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Characterization of the Flavor Profile in Sausages Collected from Four Typical Regions of Southern China Using Headspace Solid-Phase Microextraction-Gas Chromatography-Mass Spectrometry (HS-SPME-GC-MS) Combined with Electronic Nose and Electronic Tongue

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Abstract   Fermented sausage has attracted attention from consumers due to its pleasant flavor. In our study, the physicochemical characteristics, volatile compound (VOC) composition, and electronic sensory indices of sausages from four typical regions of southern China were investigated and compared, and the potential relationship between differential VOCs and odor/taste attributes was explored. The samples from Jiangsu (JS) and Zhejiang (ZJ) provinces displayed similar composition of VOCs with dominant aldehydes (hexanal and nonanal) and alcohols (1-octen-3-ol, 1-butanol, 2-ethyl-, 1-octanol, and 2-octen-4-ol), while phenols (mequinol, creosol, phenol, 4-ethyl-2-methoxy-, and phenol, 2,6-dimethoxy-) and terpenoids (D-limonene and linalool) were dominant in the samples from Sichuan (SC) and Hunan (HN) provinces, respectively. Furthermore, the Spearman rank correlation analysis revealed the key VOCs (relative odor activity value>1) causing the differences in odor and taste scores among various groups. 1-Octen-3-ol, hexanal, heptanal, nonanal, decanal, and (E)-2-octenal provided floral, green, fatty, and fruity odors and enriched the umami, saltiness, bitterness, and astringency of the JS and ZJ groups, while D-limonene and linalool brought citrus, rose, and green flavors and enhanced the umami and saltiness of the HN group. Besides, phenol, 4-ethyl-2-methoxy- and phenol, 2,6-dimethoxy- imparted smoky and spicy flavors and enhanced the sourness and richness of the SC group. These findings provide valuable insights for product improvement and quality assessment of fermented sausage.

Keywords   sausage, volatile compounds, taste, odor, correlation analysis

Introduction

Fermented sausage is a popular meat product due to its characteristic flavor, texture, and color (Wang et al., 2022). It is usually made by filling a mixture of pork back fat, lean pork, and various excipients into the small intestine of pigs, and then allowing them to undergo spontaneous fermentation under the promotion of microorganisms in raw meat and surroundings (Wang et al., 2022). The sensorial characteristics of fermented sausages are affected by the sources of raw meat and spices, ingredient proportion, processing technology, and complex microbial composition (Chen et al., 2021; Wang et al., 2018). Chinese smoke-cured sausage and Chinese dry-cured sausage are two types of traditional fermented sausages (Wang et al., 2018). Chinese smoked-cured sausage suffers a cold-smoked process with raw firewood at a temperature of 25℃–30℃ for 20–30 d until it dries (Guo et al., 2016), while Chinese dry-cured sausage has an air-dried process at ambient temperature for a few weeks (Chen et al., 2021). The drying procedure with wood smoke or air-dried can accelerate the hydrolysis of carbohydrates, proteins, and lipids through microbial metabolism and endogenous enzymes, promoting the formation of volatile compounds (VOCs) and firm texture (Flores, 2018). It was reported that the microbial composition and diversity were different between these two types of sausages (Wang et al., 2018). The smoked sausages are more easily accepted by consumers from Sichuan (SC) and Hunan (HN) provinces, while the air-dried sausages are more popular in Jiangsu and Zhejiang (ZJ) provinces, which may be attributed to their different sensory attributes. However, the characteristic VOCs of these two kinds of sausages remain unclear. Therefore, a comprehensive analysis of the flavor profiles of sausages from these four typical regions of southern China is of great significance for its geographical origin identification and quality assessment.

In recent years, headspace solid-phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC-MS) has been successfully applied to characterize the flavor profiles of fermented meat due to its distinctive advantages in identifying and quantifying VOCs (Chen et al., 2024). Saldaña et al. (2019) identified and quantified 39 VOCs of smoked bacon using HS-SPME-GC-MS, and Chen et al. (2021) detected 62 VOCs in dry fermented sausages with different NaCl substitutes using HS-SPME-GC-MS, of which 26 VOCs were considered key VOCs based on their odor activity values. Besides, the taste and odor are two important indicators that determine the overall flavor of fermented meat (Hou et al., 2024). Electronic nose (E-nose) and electronic tongue (E-tongue) can mimic the human olfactory system and gustatory sensation, facilitating to distinguish flavor features and characterize the differences in odor and taste without subjective judgements (Duan et al., 2021). The application of these two technologies have been proved to be invaluable in various aspects, including flavor analysis, quality control, and process monitoring (Di Rosa et al., 2017; Duan et al., 2021). It was reported that the distinct differences in flavor quality of bacon smoked with different woodchips (Du et al., 2021), as well as the changes of odor and taste profiles in sausages with different sodium substitutes could be distinguished by E-tongue and E-nose (Chen et al., 2021). However, these systems cannot identify specific VOCs causing the nuances in flavor unless multivariate analysis was conducted to analyze the correlation between electronic sensory indexes and VOCs (Duan et al., 2021). For example, the Spearman correlation analysis showed that the signal intensities of E-nose and E-tongue were highly correlated with the alcohols, aldehydes, and ketones content of bacon smoked with different woodchips (Du et al., 2021). Hence, HS-SPME-GC-MS combined with E-nose and E-tongue can be used to characterize the flavor profiles of sausages from different regions of southern China, which may provide a reference for establishing a comprehensive and rapid method to distinguish different flavor characteristics of fermented sausage.

This study selected representative air-dried and smoked sausages from four typical regions of southern China and compared their physicochemical characteristics, VOC composition, and electronic sensory indices, and the key VOCs closely

related to sensory quality were explored. These results provide some theoretical support for the scientific description of sensory differences in fermented sausage from varied regions, and lay the foundation for product improvement and quality assessment of fermented meat.

Materials and Methods

Reagents and standards

Potassium chloride and tartaric acid were of analytical grade and supplied by Sinopharm Chemical Reagent (Beijing, China). Chromatographic grade of 2-octanol was purchased from Aladdin (Shanghai, China).

The collection of sausage samples

The sausage samples purchased from local wet markets in HN and SC provinces, namely Gaoqiao Market (Changsha, China), Agricultural Products Wholesale Center (Chengdu, China), and Baijia Market (Chengdu, China), were representative of the classic smoked sausage. The sausage samples purchased from local wet markets in Jiangsu (JS) and ZJ provinces, namely Zhongcai Market (Nanjing, China) and Aquatic and Meat Trading Market (Jiaxing, China), were popular styles of dry sausage. They were totally made by households and sold on local wet markets, but the ingredients slightly differed (Supplementary Table S1).

Physicochemical characteristic analysis

The pH values were measured with a digital pH meter (Mettler Toledo, Shanghai, China) according to the Chinese National Standard GB 5009.237-2016 (Yang et al., 2023). The water activity (a_w) values were measured with a LabMaster-a_w meter (Novasina, Switzerland) at room temperature (Wang et al., 2021c). According to the method of Yang et al. (2023), samples (10 g) were thoroughly minced, and the CIE L*, CIE a*, CIE b*, and ΔE (total color difference) values were determined using a chroma meter WR-18 (Shenzhen Wave Optoelectronics Technology, Shenzhen, China). As described by our previous study (Hao et al., 2024), the textural indexes (cohesiveness, gumminess, hardness, springiness, and chewiness) were detected using a TMS-PRO texture analyzer (Food Technology, Sterling, VA, USA) equipped with a 250 N load cell. The sample was cut into slices of 2×2×1 cm, and ten independent batches of each sample were prepared to ensure the accuracy of the test. The texture profile analysis was conducted using a 40 mm cylindrical probe at a speed of 1 mm/s, and the compression ratio was set at 60%.

