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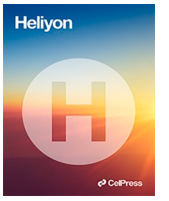

Research article

GC-IMS and multivariate analyses of volatile organic components in different Chinese breeds of chickens

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ABSTRACT

This study examined the difference in volatile flavor characteristics among four different local breeds of chicken by headspace gas chromatography-ion mobility spectrometry (HS-GC-IMS) combined with multivariate analysis. In total, 65 volatile organic compounds (VOCs) were identified (17 aldehydes, 12 alcohols, 7 ketones, 5 esters, 2 acids, and 22 unidentified, i.e., 26.15% aldehydes, 18.46% alcohols, 10.77% ketones, 7.69% esters, 3.08% acids, and 33.84% unidentified), of which 43 were annotated. The chicken meats from the four breeds exhibited good separation in topographic plots, VOC fingerprinting, and multivariate analysis. Meanwhile, 20 different volatile components, with variable importance in projection value > 1, were selected as potential markers to distinguish different breeds of chicken by partial least squares discriminant analysis (PLS-DA). These findings provide insights into the flavor traits of chicken meat. Also, HS-GC-IMS combined with multivariate analysis can be a convenient and powerful method for characterizing different meats.

1. Introduction

With the improvement in living standards, consumers are looking for higher meat quality and flavor. Local breeds of chickens are gradually occupying the dominant position in the world chicken market and becoming the mainstream of poultry production [1,2]. In addition, research has shown that poultry gelatin can be used as a sustainable alternative to mammalian gelatin [3]. Nevertheless, chicken meat quality is influenced by multiple factors, including genetics, nutrition, and environmental factors. Among them, genetic factors are the most important influencing factors, influencing chicken flavor among different broiler breeds [4,5]. Meat flavor is the biggest determinant of consumer choice. Volatile organic compounds (VOCs) are flavor compounds that are formed during the cooking process of meat and are often used as indicators of chicken flavor [6]. In 1965, Minor et al. [7] found that VOCs in chicken were mainly composed of carbonyl and sulfur-containing compounds, and there are 13 VOCs with a relatively high content (>10 ng/g) in chicken soup. Jin et al. [8] analyzed three local varieties of chickens, Jingxing Yellow chicken, Tiannong Partridge chicken, and Wenchang chicken, for differences in VOCs and identified 9 kinds of common VOCs, including hexanal, 2-nonen-1-ol, 2,4-nonadienal,

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1-octen-3-ol, heptanal, octanal, nonanal, decanal, and *trans*-2-undecene-1-ol. Using principal component analysis (PCA), they concluded that hexaldehyde and 1-octene-3-alcohol contributed the most to the overall flavor of Chinese chicken. Similarly, Xu et al. [9] found that aldehydes and alcohols, including (E) -2-octanol, 3-octanol, 1-octene-3-ol, phenylacetaldehyde, and benzaldehyde, are the main volatile flavor components in Qingyuan partridge chicken and White sliced chicken.

Currently, gas chromatography-mass spectrometry (GC-MS), gas chromatography-olfactory-mass spectrometry (GC-*O*-MS), and electronic nose are the most commonly used analytical methods for examining VOCs in chicken or meat products [10,11]. However, methods like GC-MS and GC-*O*-MS require complex sample pre-treatments, such as heating, distillation, and extraction, and require long detection times [12]. This limits these techniques for delaying sample processing. The electronic nose also has some limitations, such as poor sensor stability and reproducibility and complicated signal processing methods [13]. Headspace-gas chromatography-ion mobility spectrometry (HS-GC-IMS), with combined features of high GC separation efficiency and IMS sensitivity, can be used to detect trace volatile compounds [14,15]. HS-GC-IMS, equipped with an automatic headspace sampler, can generate fingerprints of VOCs in both liquid and solid samples without any pretreatment [16]. In addition, it is operated under normal pressure and uses nitrogen as a carrier gas, which reduces the operation cost. Furthermore, a wide array of VOCs in different samples can be screened by these technologies when combined with chemometrics such as PCA, partial least squares-discriminant analysis (PLS-DA), orthogonal partial least squares-discriminant analysis (OPLS-DA), etc [17–19]. Meanwhile, several studies have used GC-MS and GC-IMS to examine differences in volatile flavor substances in various food products [20].

To date, the GC-IMS technology has been employed to characterize VOCs in various foods, such as grain, dairy, meat, etc., to determine the effects of varieties, processing methods, and storage conditions. For instance, Li et al. [21] used GC-IMS combined with PCA to investigate differences in volatile flavor profiles in raw milk during refrigerated storage. Man et al. [22] studied the volatile flavor profiles in different breeds of donkey meat by GC-IMS and chemometrics. Altogether, it is clear that GC-IMS and multivariate statistical analysis can collectively examine VOC profiles in meat.

Recently, several studies have reported differences in chicken quality based on diverse feeding cycles, flavor-related genes, and metabolic differences [11,23,24]. These studies indicated that flavor substances are important characteristics of chicken, influencing consumer choice. However, there are few reports on GC-IMS identification and fingerprint and differential flavor analysis of VOCs in different chicken varieties. Accordingly, in this study, we characterized differences in VOCs in four different regional breeds of chickens from Zangxiang (ZX), Xinghua (XH), Heimao black (HM), and Xinjiang black (XJ) chickens by GC-IMS. Meanwhile, the differential VOCs were screened by PCA and PLS-DA. These findings improve our understanding of characteristic VOCs in chicken meat and provide a novel flavor authentication strategy.

