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GC × GC-ToF-MS and GC-IMS based volatile profile characterization of the Chinese dry-cured hams from different regions

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ABSTRACT

Chinese dry-cured hams have unique aroma characteristics appreciated by local consumers. The volatile organic compounds (VOCs) of six selected Chinese dry-cured hams (*Mianning, Nuodeng, Saba, Sanchuan, Wanhua,* and *Xuanen*) were analyzed by solvent assisted flavor evaporation (SAFE) combined with GC × GC-ToF-MS and head-space (HS) injection combined with GC-IMS. To visualize VOCs and differentiate samples, principal component analysis (PCA) and multiple factor analysis (MFA) were performed. GC × GC-ToF-MS resulted in over five times more VOCs (265) than GC-IMS (45). However, PCA and MFA indicated similar results using GC-IMS or GC × GC-ToF-MS data, suggesting HS-GC-IMS as a good choice to differentiate dry-cured hams from different regions. *Xuanen ham* from Yunnan Province having smoky aroma was significantly different from other hams, likely due to its unique process. Many aldehydes (heptanal, nonanal, etc.) played an important role in *Sanchuan ham*. Ketones were related to other four dry-cured hams, though they came from different regions. This study provides valuable analytical data to characterize and discriminate the flavor profile of Chinese dry-cured hams.

1. Introduction

Dry-cured ham is a traditional Chinese meat product with palatable flavor, particular shape, and fascinating color (Ramírez & Cava, 2007; Zhou & Zhao, 2007). Due to the different manufacture process conditions in salting, pressing, drying and ripening, dry-cured hams from different regions show distinct chemical and sensory characteristics (Li, Feng et al., 2019). The unique volatile organic components (VOCs) have a significant impact on the sensory profiles of the Chinese dry-cured hams (Zhang et al., 2018), thus resulting in smoky, acorn-like, rancid, salty, fatty, and pungent notes (García-González, Tena, Aparicio-Ruiz, & Morales, 2008). The preferred sensory attributes trigger “pleasant” emotions to consumers (Lorido, Pizarro, Estevez, & Ventanas, 2019). VOCs are generated during processing by protein hydrolysis, lipid degradation and oxidation, Maillard-type reactions, and Strecker degradation (Bermúdez, Franco, Carballo, Sentandreu, & Lorenzo, 2014). Previous GC–MS work (Li, Feng et al., 2019) resulted in 83, 95, 109, 119 VOCs identified in *Mianning, Nuodeng, Saba,* and *Sanchuan*, respectively, including aldehydes, alcohols, ketones, acids, and esters. However, due to limited separating capacity, some compounds at trace level could not be found by GC–MS.

Combined with two-dimensional gas chromatography (GC × GC), time-of-flight mass spectrometry (ToF-MS) is the most commonly adopted detector (Wang, Chen, & Sun, 2020). Due to its higher separation capacity, GC × GC-ToF-MS has been widely used to study various complex food matrices, such as wine (Song et al., 2020; Suklje et al., 2019), cream (Schutt & Schieberle, 2017), black tea (Magagna et al., 2017), and hams (Wang et al., 2018). Previous studies on palm and palmist oils have resulted in more VOCs detected by GC × GC-ToF-MS as compared to GC–MS, in particular fatty acid methyl esters (Kamatou & Viljoen, 2017).

Ion mobility spectrometry (IMS) is a powerful analytical technique based on the different mobility of gas phase ions in a constant electric field (Martín-Gómez, Arroyo-Manzanares, Rodríguez-Estévez, & Arce, 2019; Vautz, Seifert, Liedtke, & Hein, 2014). Because of its high efficiency and simplicity, GC-IMS has been used as a fast method to profile VOCs in olive oil (Gerhardt et al., 2019), eggs (Cavanna, Zanardi, Dall’Asta, & Suman, 2019), baby formula (Kamalabadi, Ghaemi,

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Table 1
Manufacture processing and physico-chemical analysis (pH, water, salt, protein, fat, and TBARS) of the Chinese dry-cured hams produced at different regions.

Types	Processing	Origin	pH	Salt (%)	Water (g/100 g)	Protein (g/100 g)	Fat (g/100 g)	TBARS (MDAmg/kg)
Mianning (H1)	(1) Pig hind legs; (2) natural cooling and trimming; (3) salting (4 times, 40 d); (4) washing and sun-drying (3–4 d); (5) ripening (spread with oleum sesame)	Sichuan	5.96	13.44	54.03	14.06	12.31	0.04
Nuodeng (H2)	(1) Pig hind legs; (2) natural cooling and trimming; (3) spraying the corn wine; (4) salting (20 d); (5) spreading salt mud; (6) ripening	Yunnan	5.78	9.34	43.21	19.11	8.58	0.25
Saba (H3)	(1) Pig hind legs; (2) natural cooling and trimming; (3) salting and stacking (3 times, 3–5 d); (4) ripening (6 months)	Yunnan	5.74	14.05	38.76	16.62	12.38	0.35
Sanchuan (H4)	(1) Pig hind legs; (2) natural cooling and trimming; (3) salting (25 d); (4) wrapping with tissue paper and hanging (2 months); (5) ripening	Yunnan	5.38	16.17	35.22	16.21	8.94	0.25
Wanhua (H5)	(1) Pig hind legs; (2) natural cooling and trimming; (3) salting (twice); (4) press with stone and turn every two days (10 d); (5) hanging and ripening	Anhui	5.78	13.71	50.39	26.84	14.63	0.19
Xuanen (H6)	(1) Pig hind legs; (2) natural cooling and trimming; (3) salting (7 times, 1 month); (4) washing and drying (slow fire, 7–8 d); (5) ripening	Hubei	5.86	14.84	54.20	28.51	13.09	0.18

Mohammadi, & Alizadeh, 2015), and bacteria (Gallegos, Arce, Jordano, Arce, & Medina, 2017; Wang et al., 2019). It is a low-cost, real-time, and rapid analytical method to discriminate samples from different regions (Li, Yang et al., 2019).