Electronic nose analysis

According to the methods of Hou et al. (2024), the E-nose analysis was carried out by using a PEN3 E-nose system (Airsense Analytics, Schwerin, Germany). This instrument composed of a sampling device and a detector unit with a sensor array. The sensor array contained 10 chemical sensors, namely W1C (aromatic compounds), W3C (aromatic compounds), W5C (arom-aliph), W1W (sulfur-organic compounds), W2W (sulph-chlor), W1S (broad-methane), W2S (broad-alcohol), W3S (methane-aliph), W5S (broad-range compounds), and W6S (hydrogen). The resistivity (measured in Ohms) was used to quantify the response of sensors.

The samples (5 g) were thoroughly minced and maintained it sealed with cling film for 1 h. The specific parameter settings of the instrument were as follows, a gas flow rate of 200 mL/min; zero setting time of 5 s; preparation time of 5 s;

measurement phase of 90 s; and cleaning time of 30 s. All samples were tested for 5 independent replicates.

Electronic tongue analysis

Based on the method of Li et al. (2022), the E-tongue analysis was performed using a SA402B taste-sensing system (Intelligent Sensor Technology, Atsugi, Japan) equipped with a set of multichannel lipid/polymer membrane electrodes, reference electrodes, automatic sampler, and electronic unit. There were five sensors that were sensitive to the taste properties of the samples, including richness, astringency, sourness, bitterness, umami, saltiness, aftertaste-A (aftertaste astringency), and aftertaste-B (aftertaste bitterness).

The minced samples (20 g) were mixed with deionized water (200 mL) and subjected to a water bath at 50℃ for 20 min. After centrifugation at 10,000×g for 15 min, the supernatant was filtered and poured into a special beaker prior to E-tongue analysis. The specific parameter settings of the instrument were as follows, stirring rate of 60 rpm; collection time of 120 s; and cleaning time of 30 s. One measurement was performed per second, and the average value of the last 20 s (100–120 s) was taken as the output value.

Analysis of volatile compounds

The VOCs were extracted by HS-SPME and analyzed by a TSQ 8000 EVO GC-MS coupled with a TG-5 MS capillary column (30 m×0.25 mm×0.25 μm; Thermo Fisher Scientific, Waltham, MA, USA). The specific parameters were obtained referring to our previous study (Han et al., 2022). The VOCs were identified by comparing their mass spectral data with the NIST 17.0 library, and semi-quantified by calculating the ratio of their mass spectral peak area to the peak area of 2-octanol.

Sensory evaluation

According to the method described by Yang et al. (2023), sensory evaluation was conducted by a trained team of 12 panel members, who were trained on the evaluation of sausages before starting the test. The samples were scored by 1–5 points to evaluate the quality of sausages from 5 aspects (texture, color, odor, state of fat, and taste). Each set of samples was presented to each panel member in a random order.

Statistical analysis

The semiquantitative results of VOCs content in sausages from different regions were expressed as mean±SD. Significance was performed at $p < 0.05$ by one-way analysis of variance (ANOVA) using SPSS 25.0 (IBM, Armonk, NY, USA). The results of physicochemical characteristics, E-nose, and E-tongue were represented by Violin plots, and the results of the VOC profiles were represented by chord diagrams. Violin plots were visualized by GraphPad Prism 8.0 (GraphPad Software, San Diego, CA, USA). The chord diagrams, Spearman correlation analysis and heatmap were visualized by Origin 2018 (OriginLab, Northampton, MA, USA). The orthogonal partial least-squares discrimination analysis (OPLS-DA) and hierarchical clustering analysis (HCA) were visualized by SIMCA 14.0 (Umetrics AB, Malmo, Sweden).

Results and Discussion

Physicochemical characteristics of sausages from different regions

The pH, a_w, color, and texture properties of sausages from different regions were shown in Fig. 1. pH value is a curial
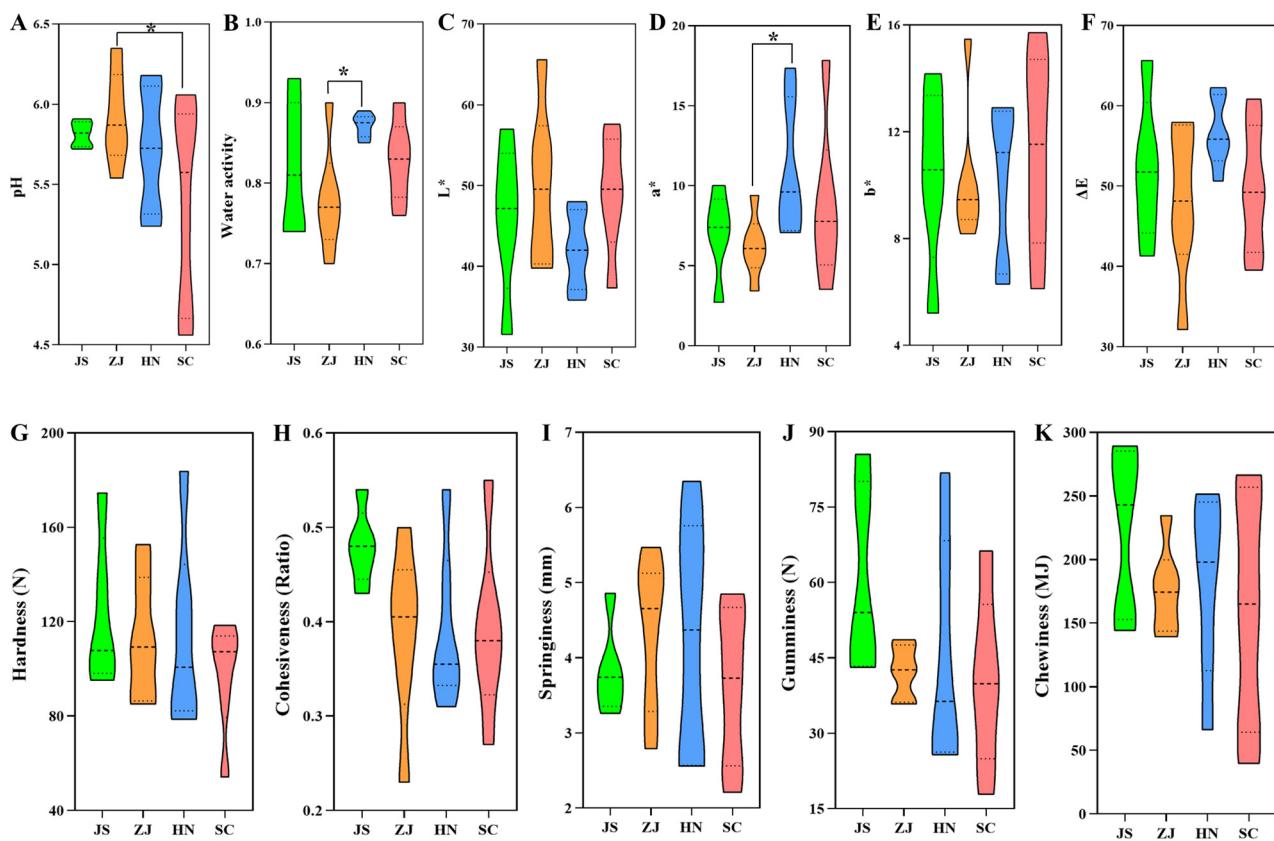
Fig. 1. Physicochemical characteristics of sausages from different regions. (A) pH values, (B) water activity values, (C) CIE L*, (D) CIE a*, (E) CIE b*, (F) ΔE (total color difference), (G) hardness, (H) cohesiveness, (I) springiness, (J) gumminess, and (K) chewiness. JS, ZJ, SC, and HN represented the sausage samples collected from Jiangsu, Zhejiang, Sichuan, and Hunan, respectively. Significant differences are represented by * p<0.05 and ** p<0.01.