2. Methods

2.1. Animals and sample preparation

This study was conducted following the Guidelines for the use of Experimental Animals established by the Ministry of Science and Technology (Beijing, China). All experimental protocols were approved by the Animal Care and Use Committee of Gansu Agriculture University (Lanzhou, China) (No. GSAU-ETH-AST-2021-02).

2.2. Sample collection

All four breeds of chickens used in this study were obtained from Gansu Qinyuanchun Art Agricultural Ecology Co., Ltd., China. 14-week-old female local breed chickens (ZX/XH/HM/XJ; 6 each) were selected. The chickens were fed basal diets formulated according to the National Resource Council (1994) requirements and the Feeding Standards of Chickens established by the Ministry of Agriculture, Beijing, China (2004). During the experimental period, different breeds of chickens were fed separately and freely throughout the entire period. After slaughter, aminal pectoral muscles were isolated and placed in a liquid nitrogen tank for transport to the laboratory. The muscle samples were stored at −80 °C for subsequent flavor substance testing.

2.3. VOC detection by GC-IMS

The chicken meat sample was evenly homogenized (Mixer B-400, Buchi, Switzerland), and about 2 g of it was put into a 20-mL headspace vial for GC-IMS (FlavourSpec®, G.A.S., Germany) analysis. Automatic injection conditions were as follows: headspace temperature, 60 °C for 15 min; headspace injection needle temperature, 85 °C; oscillating heating with incubation speed, 500 rpm; headspace injection volume, 500 μL; mode, non-diversion mode. Analytical conditions were as follows: chromatographic column, FS-SE-54-CB-1 (15 m × 0.53 mm, 1.0 μm); column temperature, 60 °C; operating time, 25 min. The carrier gas was high-purity nitrogen, with an initial flow rate of 2 mL/min, maintained for 2 min. Subsequently, the flow was linearly increased to 10 mL/min within 8 min, then to 100 mL/min within 10 min, and finally to 150 mL/min within 5 min. The temperature of the IMS detector was 45 °C, the flow rate of the IMS drift tube was 150 mL/min, the length of the drift tube was 5.3 cm, the linear voltage inside the tube was 500 V/cm, and the drift gas was nitrogen.

2.4. Data processing and statistical analyses

The VOCs in chickens were determined and identified using the built-in Laboratory Analytical Viewer (LAV) analysis software and

Library Search qualitative software. The GC-IMS fingerprint was compared using the plugin Gallery Plot in LAV. By comparing the retention time and drift time, the retention index (RI) of each compound was calculated using *n*-ketones C4–C9 (Sinopharm Chemical Reagent Beijing Co., Ltd., China) as an external standard reference. The compounds were qualitatively matched using a GC-IMS library, and the compound content was the normalized relative content.

The results were analyzed using SPSS version 22.0 (IBM, Armonk, USA) with one-way analysis of variance (ANOVA) and the differences were evaluated using Tukey's test. Data with a p-value <0.05 were deemed significant. PCA, PLS-DA, and heatmap analysis were performed using MetaboAnalyst software. Differential VOCs were determined based on the criteria of variable importance in projection (VIP) greater than 1.

3. Results

3.1. Comparison of VOCs among four breeds of chickens

The VOCs in the meats of different chicken breeds (ZX, XH, HM, and XJ) were detected by HS-GC-IMS. For direct observation and comparison, 3D topographical results were used to characterize VOCs. As shown in Fig. 1A, the X, Y, and Z axes represent ion mobility time, gas chromatography retention time, and peaks used for quantification, respectively. The 3D topographic plot results show clear differences in peak signal intensity among different chicken breeds, indicating differences in their VOC content.

HS-GC-IMS was used to obtain two-dimensional topographic plots of VOCs in different chicken breeds (Fig. 1B). The total compounds in the headspace for each sample are shown by the whole spectrum. Each point on the right of the reactive ion peak (RIP) indicates a specific volatile molecule. The drift time of most signals was within the range of 1.0–2.0 s, while the retention time was within the range of 100–800 s. Substance signal intensity is marked by white and red colors, indicating low and high intensity, respectively. Among the four types of chicken meat samples, XJ exhibited the highest variety and concentration of volatile substances (Fig. 1B). Meanwhile, ZX and XH samples demonstrated fewer volatile compounds and relatively lower concentrations compared to XJ and HM samples.