In our previous investigations (Wang et al., 2018), we have identified in total 165 VOCs from *Jinhua*, *Xuanwei*, and *Rugao hams* by GC × GC-ToF-MS and GC-IMS achieving a rapid discrimination of *Jinhua* dry-cured ham with different aging times. Besides the three most famous ones (*Jinhua*, *Xuanwei*, and *Rugao*), there are many other kinds of dry-cured ham in China, such as *Nuodeng*, *Sanchuan*, *Saba*, *Mianning*, *Xuanen*, and *Wanhua*. Other dry-cured hams such as *Mianning ham* (Sichuan), *Wanhua ham* (Anhui), *Xuanen ham* (Hubei), *Nuodeng, Saba* and *Sanchuan ham* (Yunnan) also have their unique flavor due to their different processing, regions, etc. Hence, this study aimed to apply GC × GC-ToF-MS and GC-IMS for volatiles discrimination of six selected dry-cured hams (*Mianning, Nuodeng, Saba, Sanchuan, Wanhua,* and *Xuanen*).

2. Material and methods

2.1. Sample preparation

Six types of Chinese dry-cured hams, *Mianning* (Mianning Jixiang Food Co. Ltd., Liangshan, Sichuan, China), *Nuodeng* (Nuodeng Ham Food Factory, Dali, Yunnan, China), *Saba* (Jianguo Brand Saba Ham Co., Ltd., Yunnan, China), *Sanchuan* (Lijiang Sanchuan Industrial Group, Lijiang, Yunnan, China), *Wanhua* (Xiuning Lantian Fang xinyu Family Farm, Huangshan, Anhui, China), *Xuanen* (Dapai Food Co. Ltd., Hubei, China) were used for the experiments. The detailed information about these dry-cured hams is shown in Table 1 compiling analytical data obtained in our lab.

The *Biceps femoris* muscle of dry-cured hams were sampled and cut into 1 cm^3 cubes. Samples were minced by a grinding machine (A11 basic, Germany), transferred into tubes and stored at −20 °C before analyzing.

2.2. Solvent-assisted flavor evaporation (SAFE)

The solvent-assisted flavor evaporation (SAFE) apparatus (Kimble Bomex Labware Co. Ltd., Beijing, China) was used to extract volatiles (Engel, Bahr, & Schieberle, 1999). Each ham sample (50 g) was mixed with 150 mL dichloromethane (analysis grade, Shanghai Macklin Biochemical Co., Ltd). The mixture was vibrated for 12 h with a shaker (SHZ-88, Jinyi Instrument Technology Co., Ltd., Jiangsu, China) and then stratified in a separating funnel. The organic layer was distilled with SAFE under vacuum (10^{-3} Pa). After distilling, the solution collected was dried with anhydrous Na_2SO_4 (analysis grade, Shanghai Macklin Biochemical Co., Ltd) for 12 h. Then the solution was concentrated to 4 mL by a Vigreux column and purged to 1 mL under a nitrogen gas-stream.

2.3. GC × GC-ToF-MS analysis

Samples prepared by SAFE were used directly for analyzing VOCs by GC × GC-ToF-MS. The experiment was conducted at the Instrumental Analysis Center, Shanghai Jiao Tong University. The GC × GC-ToF-MS system contains an Agilent 7890 gas chromatography (Agilent Technologies, Palo Alto, CA, USA), a cold-jet modulator and a time-of-flight mass Spectrometer (Zoex Corp., NE, USA). DB-WAX (30 m × 250 μm × 0.25 μm) and DB-5 (1 m × 100 μm × 0.10 μm) were respectively used as the first and the second dimension columns. Helium (99.999%) was used as carrier gas at a flow rate of 1 mL/min and the injection volume was 1 μL. The initial temperature was held for 2 min at 40 °C. After that the temperature was increased to 130 °C at 6 °C/min, to 200 °C at 2 °C/min and held for 1 min, to 250 °C at 25 °C/min and then held for 5 min. The modulator was offset by 5 + 15 °C in relation to the secondary oven and the modulation time was 4 s. The ion source temperature was set at 220 °C and ionization potential of MS was at 70 eV. Spectra were collected in a mass range of 33–550 amu with an acquisition rate of 50 spectra/s. The final results were the averages of three replicates.

2.4. GC-IMS analysis

Ham samples (5 g) were put into a 20 mL headspace (HS) vial and closed with a magnetic cap before analyzing. Subsequently, samples were incubated at 60 °C for 20 min. After incubation, a constant headspace (500 μL) was injected into the injector automatically by a heated syringe (85 °C). Then the samples were transferred into a FS-SE-54-CB-1 (15 m × 0.53 mm) capillary column by nitrogen (99.99%) at a programmed flow as follows: initially 2.0 mL/min, 15 mL for 10 min, 80 mL for 10 min, 130 mL for 5 min and eventually 145 mL for 5 min. The ions of analytes ionized were directed to the drift tube with a constant temperature 45 °C and the drift gas (nitrogen gas, 99.99% purity) was set at 150 mL/min. The final results were the averages of six replicates.