index for predicting the quality of fermented meat. The metabolism of lactic acid bacteria and the accumulation of organic acids could lead to a decrease in the pH value of fermented meat, which was critical for inhibiting the growth of undesirable microbes (Ashaolu et al., 2023). As shown in Fig. 1A, the pH values of the sausage samples ranged from 4.56 to 6.35. The SC group possessed significantly lower average pH value (5.40±0.65) than that of the ZJ group (5.92±0.29; p<0.05), and the pH values of all samples in the JS group were below 6.0, indicating potential differences in the safety and quality of sausages from different regions. a_w could affect the nutrient solubility, microbial colonization and their enzyme activity in the fermented meat, and the typical a_w of the southern Chinese fermented sausages was 0.75–0.78 (Yang et al., 2023). As shown in Fig. 1B, the a_w values of the sausage samples ranged from 0.70 to 0.93, and the ZJ group showed significantly lower average a_w (0.78±0.07) than that of the HN group (0.87±0.01; p<0.05). The difference in a_w could be attributed to the product formulations, fermentation conditions, and the utilization of free water by microbial growth and reproduction (Yang et al., 2023). It was reported that an a_w value of <0.7 was usually unfavorable for microbial growth, and low water activity may lead to increasing shear force and decreasing tenderness of the fermented meat products (Mediani et al., 2022). Color is a vital indicator for customers to perceive the quality of the meat products without touching (Suman and Joseph, 2013). As shown in Figs. 1C, D, E, and F, no significant difference in the CIE L*, CIE b*, and ΔE was observed among all groups. CIE a* was positively correlated with the content of nitroso myoglobin pigment produced by nitrate reduction in the coagulase-negative *staphylococci* (CNS; Majou and Christieans, 2018). The SC and HN groups displayed relatively higher CIE a* than the JS

and ZJ groups. Previous studies have reported that CIE a* and CIE b* were important parameters affecting the color of fermented meat (Posthuma et al., 2018), and the samples of the HN group may be more attractive due to the higher CIE a* and lower CIE b*. As shown in Figs. 1G, H, I, J, and K, there was no significant difference in the texture properties among the groups. Previous study has reported that with the decrease of pH value during fermentation, the myofibrillar proteins aggregated to produce an acid-induced gel, which could enhance the springiness and cohesiveness of the fermented meat (Yang et al., 2023). On the other hand, the pH value had a positive correlation with the a_w, which may affect the texture of the fermented meat (Yang et al., 2023). In this study, the Spearman rank correlation analysis showed the negative correlation between pH and cohesiveness, hardness, gumminess, and chewiness, which was consistent with the previous report (Fig. 2C).

Comparison of volatile compounds in sausages from different regions

As shown in Table S2, a total of 69 VOCs were detected in all samples, including 22 aldehydes, 17 alcohols, 6 phenols, 5 terpenoids, 5 acids, 5 alkanes, 5 esters, and 4 ketones. Briefly, the JS and ZJ groups had a great similarity in their VOC composition, with alcohols and aldehydes accounting for more than 40% of the total VOCs in these two groups. The HN group contained similar proportions of alcohols, aldehydes, esters and terpenoids, while in the SC group, phenols were the most abundant class, followed by aldehydes and terpenoids (Fig. 3A). As shown in Fig. 3B, the content of aldehydes in the JS and SC groups was higher than the other two groups. The highest total content of VOCs was found in the SC group, and most of which were phenols but the alcohols content was the lowest. However, the HN group possessed the highest content of alcohols.

The OPLS-DA biplot of VOCs released from sausages of different regions was shown in Fig. 3C. The model was explained and predicted based on $R^2X=0.797$, $R^2Y=0.732$, and $Q^2=0.605$. The differences in VOC composition of sausage samples caused variations in the distribution of the four groups (Figs. 3D, E, F, and G). D-limonene, linalool, benzeneacetaldehyde, nonanoic acid, n-hexadecanoic acid, and decanoic acid, ethyl ester were the characteristic compounds that distributed the HN group in quadrant II, whereas phenols (mequinol, creosol, phenol, 4-ethyl-2-methoxy-, phenol, 2,6-dimethoxy-, and eugenol), benzaldehyde, 4-ethyl-, and cedrol were the main reasons for the distribution of the SC group in quadrant III. The samples of the JS and ZJ groups were distributed in the positive x-axis, which may be related to its different performance in the content of hexanal, nonanal, 1-octen-3-ol, 1-butanol, 2-ethyl-, 2-octen-4-ol, 1-octanol, and 3-octanone compared to the other two groups. Besides, the model had an adequate fit with the data according to the permutation test, with intercepts of $R^2=0.248$ and $Q^2=-0.349$, respectively (Fig. 3H). Then, 14 important differential VOCs were screened and identified according to the variable importance for the projection (VIP) analysis, including 1 ester, 2 aldehydes, 5 alcohols, 1 terpenoids, 4 phenols, and 1 ketone (Fig. 3I). Compared with previous studies, 1-octen-3-ol, 1-octanol, 1-butanol, 2-ethyl-, 2-octen-4-ol, hexanal, nonanal, and 3-octanone were found to be the predominant VOCs in the JS group, while linalool, D-limonene, and decanoic acid, ethyl ester were found to be the most important VOCs in the HN group. In addition, creosol, mequinol, phenol, 2,6-dimethoxy-, and phenol, 4-ethyl-2-methoxy- were found to be the dominant VOCs in the SC group, which could contribute to the typical aroma of dry-cured meat products (Yang et al., 2023). Moreover, the variated distribution of these differential compounds was observed in sausage samples from different regions, of which the JS and ZJ groups were more similar (Fig. 3J).