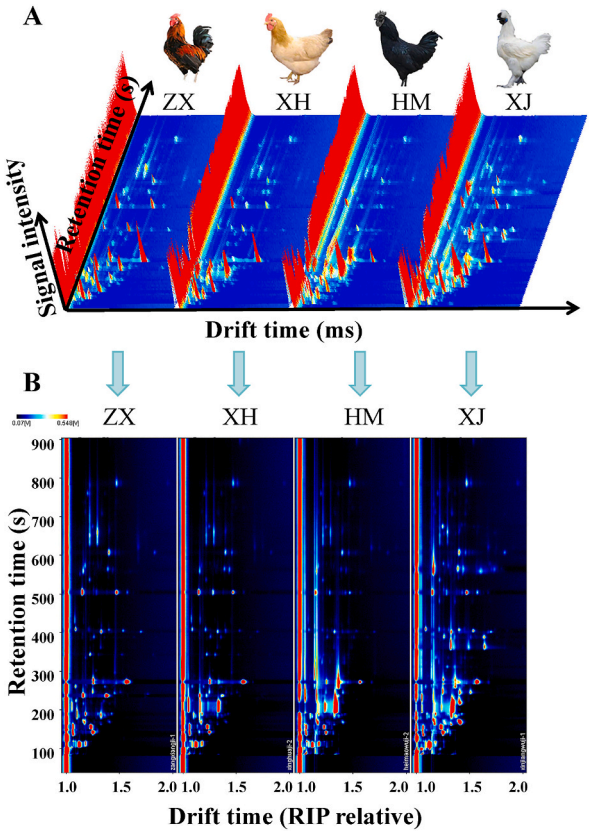

Fig. 1. Comparison of the volatile components in chicken meats from four chicken breeds. (A) 3D topographic plot and (B) topographic representations of the spectra for volatile compounds. Brighter signal peaks indicate higher component concentrations.

3.2. VOC profiles of chicken meats

VOCs were identified by comparing the IMS drift time and retention time with those of authentic reference compounds. Certain compounds produced spots (dimers or even trimers) or multiple signals that were derived from varying concentrations of the compound. As shown in Fig. 2 and Table 1, a total of 65 VOCs were detected (17 aldehydes, 12 alcohols, 7 ketones, 5 esters, 2 acids, and 22 unidentified, i.e., 26.15% aldehydes, 18.46% alcohols, 10.77% ketones, 7.69% esters, 3.08% acids, and 33.84% unidentified), 43 of which were identified in all four breeds. Thus, ketones, aldehydes, and alcohols were the most abundant VOCs in chicken meat (Fig. 2C). Ketones and alcohols were more significantly abundant in XJ than in the other three chickens ($p < 0.05$). Additionally, the contents of aldehydes in XJ and ZX were significantly higher than those in HM ($p < 0.05$; Fig. 2D).

3.3. Fingerprints and heat maps of VOCs in different chicken meats

Visual plots, together with the fingerprint gallery plot, were constructed for intuitive comparison. The signal intensity of each aroma compound represents its concentration level. The gallery plots demonstrate that the concentration of aroma compounds varied among the four chicken meat samples (Fig. 3A). Each row represents a volatile compound, and each column represents a sample. The color brightness indicates the VOC content; the brighter the color, the higher the content. Unidentified compounds are represented by numbers on the fingerprint gallery plot. VOCs in the red frame (3-methylbutyl acetate, 2-methylpropanoic acid, E−3-hexen-1-ol M, E−3-hexen-1-ol D, 5 and 3) were the main volatile components in ZX and XH, and their concentration was higher than those in other samples. The concentration of VOCs in the pink frame (ethyl acetate M, ethyl acetate D, 2_3-butanediol, 3-methylbutanoic acid, 2-pentanone D, 2-heptanone D, 1-hexanol, phenylacetaldehyde, 2-butanone, and 6–16) was very low or undetectable in ZX and XH, which were mainly present in XJ. Although there were more volatile compounds in HM, their concentration was relatively low. In addition, there were also differences between different individuals of the same breed.

Cluster heat map analysis of VOCs better represented the differences and similarities among different compounds. As shown in Fig. 3B, chicken meat samples were classified into two clusters: 1) XJ groups and 2) HM, ZX, and XH groups. VOCs in the chicken meat samples were classified into four clusters. These results demonstrated that VOCs in chicken meat vary depending on the chicken breed. The flavor characteristics of XJ were more pronounced.

