

## Effect of chilled storage period on the volatile organic compounds and bacterial community in goose meat

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### ABSTRACT

Storage time is considered to be one of the most important factors affecting the obnoxious odor and microbial spoilage of fresh meat. In this study, volatile organic compounds (VOCs) and bacterial community structure of chilled goose meat during storage were investigated. The results showed that numerous VOCs were produced during the fresh goose meat storage, including aldehydes (nonanal, (E)-2-octenal, hexanal, tetradecanal), alcohol (1-octen-3-ol), furan (2-pentylfuran), and carboxylic acids (methyl diethyldithiocarbamate), which might be a breakdown product during spoilage. In addition, there were slight fluctuations in fatty acid profiles and amino acid contents. Furthermore, bacterial community diversity decreased with prolonged storage. Also, *Pseudomonas* and *Acinetobacter* were the dominant spoilage bacteria contributing to nonanal and methyl diethyldithiocarbamate generation. Taken together, these data provide insights into the characterization of VOCs and the bacterial community of chilled goose meat, which will help to further control the microbial quality of chilled meat.

### 1. Introduction

Goose, the third-largest poultry source, has expanded consumer choices for meat (Kozák, 2021). Global production of fresh or chilled goose meat has reached 4.3 billion tons in 2022 in China (FAO-STAT, 2022). Goose is rich in nutrients, especially those with high protein and low-fat content, which are beneficial unsaturated fatty acid (Biesek et al., 2020; Orkusz et al., 2021; Wu et al., 2014). From a nutritional perspective, the demand for goose meat continues to increase worldwide.

Chilling and superchilling are the most commonly used storage techniques in the market (Zhao et al., 2022). Chilled meat is becoming increasingly popular because of its freshness (Cao et al., 2023). However, various abnormal phenomena inevitably occur in chilled meat, which reduces its edible quality and economic value. Storage time is considered one of the most important factors responsible for the presence of undesirable compounds that affect the obnoxious odor resulted in the rejection of products and microbial spoilage of fresh meat (Schuster et al., 2018).

Volatile organic compounds (VOCs), generated principally by microbial activity, which contribute the most to sensory changes can be used as potential marker compounds for evaluating beef spoilage during chilled storage (Mansur et al., 2019; Shao et al., 2023). In general, the microbial community and VOCs of chilled chicken, pork, and beef change significantly during storage (Sun et al., 2024; Yang et al., 2018). Goose meat, which is rich in unsaturated fatty acids, has characteristics similar to those of VOCs, and the bacterial community of goose meat during chilled storage has not been fully elucidated.

In this study, we investigated the changes in the VOCs and bacterial community structure of 70-day-old Yangzhou goose breast muscles during chilled storage (0, 3, 5 and 7 d). Studying the dynamic changes in VOCs and spoilage-related microbial communities, as well as their relationship, contributed to our understanding of spoilage mechanisms and the recommendation of goose meat storage to allow meat-spoilage control and evaluation. This study provides a theoretical basis for the characterization of VOCs and bacterial communities of goose meat during chilled storage, which will help further control the microbial quality of chilled goose meat.

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## 2. Materials and methods

### 2.1. Sample collection

All animal procedures in this study were approved by the Yangzhou Institutional Animal Committee (permit Number: YZUDWSY2021–239, Jiangsu Province, China). Ten-week-old healthy Yangzhou geese with similar average live weights were purchased from Tiange Goose Industry Co., LTD. (Jiangsu, China). All geese were slaughtered in a stun bath (900 Hz, 40 V) for 5 s, and the jugular vein and carotid artery on one side of the neck were severed and exsanguinated. After slaughter, the breast muscles of all geese were immediately collected in plastic wraps. Pieces of goose breasts were further partitioned into two parts, one for volatile organic compound, amino acid, and fatty acid composition analysis ( $n = 3$ ) and the other for 16S rRNA amplicon sequencing to measure microbial composition ( $n = 10$ ). Randomly selected Yangzhou goose breast muscle samples were used as the control group (day 0) on the day of slaughter. The remaining samples were stored in plastic wraps under constant temperature (+4 °C) and were randomly taken for testing at 3, 5, and 7 d, respectively. Samples within the same group come from different geese. All the test samples were selected from the right breast meat of the geese.

### 2.2. 16S rRNA amplicon sequencing

Total DNA was extracted from each breast muscle sample. DNA was also quantified using a Nanodrop, and the quality of the extracted DNA was detected by 1.2% agarose gel electrophoresis. The V3-V4 region of the 16S rRNA gene was used for PCR amplification. The corresponding primers were designed according to the conserved regions in the sequence, and sample-specific barcode sequences were added. Briefly, PCR was performed using Pfu High-Fidelity DNA polymerase (TransGen Biotech), and the number of amplification cycles was strictly controlled. Magnetic beads (Vazyme VAHTSTM DNA Clean Beads) were added to the PCR product for purification and recovery. The PCR amplification recovered products were subjected to fluorescence quantification using a fluorescence reagent (Quant-iT PicoGreen dsDNA Assay Kit) and a quantification instrument (Microplate reader (BioTek, FLx800)). Based on the fluorescence quantification results, each sample was mixed at the corresponding ratio, according to the sequencing volume requirement of each sample. Sequencing libraries were prepared using a TruSeq Nano DNA LT Library Prep Kit (Illumina). Finally, the samples were analyzed using high-throughput sequencing.