In detail, alcohols could impart floral aromas of rose and citrus and serve as good solvents for other aromatic compounds (Wang et al., 2021a). Based on the VIP analysis, 1-octen-3-ol, 1-butanol, 2-ethyl-,1-octanol, and 2-octen-4-ol were found to be the predominant alcohols in the JS and ZJ groups, while linalool was found to be the most important alcohols in the HN group. These alcohols were the typical aromas that differentiated cured and smoked meat in agreement with previous studies
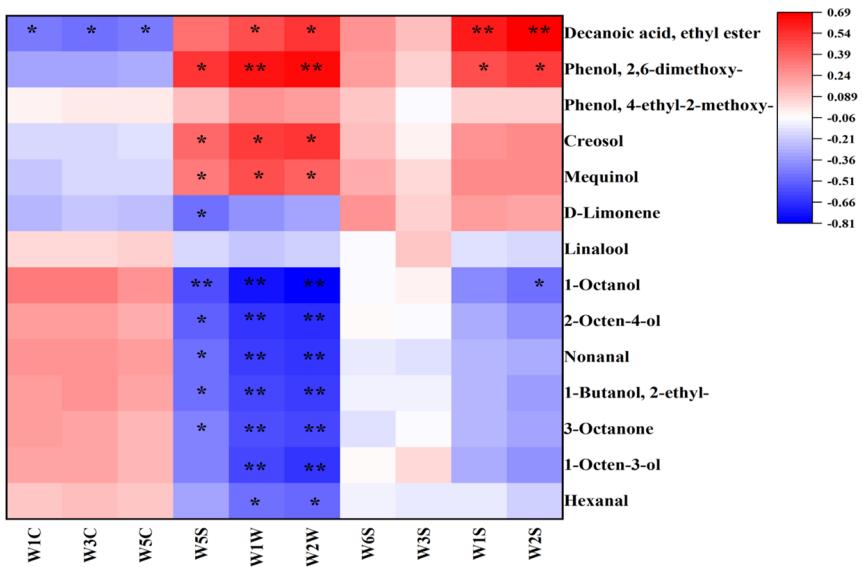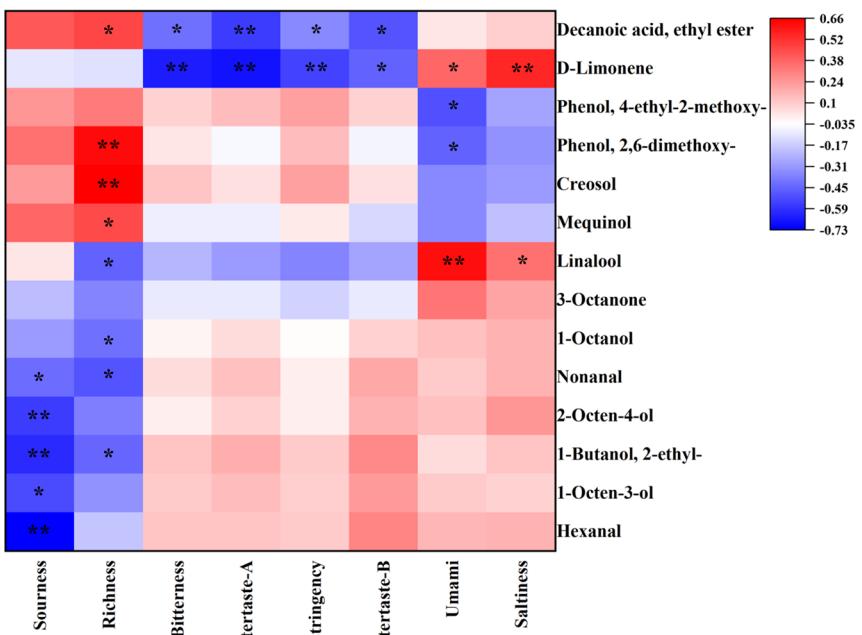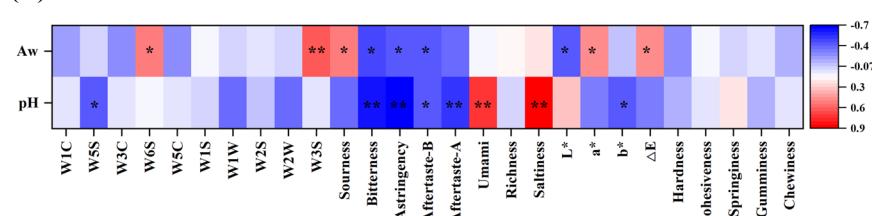
Fig. 2. The Spearman rank correlation analysis among differential volatile compounds, aroma and taste profiles of sausages from different regions. (A) Heatmap of the correlation between differential volatile compounds and aromatic profiles, (B) heatmap of the correlation between differential volatile compounds and taste profiles, and (C) heatmap of the correlation between pH and a_w with various physicochemical and flavor indicators. Significant differences are represented by * $p<0.05$ and ** $p<0.01$.
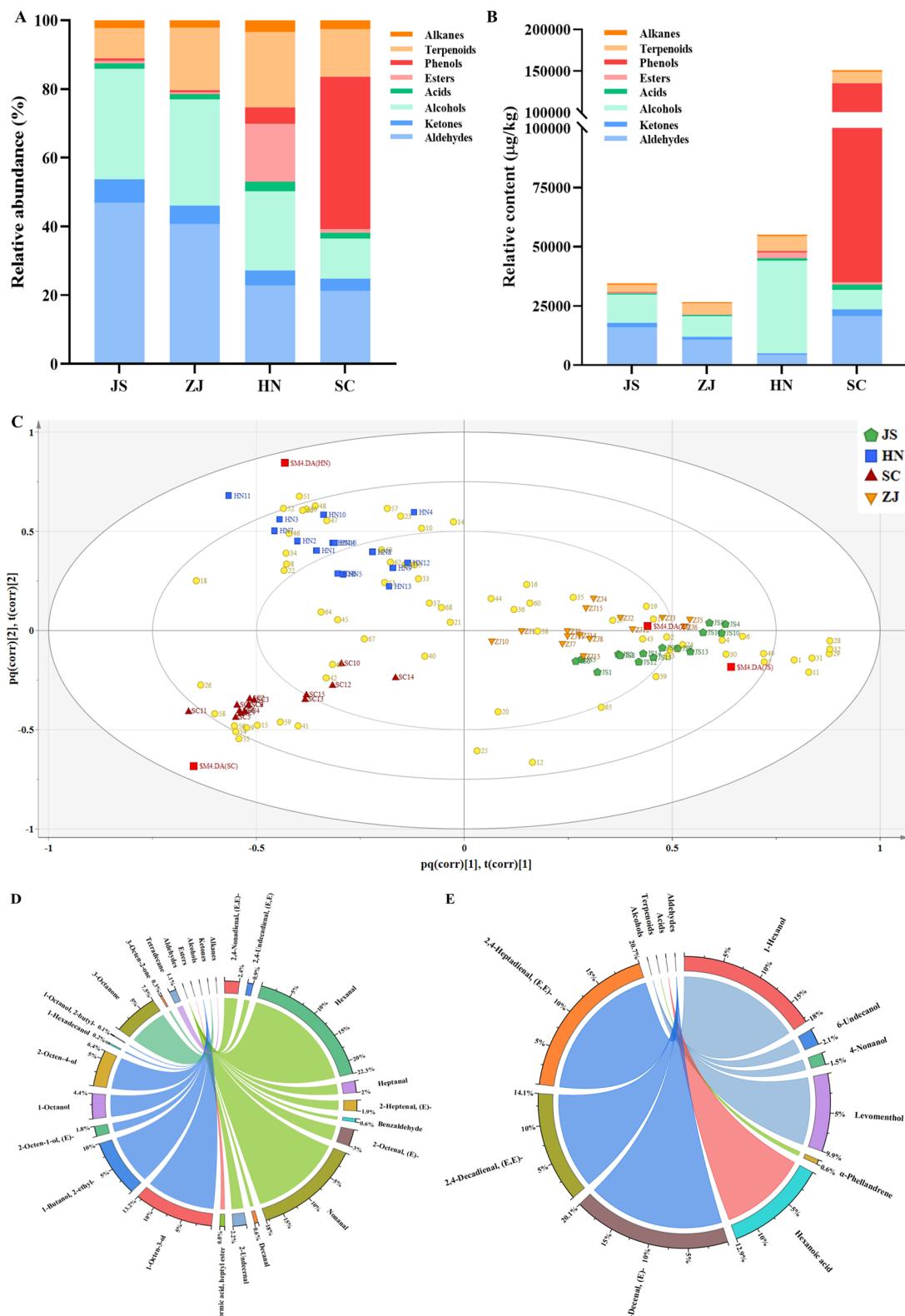
Fig. 3. Comparison of volatile compounds in sausages from different regions. (A) The relative abundant and (B) the relative peak area of different types of volatile compounds, (C) the biplot of OPLS-DA. The characteristic volatile compound profiles of the (D) JS, (E) ZJ, (F) HN, and (G) SC groups obtained by OPLS-DA. (H) Validation plot of the OPLS-DA model, (I) VIP plot, (J) the heatmap of differential volatile compounds content (VIP>1, p<0.05), and (K) hierarchical clustering analysis. JS, ZJ, SC, and HN represented the sausage samples collected from Jiangsu, Zhejiang, Sichuan, and Hunan, respectively. The serial numbers of volatile compounds were consistent with the No. column in Supplementary Table S2. OPLS-DA, orthogonal partial least-squares discrimination analysis; VIP, variable importance for the projection.
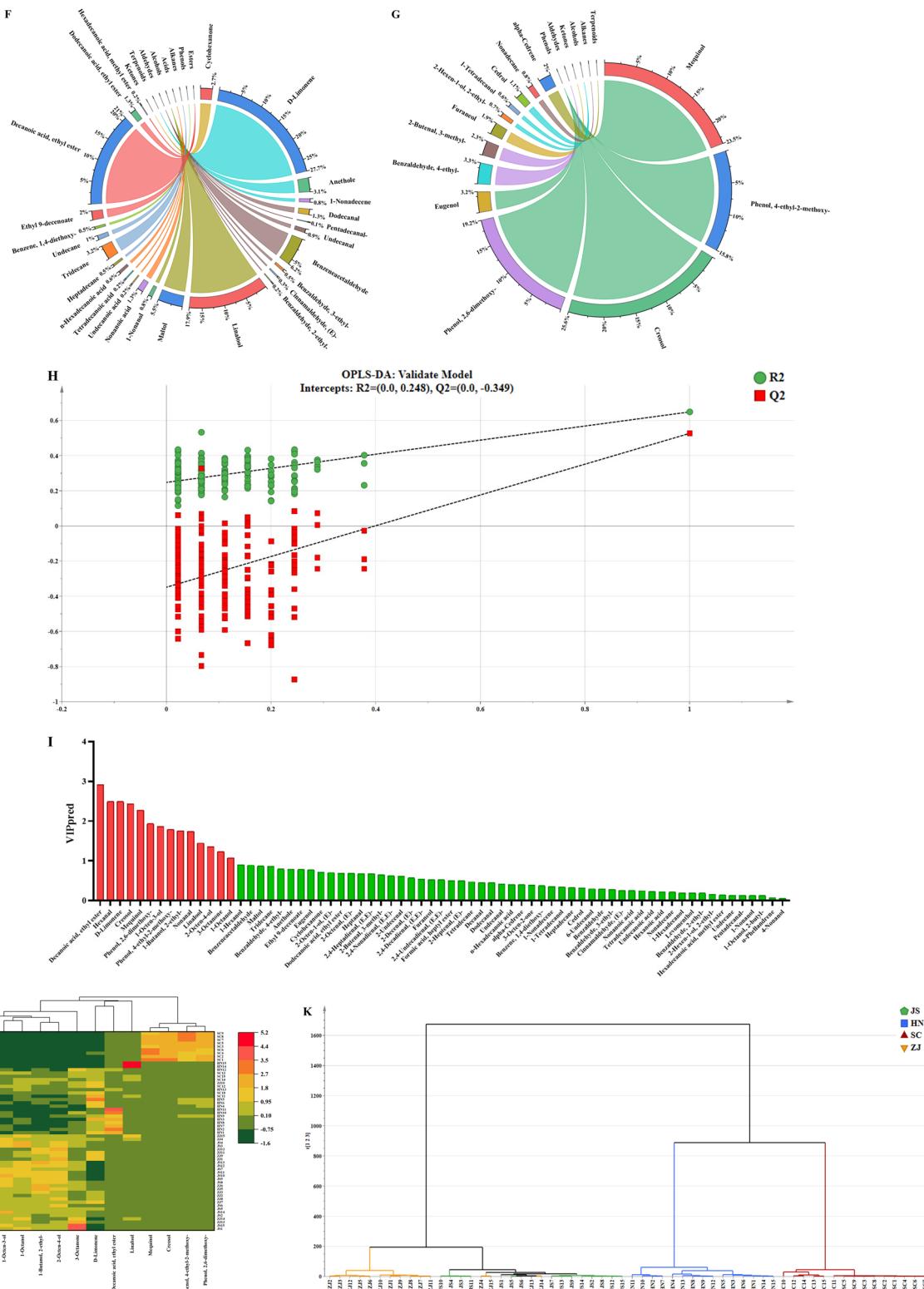
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(Merlo et al., 2021; Yang et al., 2023), and their production was positively correlated with the activities of *Staphylococcus*, *Bacilli*, and *Serratia liquefaciens* (Supplementary Fig. S1). As shown in Table S3, 1-octen-3-ol and linalool were detected in all four groups with a relative odor activity value (ROAV)>1, which could impart typical floral and green odors to the sausage (Xiong et al., 2024). Aldehydes were the main VOCs of the fermented meat that contributed to a rancid aroma at higher contents and a grass aroma at lower contents (Merlo et al., 2021). Some aldehydes with a ROAV>1, such as hexanal, heptanal, nonanal, decanal, and (E)-2-octenal (Pu et al., 2020), were detected in all four groups, and they could bring the flavors of grass, fat, fruity, and potato to the sausage (Yang et al., 2023). Among them, the contents of hexanal and heptanal in the JS and ZJ groups were distinguished from the HN and SC groups (VIP>1), which were positively correlated with the activities of *Bacilli* and *Serratia liquefaciens* (Supplementary Fig. S1). It was reported that non-smoked bacon could improve its flavor characteristics by increasing the contents of hexanal and heptanal (Zhang et al., 2021), which was consistent with the above results. Ketones were mainly produced by Maillard reactions or the microbial metabolism of lipids