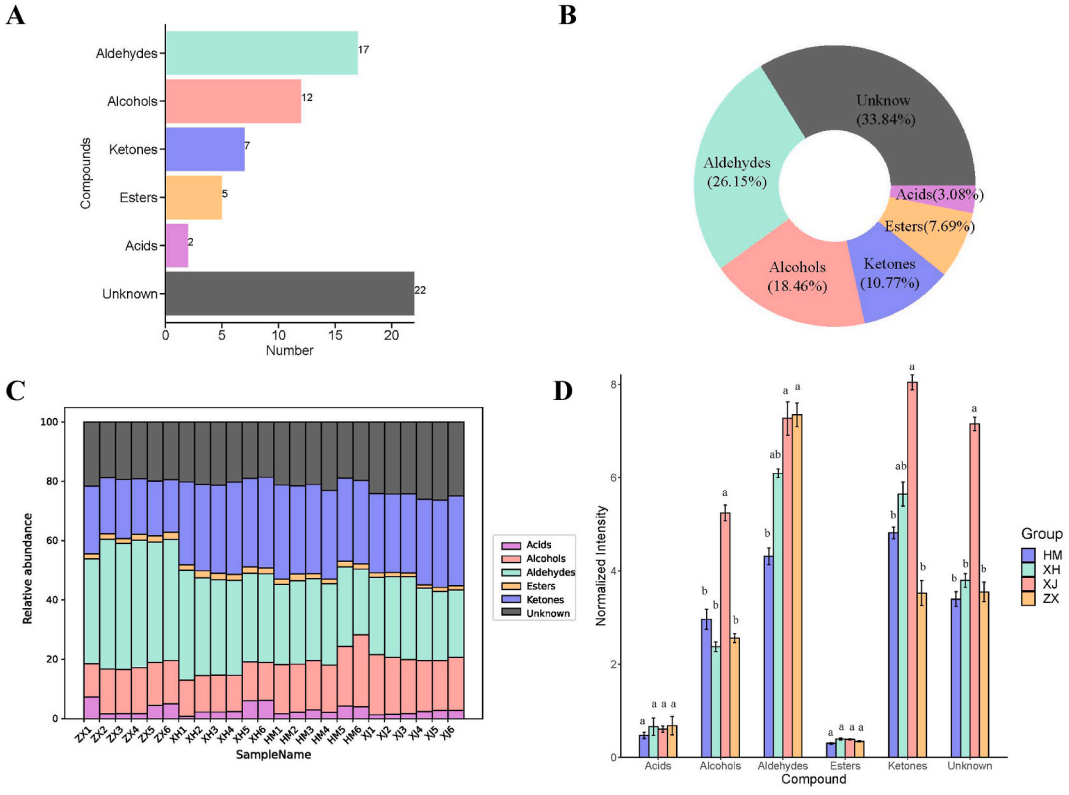

Fig. 2. VOC profiles of chicken meats from four chicken breeds. (A) Number and (B) percentage of volatile compound categories. (C) Percentages and (D) concentrations of volatiles compound typed in four chicken breeds. Data are mean ± SD (n = 6), different letters in the same class of compounds indicate significant differences at $p < 0.05$.

Table 1
Volatile compounds in chicken were identified from four chicken breeds.