2.5. Statistical analysis

VOCs about GC × GC-ToF-MS data were tentatively identified after comparing their linear retention indices (RI), 1st and 2nd dimension retention times and mass spectra with NIST 17 database. In the following results and discussion, H1, H2, H3, H4, H5, H6 separately represented *Mianning*, *Nuodeng*, *Saba*, *Sanchuan*, *Wanhua*, and *Xuanen ham.*

IMS data were analyzed by the instrumental analysis software including LAV (from G.A.S., Dortmund, Germany version 2.0.0), Reporter, Gallery Plot as well as GC × IMS Library Search, which can be used for sample analysis from different angles. The identified compounds were characterized combing retention index (RI) and drift time

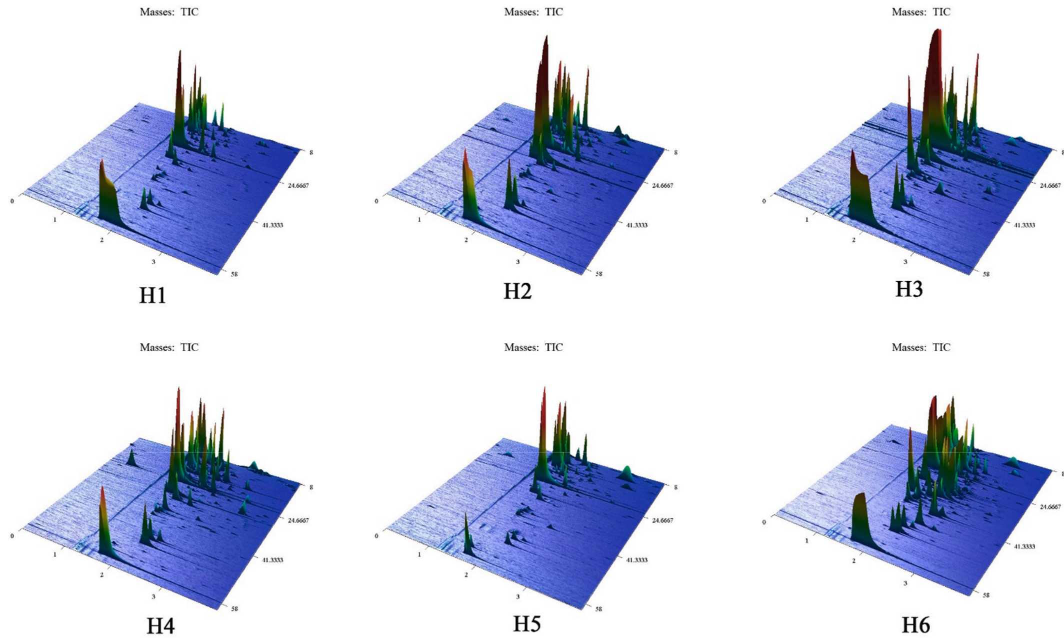

Fig. 1. **3D-chromatograms of VOCs isolated from six selected Chinese dry-cured hams from four regions using GC × GC-ToF-MS.** Column I axis represents the retention time of the compounds and the column II refers to the chemical polarity. H1 (*Mianning*), H2 (*Nuodeng*), H3 (*Saba*), H4 (*Sanchuan*), H5 (*Wanhua*), H6 (*Xuanen*).

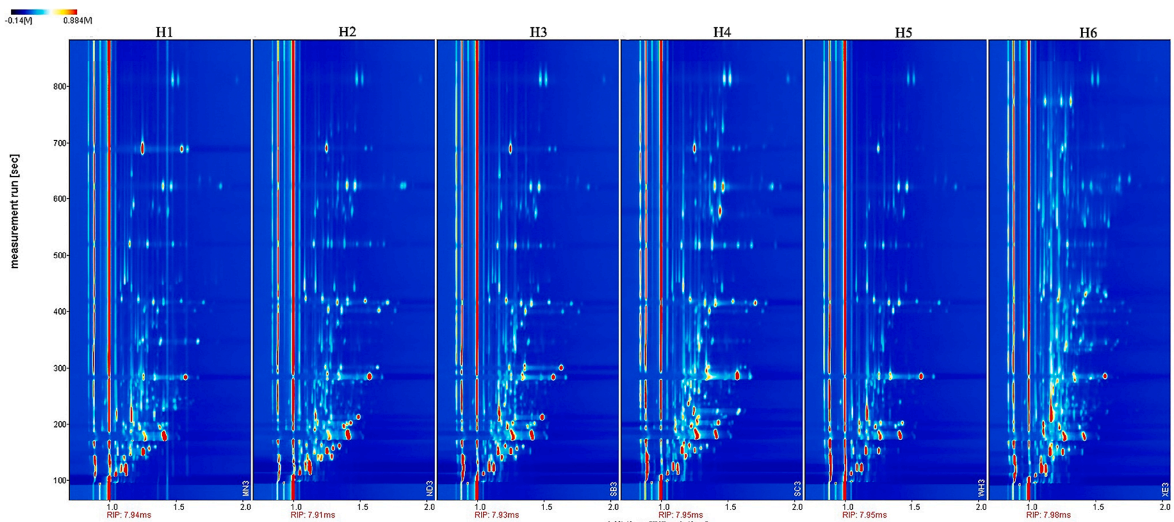

Fig. 2. **Fingerprints of VOCs isolated from six selected Chinese dry-cured hams from four regions using GC-IMS.** H1 (*Mianning*), H2 (*Nuodeng*), H3 (*Saba*), H4 (*Sanchuan*), H5 (*Wanhua*), H6 (*Xuanen*). Each colored point represents a VOC, and different colors indicate varying concentrations, red representing higher intensity, blank meant not present. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

with NIST Library and IMS database retrieval software from G.A.S.