and amino acids, which conferred butter, spice, and blue cheese aromas of the fermented meat (Merlo et al., 2021). Although ketones were detected in all groups, their contribution to flavor may be minor due to the high thresholds (Wang et al., 2021b). In this study, the JS and ZJ groups were distinguished from the other two groups due to the presence of 3-octanone (VIP>1). In addition, esters were produced by the microbial esterification of fatty acids and alcohols in fermented meat. Short-chain (C1-C10) esters could contribute the fruity and fatty aromas at low thresholds (Flores, 2018). Decanoic acid, ethyl ester was a short-chain ester that significantly differentiated the HN group (VIP>1), and its content possessed positive correlations with *Bacilli*, *Serratia liquefaciens*, and *Chryseobacterium* (Supplementary Fig. S1). Besides, acids producing by microbial fermentation of carbohydrates could impart sour and pungent odors of fermented meat (Flores, 2018). All detected acids, except for hexanoic acid, were the characteristic acids that distinguished the HN group from the other groups, and they may contribute less to the flavor profiles of the sausage samples due to high flavor thresholds. Notably, n-hexadecanoic acid could be used as the substrate of esterification to generate esters with special flavors (Li et al., 2022), which may be the reason causing the significantly higher hexadecanoic acid, methyl ester content in the HN group. Smoking is an important way to change the flavor profiles of meat products. Phenols were the main VOCs that imparted spicy, sour, and toasty flavors to smoked meat products, and were mainly produced by the decomposition of lignin in fumigating materials (Xi et al., 2021). Creosol, mequinol, phenol, 2,6-dimethoxy-, and phenol, 4-ethyl-2-methoxy- were the differential phenols (VIP>1), with the highest levels in the SC group. Among them, the ROAVs of phenol, 2,6-dimethoxy- and phenol, 4-ethyl-2-methoxy- in the HN and SC groups were higher than 1.0, which could provide a unique smoky flavor to the sausage (Yang et al., 2023). Besides, compared with previous studies, creosol and mequinol were found to be the dominant phenols in the SC group, which has not been reported in SC cured meat products (Yang et al., 2023). It was reported that phenol and methoxyphenol aromatic compounds were generated when using spices as ingredients, while the content of phenol, 4-ethyl-2-methoxy- and phenol, 2-methoxy- may be related to microbial activity (Li et al., 2023), which was consistent with the results that mequinol, creosol, phenol, 2-methoxy- and phenol, 4-ethyl-2-methoxy- were positively correlated with the activities of multiple microorganisms, such as *Vibrio palustris*, *Acinetobacter*_sp._WCHAc010034, *Soonwooa buanensis*, and *Acinetobacter johnsonii* (Supplementary Fig. S1). Terpenoids, such as D-limonene, anethole, and 1-nonadecene, were mainly provided by the addition of spices and provided fruity, floral, and green herbal aromas of meat products (Javed et al., 2019). Notably, D-limonene could provide citrus and lemon flavors of all four groups with a ROAV>1 (Li et al., 2022), and the highest content of D-limonene in the SC group was responsible for its differentiation from the other groups (VIP>1). Furthermore, HCA indicated that the samples of the ZJ and JS groups had the highest aroma similarity and were first clustered into one group at