Compounds	RI[a]	Rt[b][s]	Dt[c]	Signal intensities			
				ZX	XH	HM	XJ
3-methylbutyl acetate	857.7	343.98	1.306	0.1 ± 0.03[a]	0.14 ± 0.04[a]	0.04 ± 0.02[b]	0.04 ± 0.01[b]
ethyl acetate M	611.6	148.78	1.101	0.08 ± 0.01[b]	0.08 ± 0.01[b]	0.08 ± 0.01[b]	0.13 ± 0.02[a]
ethyl acetate D	610.4	148.21	1.342	0.02 ± 0.01[b]	0.02 ± 0.01[b]	0.03 ± 0.01[c]	0.08 ± 0.01[a]
butyrolactone	925.5	436.02	1.084	0.12 ± 0.01[a]	0.13 ± 0.02[a]	0.08 ± 0.01[b]	0.07 ± 0.01[b]
methyl 2-methyl butanoate	738.4	227.95	1.537	0.02 ± 0.01[a]	0.03 ± 0.01[a]	0.08 ± 0.05[b]	0.06 ± 0.02[ab]
esters (5)				0.34 ± 0.04[ab]	0.39 ± 0.06[a]	0.3 ± 0.04[b]	0.39 ± 0.03[a]
1-hexanol	873.8	363.87	1.324	0.09 ± 0.02[b]	0.07 ± 0.01[b]	0.08 ± 0.01[b]	0.49 ± 0.08[a]
1-octen-3-ol	997.9	562.77	1.163	0.27 ± 0.05[b]	0.27 ± 0.04[b]	0.28 ± 0.04[b]	0.67 ± 0.04[a]
1-pentanol D	762.2	247.26	1.511	0.17 ± 0.09[a]	0.06 ± 0.02[c]	0.05 ± 0.01[c]	0.42 ± 0.04[b]
1-pentanol M	763.8	248.62	1.251	0.48 ± 0.13[a]	0.28 ± 0.07[b]	0.2 ± 0.04[b]	0.35 ± 0.15[ab]
2_3-butanediol	803.7	285.28	1.366	0.2 ± 0.03[a]	0.22 ± 0.01[a]	0.74 ± 0.15[b]	0.57 ± 0.15[b]
2- methyl-1-pentanol	829.8	312.19	1.292	0.02 ± 0.01[b]	0.02 ± 0.01[b]	0.02 ± 0.01[b]	0.16 ± 0.07[a]
2-octanol	998.1	563.16	1.438	0.15 ± 0.05[ab]	0.18 ± 0.02[a]	0.09 ± 0.01[b]	0.16 ± 0.05[a]
2-propanol	528.5	113.49	1.185	0.53 ± 0.05[ab]	0.5 ± 0.04[a]	0.6 ± 0.06[b]	0.49 ± 0.09[a]
E−3-hexen-1-ol D	857.3	343.59	1.525	0.04 ± 0.02[ab]	0.09 ± 0.08[a]	0.02 ± 0.01[b]	0.02 ± 0.01[ab]
E−3-hexen-1-ol M	858.3	344.76	1.227	0.22 ± 0.08[ab]	0.31 ± 0.16[a]	0.12 ± 0.06[b]	0.09 ± 0.02[b]
ethanol	492.5	101.21	1.047	0.27 ± 0.05[bc]	0.26 ± 0.04[b]	0.65 ± 0.46[c]	1.71 ± 0.16[a]
furaneol	1083.2	761.28	1.203	0.13 ± 0.01[a]	0.11 ± 0.01[c]	0.11 ± 0.01[c]	0.1 ± 0.01[b]
alcohols (12)				2.56 ± 0.23[b]	2.37 ± 0.25[b]	2.96 ± 0.53[b]	5.24 ± 0.41[a]
2-butanone	594	140.41	1.253	1.39 ± 0.25[b]	1.5 ± 0.24[b]	0.95 ± 0.27[b]	2.6 ± 0.52[a]
2-heptanone D	892.1	387.85	1.637	0.03 ± 0.01[b]	0.03 ± 0.01[b]	0.04±0[b]	0.34 ± 0.09[a]
2-heptanone M	893.4	389.61	1.262	0.16 ± 0.03[b]	0.16 ± 0.02[b]	0.14 ± 0.01[b]	0.29 ± 0.04[a]
2-pentanone D	676.6	184.86	1.368	0.14 ± 0.02[b]	0.13 ± 0.03[b]	0.18 ± 0.02[ab]	0.24 ± 0.11[a]
2-pentanone M	675.7	184.27	1.122	0.21 ± 0.02[ab]	0.21 ± 0.01[ab]	0.24 ± 0.02[b]	0.17 ± 0.05[a]
3-hydroxy-2-butanone	715.7	210.98	1.338	0.38 ± 0.19[a]	1.53 ± 0.25[b]	1.86 ± 0.04[c]	1.95 ± 0.09[c]
acetone	524.2	111.92	1.125	1.21 ± 0.49[b]	2.1 ± 0.19[a]	1.4 ± 0.07[b]	2.45 ± 0.06[a]
ketones (7)				3.52 ± 0.66[b]	5.65 ± 0.63[ab]	4.82 ± 0.31[b]	8.05 ± 0.39[a]
benzaldehyde D	966.8	504.27	1.476	0.48 ± 0.14[b]	0.5 ± 0.1[b]	0.22 ± 0.09[c]	0.76 ± 0.09[a]
benzaldehyde M	966.2	503.09	1.151	0.79 ± 0.08[b]	0.76 ± 0.05[b]	0.44 ± 0.07[c]	0.65 ± 0.05[a]
E−2-octenal	1063.3	709.41	1.336	0.05 ± 0.01[a]	0.03 ± 0.01[c]	0.03 ± 0.01[c]	0.07 ± 0.01[b]
3-methylbutanal	659.8	174.72	1.162	0.35 ± 0.06[b]	0.45 ± 0.1[b]	0.45 ± 0.06[b]	0.64 ± 0.02[a]
heptanal D	903.2	403.25	1.701	0.2 ± 0.09[a]	0.09 ± 0.02[b]	0.08 ± 0.03[b]	0.2 ± 0.05[a]
heptanal M	903.5	403.65	1.332	0.51 ± 0.1[a]	0.36 ± 0.04[bc]	0.32 ± 0.04[c]	0.45 ± 0.04[ab]
hexanal	791.4	273.39	1.571	2.29 ± 0.21[a]	1.84 ± 0.13[c]	0.96 ± 0.16d	1.44 ± 0.3[b]
methional D	904.9	405.59	1.404	0.16 ± 0.03[b]	0.12 ± 0.01[bc]	0.09 ± 0.01[c]	0.37 ± 0.02[a]
methional M	907	408.72	1.093	0.27 ± 0.08[b]	0.3 ± 0.03[b]	0.16 ± 0.01[c]	0.47 ± 0.02[a]
nonanal D	1093	788.19	1.951	0.06 ± 0.02[a]	0.04 ± 0.01[bc]	0.03 ± 0.01[c]	0.05 ± 0.02[ab]
nonanal M	1093	788.19	1.475	0.31 ± 0.06[a]	0.23 ± 0.02[bc]	0.18 ± 0.06[c]	0.29 ± 0.04[ab]
octanal D	1019.8	608.01	1.831	0.11 ± 0.04[a]	0.06 ± 0.01[b]	0.05 ± 0.01[b]	0.12 ± 0.04[a]
octanal M	1019.4	607.23	1.404	0.44 ± 0.07[a]	0.32 ± 0.03[bc]	0.26 ± 0.06[c]	0.4 ± 0.06[ab]
pentanal D	685.5	190.51	1.429	0.31 ± 0.12[a]	0.09 ± 0.01[b]	0.13 ± 0.05[b]	0.29 ± 0.06[a]
pentanal M	685.8	190.71	1.184	0.58 ± 0.08[a]	0.35 ± 0.06[b]	0.36 ± 0.11[b]	0.44 ± 0.05[b]
phenylacetaldehyde	1005.5	577.98	1.258	0.03 ± 0.01[b]	0.04 ± 0.01[b]	0.03 ± 0.01[b]	0.13 ± 0.02[a]
2-methylbutanal	641.6	164.38	1.174	0.4 ± 0.05[ae]	0.52 ± 0.11[a]	0.52 ± 0.08[a]	0.5 ± 0.11[a]
aldehydes (17)				7.35 ± 0.62[a]	6.1 ± 0.23[ab]	4.31 ± 0.44[b]	7.27 ± 0.88[a]
2-methylpropanoic acid	750.9	237.91	1.377	0.65 ± 0.48[ad]	0.63 ± 0.46[a]	0.38 ± 0.14[a]	0.44 ± 0.09[a]
3-methylbutanoic acid	842.8	326.62	1.215	0.03 ± 0.01[af]	0.03 ± 0.01[ab]	0.1 ± 0.04[bc]	0.16 ± 0.08[c]
acids (2)				0.68 ± 0.48[a]	0.66 ± 0.46[a]	0.47 ± 0.17[a]	0.61 ± 0.17[a]