Raw sequences were received in FASTQ format and processed with DADA2 for primer removal, quality filtering, denoising, splicing, and chimerism removal. It no longer clusters based on similarity, but only performs dereplication or clustering based on 100% similarity. Each sequence that undergoes dereplication after using DADA2 quality control is called an amplicon sequence variant (ASV). By randomly selecting a certain number of sequences from each sample to reach a unified depth, we predicted the observed ASVs/OTUs and their relative abundance in each sample at that sequencing depth. By conducting statistics on the flattened ASV/OTU table, a subsequent analysis was performed, and the specific composition of the microbial communities in each sample at each classification level was obtained.

### 2.3. Analysis of VOCs

The PDMS/DVB extracted fibers were aged at 250 °C for 1 h at the GC injection port before testing. A total of 2.0 g of the samples were accurately removed and placed in a 20 mL headspace bottle. Before being placed in an 80 °C water bath, 3 mL of saturated sodium chloride was added, vortexed, sonicated, and sealed with a lid. After 30 min, the pre-aged solid-phase microextraction needle was inserted into a heated headspace bottle for further extraction and adsorption for 30 min and analyzed using a machine (Thermo 1300, Thermo Fisher Scientific,

American) for 5 min.

### 2.4. Analysis of amino acid profile

The steps used to determine the amino acid profile of Yangzhou goose breast muscles were as described by Chen et al. (2020), using an automated amino acid analyzer (LA 8080, Hitachi, Japan) equipped with a Hitachi high-performance cation exchange column. In short, scissored meat samples weighed accurately were integrated with 25 mL of 6 mol/L HCl in a glass tube filled with nitrogen gas, and then heated at 110 °C for 22 h to fully hydrolyze. The samples were moved to a 50 mL volumetric flask after cooling to room temperature and were diluted with ultrapure water. Afterward, a 1 mL hydrolysis product was extracted. The acid was removed and the sample was dried under vacuum. Then, the sample was completely dissolved with 1  $\mu$ L of 0.02 mol/L HCl. Finally, the solution was passed through a 0.22  $\mu$ m membrane filter before analysis.

### 2.5. Analysis of fatty acid composition

The fatty acid composition of breast meat was determined by gas chromatography (Agilent, 7890 A, CA, USA) according to the following steps. Goose meat samples were collected first and crude fat was extracted using a Soxhlet extract (FOSS Soxtec 2050, Hillerod, Denmark). The extracted lipids were dissolved in 2 mL of 2% methanolic sodium hydroxide. The solution was moved to the round bottom flask before heating at 85 °C. After 30 min, 3 mL 14% methanolic boron trifluoride was added and further heated at 85 °C for 30 min. After returning the mixture to room temperature, it was transferred to a tube. Subsequently, 1 mL of n-hexane was added to extract the nonpolar components. Finally, the solution used a 0.45  $\mu$ m membrane filter before analysis.

### 2.6. Statistical analysis

For the analysis of VOCs, amino acids, and fatty acids, the experimental results were expressed as the mean  $\pm$  standard error. The experimental data were preliminarily organized using Excel 2021, and SPSS was used to perform an ANOVA on the experimental data to examine the significant differences, which were significant differences expressed as  $P < 0.05$ . The correlation analysis between top 20 bacteria at genus and characteristic VOCs were carried out by using Pearson method.