the distance scale of 200. Then, when the distance scale was 900, sausages samples of the HN and SC groups were clustered together. The ZJ and JS groups did not group with the other two groups until the distance scale reached 1,675 (Fig. 3K). These results suggested that there were distinct differences in aromatic compound composition between Chinese air-dried sausages and smoked sausages. The air-dried sausage samples in the JS and ZJ groups performed similar VOC composition, while the VOC composition of smoked sausage samples in the HN and SC groups was different, which may be attributed to the source of the raw meat and spices, technological characteristics, and microbial composition.

Electronic nose analysis of sausages from different regions

The aroma and taste attributes determine the overall sensory quality of fermented meat (Chen et al., 2021). According to the results of E-nose sensing (Figs. 4A, B, C, D, E, F, G, H, I, and J), the HN group showed significantly lower values of the W1C, W3C, and W5C sensors ($p<0.01$), while showing significantly higher values of the W1S, W2S, W5S, W6S, WIW and W2W sensors ($p<0.05$). These results indicated lower levels of aromatic compounds, aldehydes, and ketones but higher levels of alcohols, terpenes, and sulfur organic compounds in the HN group. However, the JS and ZJ groups displayed similar aromatic properties but entirely opposite to the sensing results of the HN group, indicating higher levels of aldehydes, ketones, and aromatic compounds in those two groups. Compared to the JS and ZJ groups, the SC group showed significantly higher values of the W1W, W2W, and W5S sensors ($p<0.05$), which indicated higher levels of aromatic compounds, terpenes, and sulfur organic compounds than those two groups. The OPLS-DA biplot of E-nose analysis was shown in Fig. 4K. The explanation and prediction of the model were achieved with $R^2X=0.982$, $R^2Y=0.251$, and $Q^2=0.195$. The indictors of E-nose could distinguish the sausage odors from different regions. W1W and W2W had the greatest contribution to the distribution of the SC group in quadrant I, whereas W1S, W2S, W3S, W5S, and W6S had the greatest contribution to the distribution of the HN group in quadrant IV. The samples of the JS and ZJ groups were distributed in quadrant III, which may be related to its different performance in those odors compared to the other two groups. According to the permutation test, the model could predict the data with $R^2=0.044$ and $Q^2=-0.248$. W1W, W2W, W1S, W2S, and W5S may play a major role in the process to differentiate the sausages from different regions (VIP>1). Moreover, the petal map reflected the response values of the significantly discriminating sensors to the four sausage producing regions (Fig. 4L). The HN group had the highest response to these sensors, followed by the SC group, while the JS and ZJ groups showed the lowest response. In general, the odor profiles of smoked sausage samples deviated significantly from those of air-dried sausage samples. The SC and HN groups had higher sensing values of aromatic compounds, sulfur organic compounds, and terpenes, while the JS and ZJ groups possessed higher sensing values of arom-aliph, alkanes, alcohols, aldehydes, and ketones, which was consistent with the results of VOCs analysis that the JS and ZJ groups showed similar composition of VOCs with dominant aldehydes (hexanal and nonanal) and alcohols (1-octen-3-ol, 1-butanol, 2-ethyl-, 1-octanol, and 2-octen-4-ol), while phenols (mequinol, creosol, phenol, 4-ethyl-2-methoxy-, and phenol, 2,6-dimethoxy-) and terpenoids (D-limonene and linalool) were predominant in the SC and HN groups, respectively.

Electronic tongue analysis of sausages from different regions

E-tongue evaluation is helpful for revealing the taste profiles of fermented meat (Du et al., 2021). As shown in Figs. 5A, B, C, D, E, F, G, and H, there was no significant difference in the bitterness, astringency, umami, richness, and saltiness values among all groups. The taste characteristics of umami, richness, and saltiness could be perceived due to being higher than the odorless points of 0, 0, –6, respectively. The HN and SC groups exhibited significantly higher values of sourness in
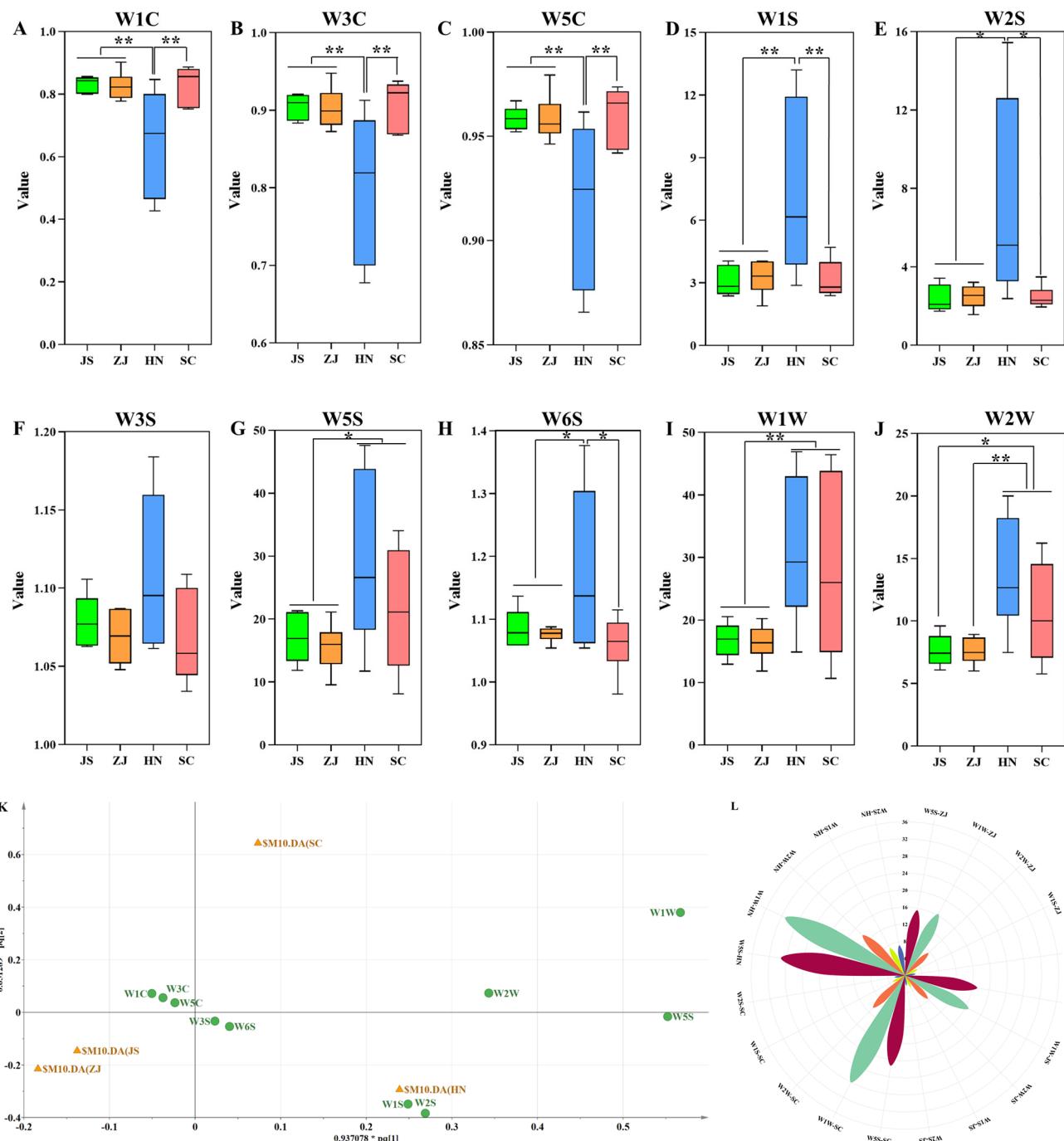
Fig. 4. E-nose analysis of sausages from different regions. (A–J) Box plots of the aromatic profiles, (K) the biplot of OPLS-DA, and (L) petal response map of differential sensors (VIP>1, p<0.05). JS, ZJ, SC, and HN represented the sausage samples collected from Jiangsu, Zhejiang, Sichuan, and Hunan, respectively. Significant differences are represented by * p<0.05 and ** p<0.01. E-nose, electronic nose; OPLS-DA, orthogonal partial least-squares discrimination analysis; VIP, variable importance for the projection.