Note.
[a] Retention index calculated using n-ketones C4–C9 as the external standard on an FS-SE-54-CB column.
[b] Retention time in the capillary GC column.
[c] The drift time in the drift tube.
[d] M presented as "Monomer", D presented as "Dimer".
[e] Data presented as "mean ± SD" indicate the mean value and standard deviation.
[f] Lowercase letters within the same row indicate significant differences between the corresponding values ($p < 0.05$).

3.4. Multivariate analysis of VOCs

Unsupervised PCA was applied to the VOC data. Fig. 4A shows a PCA score plot demonstrating a clear separation among the four chicken meats. The distribution of the two main PCA components was 56.5% and 22.3%, respectively. Overall, four distinct regions were identified within the PCA profile (Fig. 4A), indicating obvious differences in the flavor of different varieties of chicken. Furthermore, a loading plot was used to identify key VOCs that most contributed to the clustering of samples. As shown in Fig. 4B, the direction and length of the vector indicate the contribution of the variables to the two principal factors. For example, 2-butanone, acetone, and ethanol were the main characteristic flavors of XJ and primarily contributed to the PC1 variance.

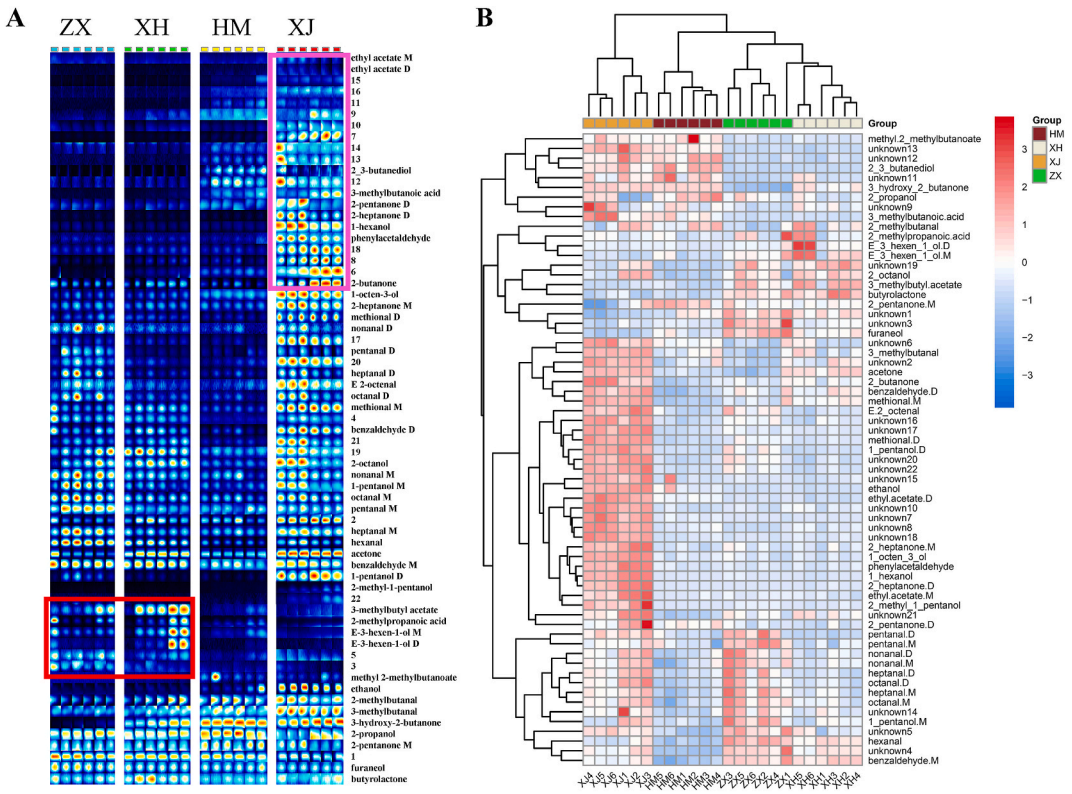

Fig. 3. (A) Fingerprints of gallery plot and (B) heat maps of VOCs in chicken meats from four breeds.

To identify potential marker volatiles to discriminate chicken meats of different breeds, we performed supervised PLS-DA on the VOC data. Fig. 4C shows the PLS-DA score plots of different chicken meats. A high level of similarity was observed in terms of VOC concentration between ZX and XH samples, while a significant difference was found between XJ and the other three breeds. These results further supported the clustering analysis of the heat map (Fig. 3B). The different breeds of chicken meat were well differentiated according to their VOCs by multivariate analysis.