Significant differences ($p = 0.05$) were analyzed using SPSS 25 version (Chicago, IL, USA), by Duncan's multiple comparison method. Principal component analysis (PCA) was performed by SIMCA 14.1 version (Umetrics, Umea, Sweden). Multiple factor analysis (MFA) was performed by XLSTAT 2016 version (Addinsoft, New York, USA).

3. Results and discussions

3.1. 3D plots of dry-cured hams from different regions by GC × GC-ToF-MS

VOCs in different dry-cured ham samples analyzed by GC × GC-ToF-MS were visualized in the form of three-dimensional chromatograms (Fig. 1). The column I axis represents the retention time of the compounds and the column II refers to the chemical polarity. H2 (*Nuodeng*) and H3 (*Saba*) showed similar peak profiles likely due to their same origin from the Yunnan Province. The number of peaks in H6 (*Xuanen*) was the highest, and that in H1 (*Mianning*) and H5 (*Wanhua*) were the lowest (Table S1). These findings indicate that the VOCs of dry-cured hams varied from different regions and more VOCs were found in *Xuanen ham* compared to the other five samples.

3.2. GC–IMS topographic plots in different dry-cured hams from different regions

Top view of six selected dry-cured hams with different characteristics is shown in Fig. 2, where drift time and retention time was represented by the X-axis and Y-axis, respectively. Most of the signals appeared in the retention time range of 100–400 s and drift time of 1.0–1.5 riprel. The red vertical line at the abscissa 1.0 is the reactive ion peak (RIP). Each colored point represents a VOC, and different colors indicate varying concentrations, red representing higher intensity, blue meant not present. Compared with other samples, the signal intensities of phenols (phenol, 2-methoxyphenol) and some *N*-containing VOCs (2-acetylpyrrole) in H6 (*Xuanen*) were much higher compared to other hams. This may be attributed to its special process and the higher content of protein which is supported by *N*-containing VOCs (such as pyrazines and amides) occurring in higher amounts in sample H6 (*Xuanen*) as shown in the supporting information (Tables S1 and S3).

3.3. Analysis of VOCs obtained by GC × GC-ToF-MS and GC-IMS

Detailed information on VOCs identified by GC × GC-ToF-MS are shown in Table S1. A total of 265 VOCs were identified in the six selected Chinese dry-cured hams, including ten classes: i.e. hydrocarbons (25), aldehydes (27), ketones (48), alcohols (43), acids (18), esters (21), phenols (11), *O*-heterocycles (28), *S*-containing compounds (4), and *N*-containing compounds (40). Detailed information on VOCs identified by GC-IMS are shown in Table S2. A total of 45 VOCs were identified in the six selected Chinese dry-cured hams, including eight classes: i.e. aldehydes (11), ketones (8), alcohols (11), acids (2), esters (7), phenols (2) and *O*-heterocycles (2), and *N*-containing compounds (2).

As shown in Table S1, hydrocarbons represent a small group of VOCs found in six selected dry-cured hams and they were not found in Table S2. Most of them were produced by the decomposition of lipids (Sirtori et al., 2019). They usually have a marginal sensory effect on the overall odor of dry-cured hams due to their high odor thresholds (Zhao et al., 2017).

As far as aldehydes are concerned, H3 (*Saba*) and H4 (*Sanchuan*) revealed to be the most abundant in aldehydes (20) as shown in Table S1, followed by H6 (*Xuanen*) (19), H1 (*Mianning*) (15), H5 (*Wanhua*) (15), and H2 (*Nuodeng*) (14). Aldehydes are major degradation products of lipid oxidation, however, some can also be generated upon Maillard-induced amino acids degradation. They usually have a great effect on the characteristic flavor in dry-cured hams due to their lower odor thresholds (Bosse Nee Danz et al., 2017; Lorenzo, Carballo, & Franco, 2013; Wang, Jin, Zhang, Ahn, & Zhang, 2012). In Table S1, 3-methyl-butanal (malty, toasted) as a branched Strecker aldehyde (Zhang et al., 2019) was present in all six selected dry-cured hams, of which the relative content in H4 (*Sanchuan*) was highest (2.91%) and in H6 (*Xuanen*) very low (0.94%) compared to other hams (1.03–1.84%). In Table S2, it had a high content in H1 (*Mianning*) (2.61%) and H2 (*Nuodeng*) (2.72%), which is most likely due to the different sampling technique applied (headspace). As recently shown, 3-methylbutanal was one of the largest contributors to the flavor of Istrian dry-cured hams (Marušić, Vidaček, Janči, Petrak, & Medić, 2014). Phenylacetaldehyde, another Strecker aldehyde, providing honey-like odor to hams (García-González et al., 2008) was identified in all samples, however dominating in H2 (*Nuodeng*) and H4 (*Sanchuan*) with 6.41% and 5.82%, respectively (Table S1). Table S2 shows phenylacetaldehyde monomer dominating in H3 (*Saba*) (1.21%) and H4 (*Sanchuan*) (1.14%). It is also remarkable to find methional, the Strecker degradation product of methionine, at relatively high levels in all hams, particularly abundant in H6 (*Xuanen*) with 3.66% (Table S1). Its cooked-potato note will most likely contribute to the overall aroma of hams, as previously reported for Parma ham by Blank, Devaud, Fay, Cerny, Steiner, and Zurbriggen (2001).

