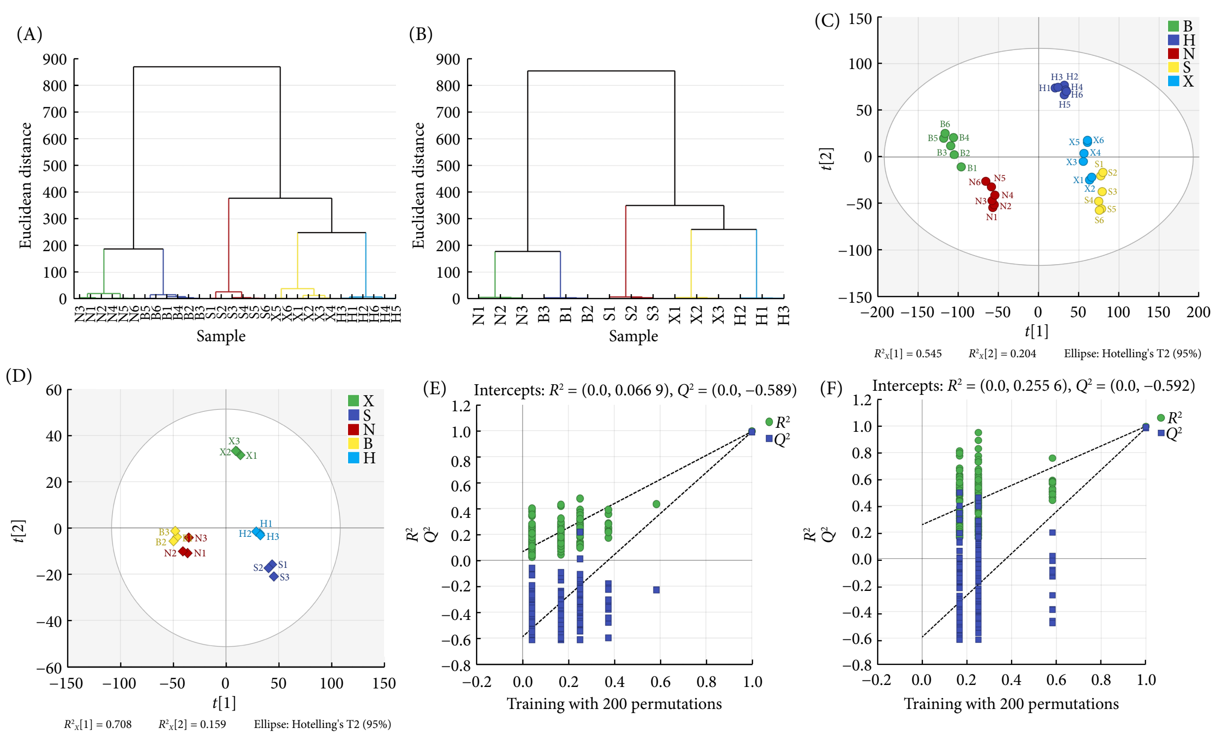

Figure 3 Multivariate statistical analysis of volatile compounds in five types of Yunnan dry-cured hams. (A) HCA, (C) PLS-DA and (E) permutations analysis based on GC-IMS data, respectively. (B) HCA, (D) PLS-DA and (F) permutations analysis based on GC-MS data, respectively.

of VOCs, which is consistent with the results of the fingerprint analysis. In addition, Xuanwei ham and Heqing ham were first clustered into one category and then clustered with Sanchuan ham. The distinctive processing of Sanchuan ham may be responsible for the specific trend it had shown. It has been proven that the processing technology is a significant contributor to the differences in VOCs in dry-cured hams[40].

PLS-DA is a supervised model, which can find the hidden characteristic variables that damage the robustness of the model and highlight the differences between groups[41]. The cross validation results of PLS-DA showed that R^2_X = 0.981, R^2_Y = 0.988, Q^2 = 0.984 in Figure 3C, and R^2_X = 0.991, R^2_Y = 0.992, Q^2 = 0.974 in Figure 3D, indicating that both models had a good classification predictive ability and stability. In order to identify whether the model was overfitting, the PLS-DA model was tested for validity using a permutation test with 200 repetitions. As shown in Figures 3E and F, both R^2 and Q^2 of the original models were greater than R^2 and Q^2 of the permutation random test model, and the intercept of the regression line of Q^2 with the vertical axis was less than 0, indicating that the original models had good stability and there was no overfitting phenomenon.