The VIP results for OPLS-DA are shown in Fig. 4D. We identified 20 differential VOCs among 4 breeds of chicken meat based on the criteria of VIP >1. These differential VOCs belong to four categories: aldehydes (n = 9), alcohols (n = 7), esters (n = 2), and ketones (n = 2). These 20 VOCs contributed the most to distinguishing ZX, XH, HM, and XJ meat. Furthermore, we conducted radar and heat map clustering analyses based on 20 differential VOCs (Fig. 4E and F). The differences in radar images to some extent reflected the differences in flavor substances of different chicken varieties. It can be inferred that the overall flavor characteristics of chicken were formed by main volatiles and modified by differential volatiles. The heat map clustering results also indicated that the 20 markers VOCs in different breeds of chickens can better classify the differences in the sample.

4. Discussion

In this study, we identified and analyzed the VOCs in meat from different local breeds of chickens using GC-IMS, along with chemometric analysis. The GC-IMS spectra and fingerprints visually represent the flavor measurement results between samples. The fingerprint can be used to intuitively and quantitatively compare the differences in VOC profiles between samples [21]. We observed distinct differences in the spectra of ZX, XH, HM, and XJ chicken meats, which were confirmed by their VOC fingerprints. Notably, 3-methylbutyl acetate, 3-methylbutanoic acid, 2-pentanone D, 2-heptanone D, 1-hexanol, phenylacetaldehyde, 2-butanone, and ethanol exhibited significant differences between the four breeds. These findings indicate that GC-IMS analysis can rapidly distinguish between chicken meat samples from the four breeds, consistent with previous studies on different pork and donkey meat varieties [22,25].

We identified 43 VOCs in raw chicken meat across four breeds of chicken using GC-IMS. This number is notably lower than the VOCs identified in cooked chicken meat using GC-MS and GC-IMS [26,27]. It is well known that VOCs are more abundant in cooked meat and are generated by the Maillard reaction between amino compounds and reducing sugars, lipid degradation, and lipid-Maillard interactions during heating [28]. Mancinelli et al. [29] found that the drastic increase in VOC content in cooked chicken meat is caused by the oxidation of polyunsaturated fatty acids, with cooked meat having 5.5 times more VOCs compared to raw meat. Additionally, our study revealed that aldehydes, ketones, and alcohols were the three VOCs with the highest content in raw chicken. Ketones and

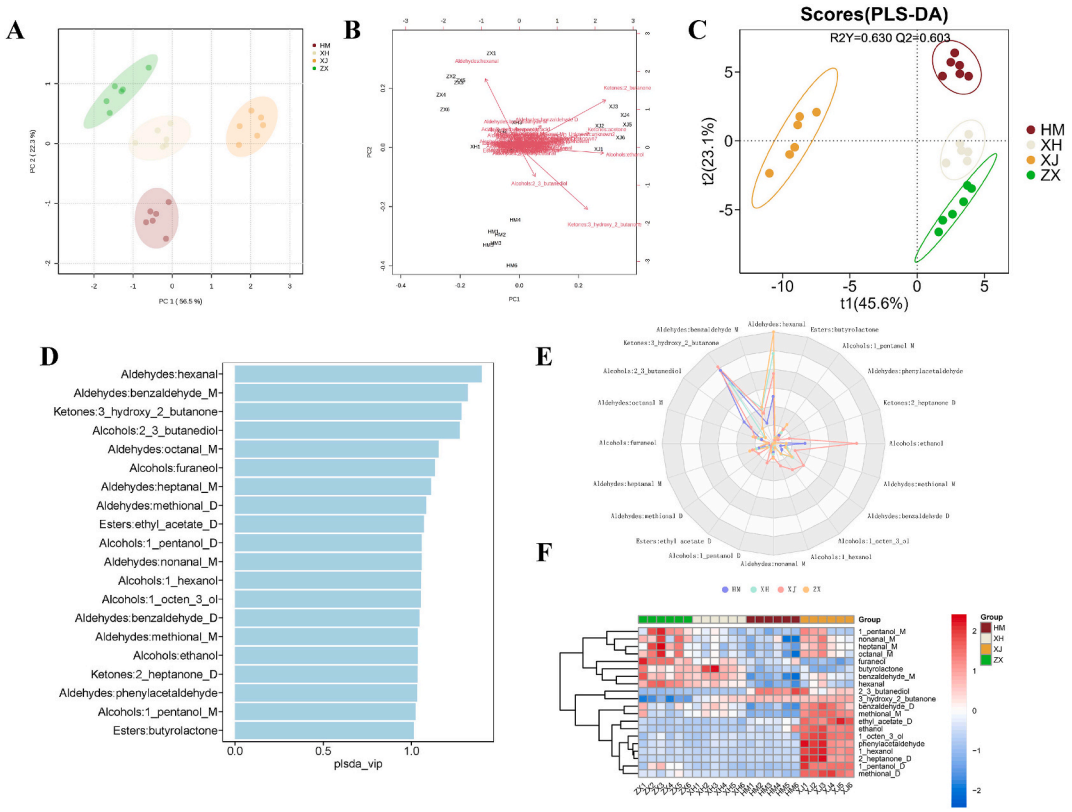

Fig. 4. Multivariate analysis of volatile components in meats from four chicken breeds. (A–B) Scores plot and biplot for PCA model. (C–D) Scores and VIP scores plots for the PLS model. (E–F) Radar plots and clustering heatmap for differential volatiles.

alcohols were notably more abundant in XJ compared to the other three chicken breeds, whereas the content of aldehydes in XJ and ZX was significantly higher than that in HM. Similar variations in VOCs have been observed in different pork breeds [30,31]. Interestingly, chicken meat contains more VOCs compared to pork, duck, and beef, and poultry meat has higher levels of aldehydes, acids, and alcohols than livestock meat [11].