The most abundant aldehyde in the hams (Table S1), was hexanal generated by lipid oxidation. It is in particular high in H4 (*Sanchuan*) at 6.52%, lowest in H1 (*Mianning*) at 0.99%. The finding is also consistent with the previous study from our lab (Li, Feng et al., 2019). High concentration of hexanal detected in these ham samples was also in accordance with investigation of Istrain hams (Marušić, Petrović, Vidaček, Petrak, & Medić, 2011). In Table S1, nonanal (soapy, fatty) was the only other alkanal occurring at higher levels (1.19% in H4). This substance as monomer was also found high by GC-IMS as shown in Table S2 (0.38%). The unsaturated aldehyde 2,4-decadienal (fatty, seaweed odor) was only detected in H5 (*Wanhua*) and H6 (*Xuanen*) (0.03%), while (*E*,*E*)-2,4-hexadienal (0.07%) (deep-fried) (Table S1) was identified in H4 (*Sanchuan*) in higher levels than in other hams. Their role as important contributors to the dry-cured ham have been reported recently (Wang et al., 2018).

The group of ketones comprised in total 48 VOCs (Table S1), mainly present in H6 (*Xuanen*) (38), H4 (*Sanchuan*) (27), and H3 (*Saba*) (24). Ketones have been considered as the results of lipid oxidation (Marušić et al., 2011). However, there abundance was rather low as indicated by 2-heptanone and 1-octen-3-one with 0.21% and 0.03% (S2, *Nuodeng*), respectively. 2-Heptanone has been described as a characteristic compound in burnt meat (Pham et al., 2008). The relative amount of 1-hydroxy-2-propanone (13.23%) and acetoin (9.69%) in H5 (*Wanhua*) were higher than in other samples (Table S1). These hydroxyketones are typical fermentation products (Huan, Zhou, Zhao, Xu, & Peng, 2005), thus indicating that ham H5 (*Wanhua*) was particularly well fermented compared to H4 (*Sanchuan*). Acetoin has been considered imparting a buttery note (Li, Feng et al., 2019). 2,3-Butandione, a Maillard reaction product, is likely to be another key contributor to the buttery character for its lower odor threshold. Its level (2,3-butandione) in Table S1 was relatively high in H1 and H6 with 1.59% and 1.98%, respectively. All other ketones identified showed low amounts (Table S1). 2,3-Methyl-2-cyclopenten-1-one, 2-methyl-3-heptanone, and 3-methyl-2-pentanone were not identified in our previous study (Li, Feng et al., 2019). In this study, 2-methyl-2-cyclopenten-1-one was mainly detected in H4 (*Sanchuan*) (0.05%) and H6 (*Xuanen*) (0.16%) (Table S1). These methyl ketones caused by auto-oxidation and β-oxidation of fatty acids or microbiological metabolism have been described as important contributors to the complex flavor and aroma attributes of pork (Lorenzo et al., 2013; Pham et al., 2008; Wang et al., 2012).

Forty-three VOCs belong to the alcohol group reported in all dry-cured hams (Table S1). In our previous study, 1-octen-3-ol was only found in *Sanchuan ham* (H4) by SPME-GC–MS, and could contribute to the sensory profiles of dry-cured hams (Li, Feng et al., 2019). But in this study, it was identified in all hams investigated (Table S1), most likely due to the better peak resolution (GC × GC) and higher sensitivity (ToF-MS). Also, 1-octen-3-ol (2.72%) contributing to mushroom attributes (García-González et al., 2008) was found at higher levels in H4 (*Sanchuan*) compared with other hams. 1-Penten-3-ol contributing to grassy aroma was found in all ham samples. Its relative amount in H4 (*Sanchuan*) was higher (1.29%), which was in agreement with our previous result (Li, Feng et al., 2019). Previous studies showed linear alcohols were originated from lipid oxidation, and branched-chain alcohols were produced by Millard-type reactions and Strecker degradation with subsequent reduction under fermentative conditions (Martínez-Onandi et al., 2017; Pérez-Santaescolástica et al., 2018). In addition, the aromatic alcohols phenylethyl alcohol and benzyl alcohol may contribute to the floral odor (Zhao et al., 2017).

The major acids were 2-methylbutanoic acid, 3-methylbutanoic acid and hexanoic acid formed by hydrolysis of triglycerides or phospholipids (Berdagué, Denoyer, Quéré, & Semon, 1991), of which only 2-methylbutanoic acid was detected in Table S2. They were particularly abundant in H4 (*Sanchuan*), which was consistent with our previous study (Li, Feng et al., 2019). As a short-chain acid, hexanoic acid accounted for 7.14% in H1 (*Mianning*) and 7.27% in H4 (*Sanchuan*), which played an important role in the dry-cured hams' aroma (Wang et al., 2018). The short chain fatty acids are known to elicit rancid, and sometimes soapy notes, some of them having low odor thresholds.