Variable importance in the projection (VIP) could be used to reflect the contribution of the variables of the PLS-DA model to the classification, with VIP ≥ 1 usually indicating a significant role in the discriminant process[42]. Based on HS-GC-IMS data, a total of 12 qualitatively differential characteristic markers with VIP > 1 were screened (Figure 4A), including 4 aldehydes, 4 alcohols, 3 ketones and 1 acid. The contents of hexanal in Saba ham, phenylacetaldehyde, butanal, 2-furanmethanol and acetic acid in Sanchuan ham, and 2-methylbutanal, ethanol and acetone in Heqing ham were higher. Meanwhile, the contents of 1-pentanol-M and 2-butanone were higher in Xuanwei ham, and 1-octanol and 2-pentanone were higher in Nuodeng ham. Based on HS-SPME-GC-MS data, a total of 26 differential characteristic markers with VIP > 1 were screened (Figure 4B), including 6 alcohols, 5 acids, 4 aldehydes, 4 ketones, 4 hydrocarbons, 1 ester and 2 other compounds. In Sanchuan ham, 2-heptanone, 1-propanol, acetic acid, 3-methyl-butanal and 2-methyl-butanal were present at high levels, with acetic acid and butanal also identified as characteristic markers for Sanchuan ham in the HS-GC-IMS analysis. 2,2-Dichloro-ethanol, butanoic acid and hexanenitrile were found in high concentrations in Xuanwei ham, while in Heqing ham, high concentrations of 2,2,4,6,6-pentamethyl-heptane, 2,2,4,4-tetramethyloctane, decane and acetoin were found. The composition of the characteristic markers for Nuodeng ham and Saba ham was similar. The contents of pentanoic acid, 5-ethyldihydro-2(3*H*)-furanone and hexanoic acid were more higher in Nuodeng ham, while *n*-caproic acid vinyl ester, hexanal and 1-pentanol were more higher in Saba ham. The hexanal was also identified as a characteristic marker for Saba ham in the HS-GC-IMS analysis. These VOCs can be used as potential markers to distinguish between the five different dry-cured hams.

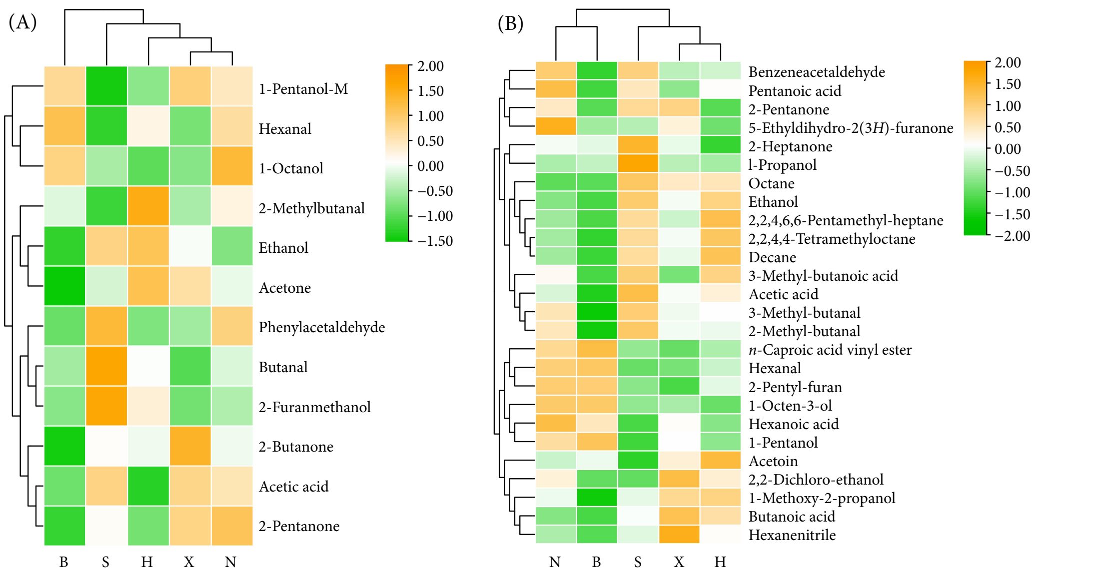

Figure 4 The cluster heat map of differential volatile compounds (VIP > 1) in five types of Yunnan dry-cured hams based on (A) GC-IMS and (B) GC-MS data.

4 Conclusion

In this study, HS-GC-IMS combined with HS-SPME-GC-MS was used to systematically investigate the aroma characteristics of dry-cured hams from five regions in Yunnan. HS-GC-IMS and HS-SPME-GC-MS identified 41 and 128 VOCs, respectively, which were mainly aldehydes and alcohols. The VOCs profiles of Nuodeng ham and Saba ham were similar. Both of them contained the most abundant aldehydes, such as hexanal and nonanal. In addition, they were also rich in 1-octen-3-ol, 2-pentyl-furan and 2-ketones, which could give Nuodeng ham and Saba ham good fruity and mushroom flavors. Sanchuan ham was rich in 3-methyl-butanal and 2-heptanone, which had a typical toasted flavor and were the result of the unique processing technology of Sanchuan ham. Based on the PLS-DA model, 12 and 26 VOCs with VIP > 1 were screened from HS-GC-IMS and HS-SPME-GC-MS data, respectively, which could be used as characteristic markers to distinguish dry-cured hams in five regions of Yunnan. The results of our work provide comprehensive information on the VOCs of five types of Yunnan dry-cured hams, which can provide a strong basis for the quality control of Yunnan dry-cured hams.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that may have appeared to influenced the work reported in this paper.

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