comparison to the other two groups (p<0.05), and the sourness in most samples of these two groups could be perceived because these values were higher than the odorless point of -13, which may be related to the increased acids produced by microbial fermentation (Flores, 2018). Besides, the JS group had significantly higher values of aftertaste-A and aftertaste-B than the HN group (p<0.05), indicating higher levels of bitter aftertaste and astringent aftertaste in this group. The OPLS-DA
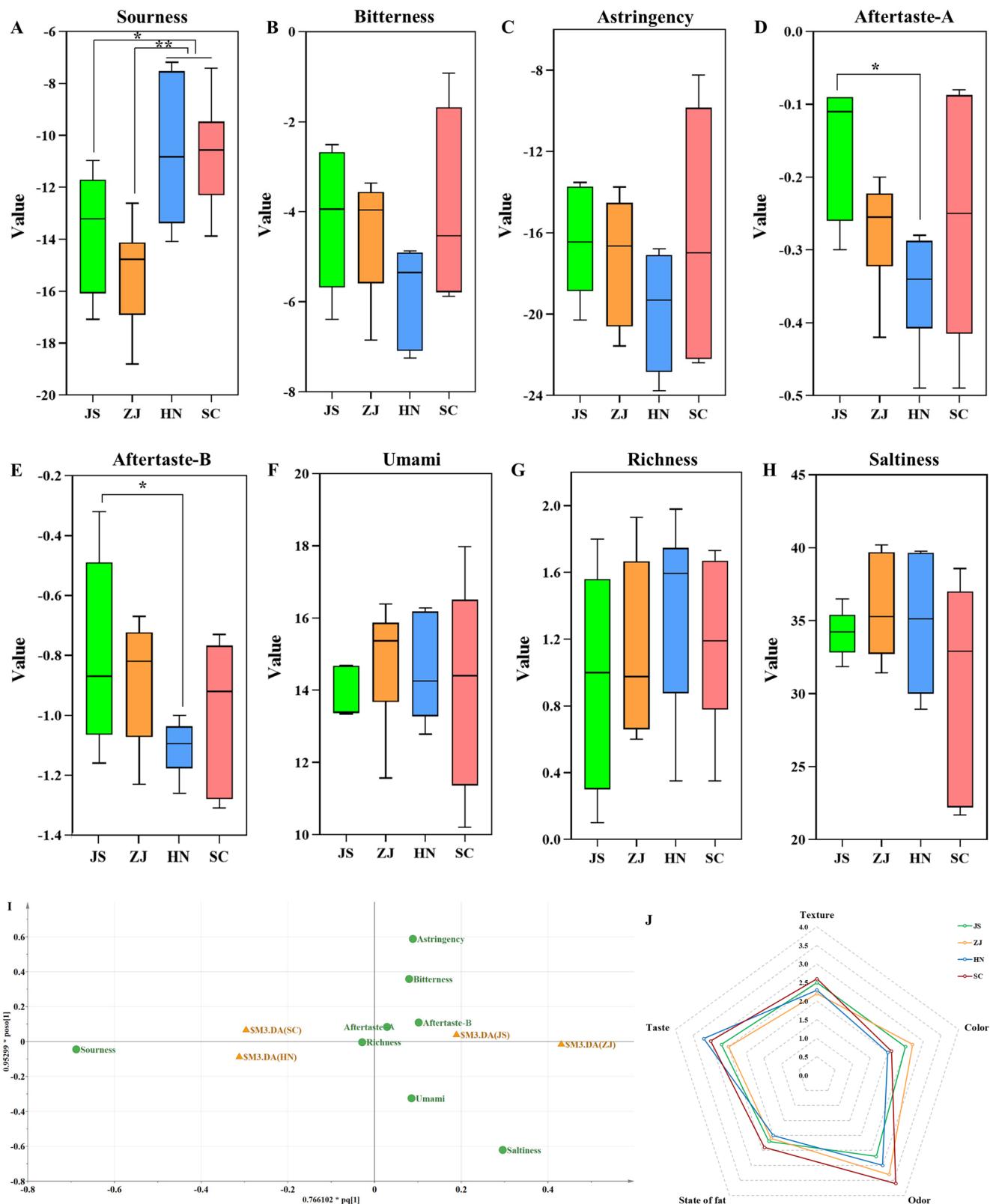
Fig. 5. E-tongue analysis of sausages from different regions. (A–H) Box plots of the taste profiles, (I) the biplot of OPLS-DA, and (J) radar response map of sensory evaluation. JS, ZJ, SC, and HN represented the sausage samples collected from Jiangsu, Zhejiang, Sichuan, and Hunan, respectively. Significant differences are represented by * p<0.05 and ** p<0.01. E-tongue, electronic tongue; OPLS-DA, orthogonal partial least-squares discrimination analysis.

biplot of E-tongue analysis was showed in Fig. 5I. The E-tongue sensors could distinguish the sausage taste from different regions with $R^2X=0.902$, $R^2Y=0.219$, and $Q^2=0.119$. Astringency, bitterness, aftertaste-A, and aftertaste-B were the principal factors affecting the distribution of the JS group in quadrant I, whereas umami and saltiness were the major factors affecting the distribution of the ZJ group in quadrant IV. The samples of the HN and SC groups were distributed in the negative x-axis, which may be related to its different performance in sourness and richness compared to the other two groups. Based on the permutation test, the model had an adequate fit with the data of $R^2=0.11$ and $Q^2=-0.149$. Sourness, astringency, and saltiness were significantly discriminating sensors based on the VIP analysis (VIP>1). Furthermore, the sensory profiles of sausages from different producing areas varied greatly based on the radar response map (Fig. 5J). The JS and ZJ groups had higher scores in color, while the HN and SC groups had higher scores in odor and taste. Studies have shown that the different compositions of *Staphylococci*, nitrate/nitrite levels, and pH values may lead to differences in the color of sausages from different places (Yang et al., 2023). Besides, the differences in taste and odor scores of sausages may result from differences in the composition and level of metabolites of protein degradation (Ashaolu et al., 2023). These results demonstrated that the HN and SC groups may display more complex taste profiles with stronger sourness and richness due to the unique flavor imparted by smoking. However, the impact of undesirable taste attributes on sausages and strategies to reduce them need further exploration.