Esters formed by esterification of alcohols and carboxylic acids impart fruity and sweet notes to dry-cured hams (Martínez-Onandi, Rivas-Cañedo, Picon, & Nuñez, 2016; Zhao et al., 2017). A large number of methyl and ethyl esters was found in this study, thus indicating

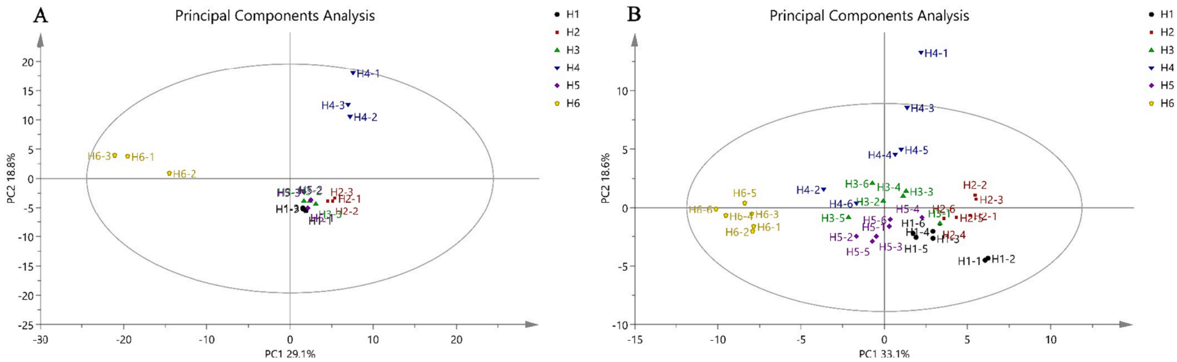

Fig. 3. Principal component analysis (PCA) of six selected dry-cured hams based on VOCs identified by GC × GC-ToF-MS (A) and GC-IMS (B). Scores plot based on the peak area by GC × GC-ToF-MS and signal intensity by GC-IMS obtained with six selected dry-cured hams. H1 (*Mianning*), H2 (*Nuodeng*), H3 (*Saba*), H4 (*Sanchuan*), H5 (*Wanhua*), H6 (*Xuanen*).

formation of ethanol upon fermentation with microorganisms (Li, Feng et al., 2019; Martínez-Onandi, Rivas-Cañedo, Nunez, & Picon, 2016). In the six dry-cured hams, decanoic acid ethylester, 2-oxo-propanoic acid methylester and n-caproic acid vinylester were the common representatives.

A total of 11 phenols were detected using GC × GC-ToF-MS (Table S1), all of them were found in H6 (*Xuanen*) but other hams contain just some. However, using GC-IMS, only two phenols were identified (Table S2). Phenols are known to significantly contribute to the smoky note in smoked dry-cured hams because of their low odor thresholds (Petričević, Marušić Radovčić, Lukić, Listeš, & Medić, 2018). However, only phenol and *p*-cresol occurred in all hams (0.17–1.26%), and they were most abundant in H6 (*Xuanen*) with 1.26%. In this study, we have also identified 4-ethyl-2-methoxyphenol, also called 4-ethylguaiacol (0.29%), having a spicy aroma and low odor threshold (Blank, Sen, & Grosch, 1992), and 2-ethylphenol (0.03%) at lower levels in H6 (*Xuanen*). This is most likely due to the process of H6, which was strongly exposed to smoke as one of the process steps.

Several cyclic compounds with an oxygen in the ring (*O*-heterocycles) were identified (28) in the six selected hams, including furans, furanones, and pyranones. They are known sugar degradation products formed by Maillard-type reactions and may contribute to the meaty, sweet flavor of cured hams (Benet et al., 2016). The major furanone was 5-ethyldihydro-2(3*H*)-furanone found in H2 (*Nuodeng*) with 1.34%.

Apart from methional mentioned in the group of aldehydes, we found in the group of *S*-containing compounds thiazoles, which were reported to impart meaty aroma notes (Benet et al., 2016). They are generated from cysteine and pentose by heat-induced degradation reactions (Benet et al., 2016; Thomas, Mercier, Tournayre, Martin, & Berdagué, 2014). Of particular interest is sulfurol described as toast-meaty that was present in all dry-cured hams at a level of 0.01–4.49%. It is known as a thermal degradation product of thiamine (vitamin B1) occurring in meat flavors. Relatively high levels of dimethyl sulfone were found in all hams, particularly in H2 (*Nuodeng*) and H6 (*Xuanen*) with 4.49% and 3.52%, respectively. Its presence is related to the metabolism of methionine via dimethylsulfoxide.

The group of *N*-containing compounds (40) was composed of pyrazines, pyridines, pyrroles, amides, and indole. They are typical degradation products of amino acids, peptides and proteins generated upon heat treatment or under fermentative conditions. Pyrazines which were found in all hams, particularly pronounced in H3 (*Saba*), e.g. 2,6-dimethylpyrazine (1.23%) and trimethylpyrazine (2.09%), contributing to the roasty, meaty character of the hams (Benet et al., 2016). As typical heat-induced Maillard reaction products, the presence of pyrazines indicate that theses hams underwent some more severe heat treatments compared to the other hams.