In the present study, multivariate analysis methods (PCA, PLS-DA, and heatmap analysis) were used to ensure the data accuracy and fingerprints obtained by GC-IMS. The VOCs of chicken meats from different breeds were found well differentiated by PCA, PLS-DA, and heatmap analysis, which is consistent with the fingerprint results. In addition, 20 differential VOCs were identified, including 9 aldehydes, 7 alcohols, 2 esters, and 2 ketones. These results demonstrate that multivariate analysis can be applied to distinguish different samples and screen biomarkers from GC-IMS data. Nevertheless, the feasibility and accuracy of these signature volatiles in actual products, as well as their aroma characteristics, must be verified by GC-O data in the future.

Previous studies have shown that the VOCs in meat are breed-dependent [22,32]. Our present study aligns with these findings and reveals that VOCs in chicken meat are also breed-dependent. The formation of VOCs is complex and their sources are extensive, among which lipids play a central role in meat flavor development [33]. Lipid degradation influences the creation of distinct meat flavor compounds, including aldehydes, ketones, and alcohols [34]. Aldehydes have a lower threshold and contribute more to the overall favor of the meat. In meat and meat products, aldehydes are mainly derived from lipid oxidation. For example, hexanal, nonanal, octanal, heptanal, pentanal, benzaldehyde, and so forth are the products of lipid oxidation [6]. Jin et al. [8] discovered that aldehydes, particularly hexal derived from linoleic acid oxidation, constitute the primary volatile flavor compounds in chicken. However, an excessive presence of aldehydes may indicate meat undergoing oxidative deterioration, which negatively impacts meat flavor [35]. Among the alcohols, 1-octene-3-ol, a significant alcohol in chicken, results from the oxidation of linoleic acid. It possesses a very low sensory threshold and a potent mushroom odor [36]. The expression levels of *HSP90AA1*, a heat shock protein (HSP), and the non-receptor protein tyrosine phosphatase 9 (*PTPN9*) were found to be significantly positively correlated with the content of 1-octene-3-ol [37]. Hexyl alcohol originates from the reduction of hexaldehydes, and amyl alcohol arises from the degradation of lipid hydroperoxides, contributing to a pungent, strong balsamic odor [38]. Ketones mainly result from fat oxidation, with 2-ketones, such as 2-pentanone, 2-heptanone, and 2-decanone, playing a significant role in meat flavor, resembling odors of ether, butter, and cheese [39]. 3-hydroxy-2-butanone, a Maillard reaction product, has a pleasant creaminess, possibly due to oleic acid oxidation or glucose decomposition [21].

In this study, while we successfully characterized and screened VOCs from various regional breeds of chicken using GC-IMS and chemometrics, our experiment had certain limitations. It was somewhat biased because we limited our subjects to four regional

chicken breeds and only characterized VOCs in the breast muscle. Research indicates that various factors, including age, diet, and environmental conditions, can influence the results of meat flavor analysis [35,40,41]. Therefore, our future research will incorporate a broader range of chicken varieties, ages, feeding patterns, and types of tissues. We aim to establish a comprehensive VOCs database for diverse chicken varieties based on GC-MS, GC-IMS, GC-*O*-MS, and other multi-group learning segments. This will provide the theoretical foundation and technical support essential for enhancing chicken quality, flavor, and variety.

5. Conclusions

In this study, VOCs in chicken meat from different breeds were comprehensively analyzed and compared using HS-GC-IMS. A total of 43 VOCs were detected in chicken meat from the four breeds. The primary volatile components in the various chicken meats were aldehydes, ketones, and alcohols. Distinct differences in volatile components in various chicken meats were successfully identified using GC-IMS data, PCA, and heatmap clustering analysis. Furthermore, PLS-DA was employed to identify 20 different VOCs as volatile markers for distinguishing among the four regional chicken varieties. However, further research with larger sample size is necessary to validate the discrimination performance of these volatile markers and elucidate the formation mechanism of aroma substances through multi-omics approaches.

Ethics statement

This study was conducted following the Guidelines for the use of Experimental Animals established by the Ministry of Science and Technology (Beijing, China). All experimental protocols were approved by the Animal Care and Use Committee of Gansu Agriculture University (Lanzhou, China) (No. GSAU-ETH-AST-2021-02).

Data availability statement

Data will be made available on request.

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CRediT authorship contribution statement

Hongqiang Li: Writing – original draft, Methodology, Investigation, Data curation. **Xiangmin Zhao:** Methodology, Investigation. **Shizhen Qin:** Software, Resources. **Jinlu Li:** Formal analysis. **Defu Tang:** Writing – review & editing, Supervision. **Bin Xi:** Software, Investigation, Data curation.

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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