Correlations between volatile compounds and odor/taste attributes of sausages

The relationship between the 14 important differential compounds (VIP>1) and the electronic sensory indices was investigated by the Spearman rank correlation analysis (Fig. 2). As shown in Fig. 2A, the differential alcohols, aldehydes, and ketones were positively correlated with the response values of W1C, W3C, and W5C, but exhibited negative correlations with the response values of W1W, W2W, W1S, W2S, W5S, and W6S. These results indicated that the flavors with rose, citrus fruit, grass, and cheese may be closely related to the W1C, W3C, and W5C sensors. In contrast, the differential phenols (except for phenol, 4-ethyl-2-methoxy), and decanoic acid, ethyl ester presented inverse correlations with the response values of aforementioned sensors, indicating that the spicy, woody, smoky flavors may be closely correlated with W1W, W2W, W1S, W2S, W3S, W5S, and W6S sensors. Briefly, decanoic acid, ethyl ester showed significantly positive correlations with W1W, W22, W1S, and W2S sensor values, while showing significantly negative correlations with W1C, W3C, and W5C (p<0.05). Additionally, the differential phenols (except for phenol, 4-ethyl-2-methoxy) exhibited significantly positive correlations with W1W, W2W, and W5S (p<0.05), while 1-octanol, 2-octen-4-ol, nonanal, 1-butenol, 2-ethyl-, and 3-octanone exhibited significantly negative correlations with those sensor values (p<0.05), indicating the importance of these three sensors in describing and distinguishing the odor of sausages from different regions.

The relationship between differential VOCs and taste characteristics were visualized in Fig. 2B. The differential alcohols, aldehydes, and ketones were positively correlated with umami and saltiness, and negatively correlated with sourness and richness, suggesting that the differential alcohols, aldehydes, and ketones may enrich the umami and saltiness tastes of the JS and ZJ groups. However, the differential phenols presented inverse correlations with the aforementioned taste sensors, indicating that the differential phenols produced by fumigation smoke may enhance the sourness and richness of the SC group. Moreover, D-limonene and decanoic acid, ethyl ester showed significantly negative correlations with bitterness, astringency, and their aftertastes (p<0.05), indicating that the HN group may possess lower undesirable taste attributes due to higher levels of these two compounds. Notably, nonanal, 2-octen-4-ol, 1-butanol, 2-ethyl-, 1-octen-3-ol, and hexanal had significantly negative correlations with sourness (p<0.05), while linalool and D-limonene had significantly positive

correlations with umami and saltiness (p<0.05), and differential phenols (except for phenol, 4-ethyl-2-methoxy-) had significantly positive correlations with richness (p<0.05). These results indicated that the SC and HN groups may exhibit better comprehensive taste with the balance of sourness, saltiness, umami, and richness. Overall, the differential VOCs (VIP>1) may cause the distinct taste and aroma responses of smoked and air-dried sausage samples from different regions.

The relationship between pH and a_w with various physicochemical and flavor indexes has attracted great attention because of their effects on the quality of fermented meat (Hou et al., 2024). Spearman rank correlation analysis revealed significantly positive correlations between a_w with W3S, W6S, sourness, CIE a*, and ΔE (p<0.05), and between pH with umami and saltiness (p<0.01). Conversely, the significantly negative correlations were observed between a_w with bitterness, astringency, aftertaste-B, and CIE L* (p<0.05), and between pH with W5S, bitterness, astringency, aftertaste-A, aftertaste-B, and CIE b* (p<0.05; Fig. 2C). The a_w may be necessary for producing fermented meat flavor, as the reaction between protein and lipid breakdown products would be stimulated as a_w increased (Mediani et al., 2022), which was consistent with the close correlations between a_w and various odor/taste attributes. Previous studies have demonstrated that pH value was a critical factor affecting color formation because the main functional microbes in fermented meat, such as *S. saprophyticus*, contributed to the color formation as the pH value decreased (Yang et al., 2024). However, the lipid oxidation products in fermented meat, such as ketones and aldehydes, may damage meat color by accelerating the oxidation of myoglobin (Yang et al., 2024), which may be the main reason causing the negative correlations between pH and various color indices.

In this study, it was found that salty-fresh taste and cured flavor was the common flavor characteristics of sausages from southern China, despite significant differences in the flavor of air-dried and smoked sausages. Both two kind of sausages had common esters, which were produced by esterification and microbial metabolism of the carbohydrate, and directly proportional to the fruity and fatty aromas (Merlo et al., 2021). The high level of hexanal and nonanal in air-dried sausages compensated for the deficiency of flavor caused by the lack of fumigation. Phenols contributed intense smoky odor to smoked sausages in SC and HN provinces, whereas linalool and D-limonene imparted a sweet aroma of those smoked sausages. Therefore, some necessary processing technologies, such as smoking, would be an effective measure to enhance the flavor profile of Chinese cured meat products.

Conclusion

This study systematically compared the characteristic VOCs, odor, and taste attributes of sausages from four typical regions of southern China by combining the chemometrics, E-nose, and E-tongue. The JS and ZJ groups had a great similarity in their VOC composition with dominant aldehydes (hexanal and nonanal) and alcohols (1-octen-3-ol, 1-butanol, 2-ethyl-, 1-octanol, and 2-octen-4-ol). Phenols (mequinol, creosol, phenol, 4-ethyl-2-methoxy-, and phenol, 2,6-dimethoxy-) were the most predominant VOCs in the SC group, while D-limonene, linalool, and decanoic acid, ethyl ester were the most predominant VOCs in the HN group. These differences may be mainly attributed to the dried processing of smoking or air-drying. The relationship between the important differential VOCs (ROAV>1) and electronic sensory indexes revealed the key VOCs causing the different odor and taste scores of sausages from various regions. 1-Octen-3-ol, hexanal, heptanal, nonanal, decanal, and (E)-2-octenal, which imparted floral, grass, and cheese flavors, had positive correlations with umami, saltiness, bitterness, and astringency of the JS and ZJ groups. phenol, 4-ethyl-2-methoxy-, and phenol, 2,6-dimethoxy-, which endowed sour, spicy, woody, toasty, and smoky aromas, had positive correlations with sourness and richness of the SC group, while D-limonene and linalool providing lemon, floral, and green herbal aromas, were positively correlated with umami and saltiness

of the HN group. These findings could provide valuable insights into the flavor characteristics of traditional Chinese fermented sausages, and be helpful for improving the quality of fermented meat in industrial production.

Supplementary Materials

Supplementary materials are only available online from: https://doi.org/10.5851/kosfa.2025.e18.

Conflicts of Interest

The authors declare no potential conflicts of interest.

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Author Contributions

Conceptualization: Wang D. Data curation: Han B, Wang X. Formal analysis: Han B, Wang X. Methodology: Han B, Liu H. Software: Han B. Validation: Han B, Wang D. Investigation: Liu H. Writing - original draft: Han B. Writing - review & editing: Han B, Wang X, Liu H, Wang D.

Ethics Approval

Sensory evaluation of this study was approved by the Ethics Committee of Jiangsu Academy of Agricultural Sciences (Authority No: PA-2025-02-005).

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