3.4. PCA and MFA of VOCs identified by GC × GC-ToF-MS and GC-IMS

To visualize the vast amount of data by reducing dimensions, PCA

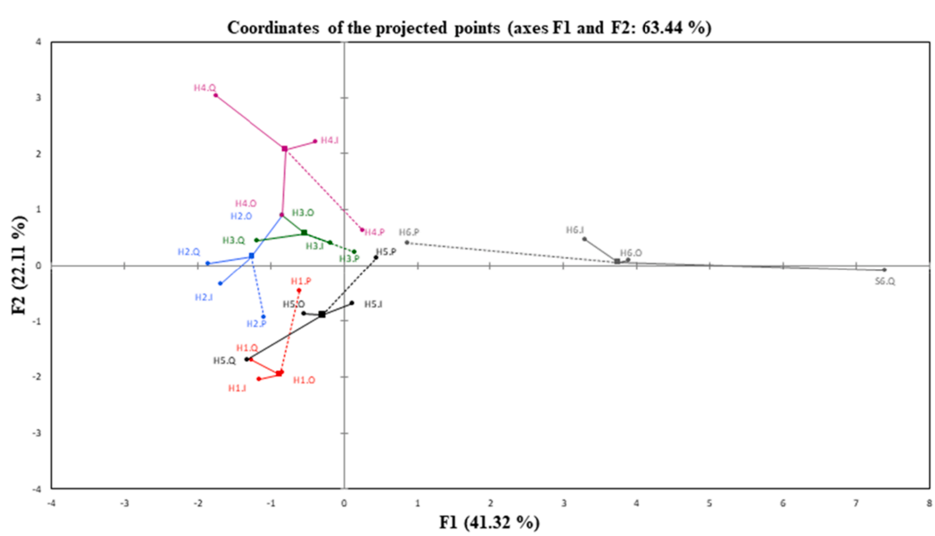

Fig. 4. Coordinates of the projected points of the variations of the multiple factor analysis (MFA). The abbreviations Q, I, P, O refer to the results identified by GC × GC-ToF-MS and GC-IMS, physical and chemical indices (pH, salt, water, protein, fat, and TBARS), origin and processing information (Table 1). H1 (*Mianning*), H2 (*Nuodeng*), H3 (*Saba*), H4 (*Sanchuan*), H5 (*Wanhua*), H6 (*Xuanen*).

Table 2
RV coefficients for the correlation of variables obtained with O, GC × GC-ToF-MS, GC-IMS and P in six selected Chinese dry-cured hams.

	O	GC × GC-ToF-MS	GC-IMS	P
O	1.000	0.801	0.818	0.628
GC × GC-ToF-MS		1.000	0.927	0.719
GC-IMS			1.000	0.809
P				1.000

O: Location and processing where the dry-cured hams were produced as shown in Table 1.
GC × GC-ToF-MS: Identified VOCs using GC × GC-ToF-MS shown in Table S1.
GC-IMS: Identified VOCs using GC-IMS shown in Table S2.
P: pH, salt, water, protein, fat, and TBARS data shown in Table 1.

(Principal Component Analysis) was performed based on the results of GC × GC-ToF-MS and GC-IMS, respectively. PCA is a statistical analysis to analyze multiple variables. The data basis for the PCA of GC × GC-ToF-MS is based on the relative content of each compound in Table S1. The data basis for the PCA of GC-IMS is based on the relative content of each compound in Table S2. Scores plots obtained by PCA are shown in Fig. 3. The first and second principal components (PC1 and PC2) explained respectively 47.9% (data of Table S1) and 51.7% (data of Table S2) of the accumulative variance contribution rate. The distribution diagram (Fig. 3) showed that six selected hams could be distinguished by PC1 and PC2, indicating the relationships of flavors among the investigated samples. Fig. 3 A showed that four dry-cured hams (H1, H2, H3, H5) were located close to each other and suggested that the VOCs in H1 (*Mianning*), H2 (*Nuodeng*), H3 (*Saba*), and H5 (*Wanhua*) were relatively similar, but that in H4 (*Sanchuan*) and H6 (*Xuanen*) varied distinctly. However, in Fig. 3B, six investigated samples were well separated from each other, indicating GC-IMS was better differentiating the six dry-cured hams although only 45 VOCs were detected. The volatiles of dry cured ham were affected by many factors, including the feeding of pigs, the species of pigs, and the manufactured processing. The samples used in our experiments were randomly selected to represent the volatile of dry cured ham. The selected hams (H4) were produced manually, so they have large variances of the volatiles. The PCA result showed the repeats of H4 samples located far away from each other (Fig. 3B), which mainly due to the fluctuated processing of H4.

The four variations were then analyzed by MFA in Fig. 4 (F1 and F2: 63.44%). Multiple factor analysis (MFA) is useful to study relationships by simultaneously analyzing observations, the variables, and the tables (Scholz, Kitzberger, Prudencio, & Silva, 2018). In Fig. 4, the centroid represents the resulting coordinates of the MFA. The points connected to the centroid represent the coordinates of the projections formed by the variations. The closer these projections are to the centroid, the greater the similarity between the descriptions. As can been seen in Fig. 4, origin and GC-IMS (the results of Table S2) variables showed close distance to centroid, indicting GC-IMS was a good choice to discriminate dry-cured hams from different regions. Additionally, the terroir showed the largest distance between the descriptions of the GC × GC-ToF-MS and physical and chemical indices, making them different from other dry-cured hams. It also confirmed that the groups had different levels of importance in describing the dry-cured hams from different regions.

The RV coefficients for six selected Chinese dry-cured hams are summarized in Table 2 based on the data such as origin and process information, physical and chemical indices (pH, salt, water, protein, fat, and TBARS), and VOCs identified by GC × GC-ToF-MS and GC-IMS. It is particularly useful for the dimension with the largest explained variance. The value of the RV coefficients varies between 0 and 1 making them easier to analyze. It has been mentioned that RV coefficients > 0.7 are considered as a good level of differentiation (Cartier et al., 2006, Moelich, Muller, Joubert, Naes, & Kidd, 2017). The RV coefficients of GC × GC-ToF-MS and GC-IMS were high (RV:0.93), thus these two methods appear to be a reliable for distinguishing dry-cured hams.

As shown in Fig. 5, it displayed correlations between variables and factors about VOCs in six selected dry-cured hams. PC1 and PC2 explained respectively 41.32% and 22.09% of the accumulative variance contribution rate, which could reflect the total information. The blue and green spots represented the VOCs in Tables S1 and S2. The red square points represented pH, salt, water, protein, fat, and TBARS. The closer their positions are, the more relevant their relationships.

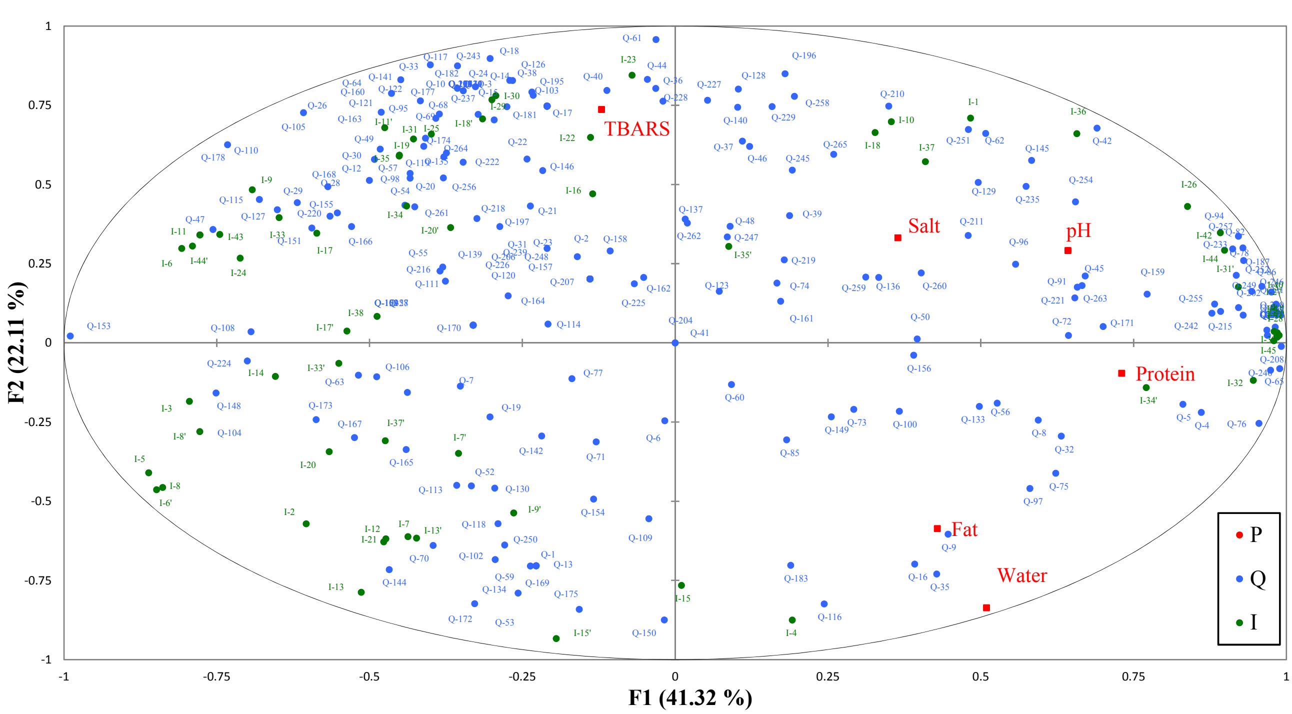

Fig. 5. Correlations between variables and factors about six selected dry-cured hams. The number prefixed by Q and I in the Fig. 5 was used to replace compounds, corresponding to the number showed in Table S1 (blue plot) and Table S2 (green plot). H1 (*Mianning*), H2 (*Nuodeng*), H3 (*Saba*), H4 (*Sanchuan*), H5 (*Wanhua*), H6 (*Xuanen*). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Combining Fig. 4 and Fig. 5, it can be seen that H6 (*Xuanen*) is located in the first quadrant being correlated with benzaldehyde and many phenols, which might be related to smoky aroma, as previously discussed. It can also be seen that salt and pH, likely to cause protein degradation, were related to some *N*-containing compounds in H6. H4 (*Sanchuan*) is located in the second quadrant as well as many aldehydes (such as heptanal and nonanal) which are related with H4. The relatively high value of TBARS (Table 1) may account for this result. H1, H2, H3, H5 are located in the similar region (Fig. 5), indicating that they had similar flavor profiles and were associated with ketones.

4. Conclusions

This study investigated the VOCs found in six selected Chinese dry-cured hams. A total of 265 and 45 VOCs was identified in the six selected Chinese dry-cured hams by GC × GC-ToF-MS and GC-IMS, respectively. GC × GC-ToF-MS was shown to result in more VOCs in the dry-cured ham, especially those compounds at trace levels. GC-IMS identified a limited number of compounds when compared with GC × GC-ToF-MS. However, GC-IMS could present similar clustering on the PCA and MFA plots, indicating that this fast method is suitable to discriminate dry-cured hams based on VOCs. PCA and MFA results indicated that H4 (*Sanchuan*) and H6 (*Xuanen*) varied from other hams distinctly, while H1, H2, H3, and H5 have similar volatile profiles related with ketones. Owing to the distinct process steps, the abundant phenol components in H6 could contribute to its smoky attribute. The data presented in this study provide valuable new information related to the flavor profile of dry-cured hams in China.

CRediT authorship contribution statement

Wenqian Li: Validation, Investigation, Writing - original draft. **Yan Ping Chen:** Writing - review & editing, Funding acquisition. **Imre Blank:** Writing - review & editing. **Fuyang Li:** Methodology, Data curation. **Chunbao Li:** Visualization. **Yuan Liu:** Supervision, Project administration.

Declaration of Competing Interest

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

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2021.110222.

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