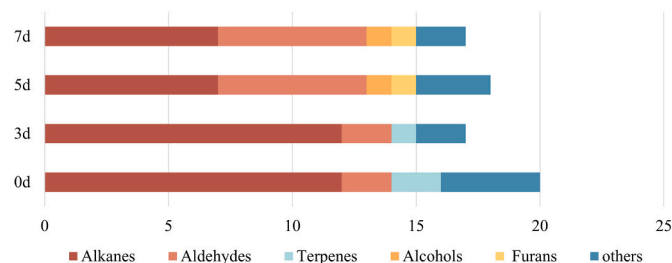
## 3. Results

### 3.1. Changes in VOCs during chilled storage

A total of 20, 17, 18 and 17 main VOCs were identified at 0, 3, 5, and 7 d after removing the outliers, which were grouped into different chemical categories, including alkenes, aldehydes, furans, terpenes, alcohols, and other compounds (Table 1). To display the profile changes in VOCs more intuitively, we created a distribution diagram of VOCs among the six classes mentioned above in goose breast muscle during chilled storage. The number of VOCs categories varied over time (Fig. 1). Chilled meat stored on day 5 could undergo a critical point for changes in the classes of VOCs, as the breast muscles began to contain alcohols and furans. In particular, aldehydes were more abundant in the late storage period (5–7 d) than in the early period (1–3 d). Furthermore, aldehydes (nonanal, (E)-2-octenal, hexanal, tetradecanal), alcohol (1-octen-3-ol), furan (2-pentylfuran) and carboxylic acids (methyl diethylthiocarbamate) increased significantly ( $P < 0.05$ ) in the late storage period than those in the early period. Terpenes began to disappear with prolonged chilling time. As the chilling time increased, a portion of VOCs significantly decreased between 0 and 7 d ( $P < 0.05$ ), for example, alkanes (3-methyltetradecane, pentadecane, 2-methylpentadecane,

**Table 1**  
The composition and relative content of VOCs of breast muscles at different chilled time (%).

Classification	Compound	0d	3d	5d	7d	
Alkanes	Tetradecane	0.74 ± 0.11	0.85 ± 0.04	0.72 ± 0.06	0.59 ± 0.30	
	3-Methyltetradecane	0.48 ± 0.03 <sup>a</sup>	0.29 ± 0.15 <sup>a</sup>	0.00 ± 0.00 <sup>b</sup>	0.00 ± 0.00 <sup>b</sup>	
	Pentadecane	5.69 ± 0.68 <sup>a</sup>	5.19 ± 0.5 <sup>a</sup>	1.38 ± 0.17 <sup>b</sup>	0.96 ± 0.5 <sup>b</sup>	
	2-Methylpentadecane	1.29 ± 0.24 <sup>a</sup>	0.79 ± 0.39 <sup>a</sup>	0.00 ± 0.00 <sup>b</sup>	0.00 ± 0.00 <sup>b</sup>	
	Hexadecane	7.67 ± 1.08 <sup>a</sup>	6.26 ± 3.01 <sup>ab</sup>	2.14 ± 0.25 <sup>ab</sup>	1.24 ± 0.63 <sup>b</sup>	
	2-Methylhexadecane	1.52 ± 0.21 <sup>a</sup>	0.96 ± 0.52 <sup>a</sup>	0.00 ± 0.00 <sup>b</sup>	0.00 ± 0.00 <sup>b</sup>	
	Heptadecane	9.31 ± 1.42 <sup>a</sup>	8.44 ± 1.9 <sup>a</sup>	3.33 ± 1.25 <sup>b</sup>	1.84 ± 0.92 <sup>b</sup>	
	Octadecane	6.12 ± 2.06 <sup>a</sup>	6.25 ± 1.22 <sup>a</sup>	1.92 ± 0.90 <sup>ab</sup>	1.55 ± 0.80 <sup>b</sup>	
	Eicosane	0.70 ± 0.31 <sup>ab</sup>	1.12 ± 0.41 <sup>a</sup>	0.00 ± 0.00 <sup>b</sup>	0.00 ± 0.00 <sup>b</sup>	
	Nonadecane	1.24 ± 0.34	2.12 ± 0.4	0.95 ± 0.24	0.89 ± 0.53	
	4-Methylpentadecane	0.73 ± 0.38 <sup>ab</sup>	1.09 ± 0.22 <sup>a</sup>	0.00 ± 0.00 <sup>b</sup>	0.00 ± 0.00 <sup>b</sup>	
	Aldehydes	Nonanal	0.54 ± 0.25 <sup>c</sup>	1.35 ± 0.18 <sup>c</sup>	10.8 ± 0.68 <sup>a</sup>	5.16 ± 1.61 <sup>b</sup>
		Benzaldehyde	0.18 ± 0.10	1.08 ± 0.61	0.78 ± 0.12	0.49 ± 0.27
(E)-2-Octenal		0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	1.94 ± 0.18 <sup>a</sup>	0.58 ± 0.13 <sup>b</sup>	
Hexanal		0.00 ± 0.00 <sup>b</sup>	0.00 ± 0.00 <sup>b</sup>	3.41 ± 1.37 <sup>a</sup>	1.47 ± 0.32 <sup>ab</sup>	
Decanal		0.00 ± 0.00 <sup>b</sup>	0.00 ± 0.00 <sup>b</sup>	0.93 ± 0.18 <sup>a</sup>	0.00 ± 0.00 <sup>b</sup>	
Tetradecanal		0.00 ± 0.00 <sup>b</sup>	0.00 ± 0.00 <sup>b</sup>	3.11 ± 0.56 <sup>a</sup>	2.20 ± 0.50 <sup>a</sup>	
Bute hydrocarbon		7.63 ± 2.23 <sup>a</sup>	4.82 ± 2.28 <sup>ab</sup>	0.00 ± 0.00 <sup>b</sup>	0.00 ± 0.00 <sup>b</sup>	
Terpenes	Phytane	5.99 ± 2.38 <sup>a</sup>	0.00 ± 0.00 <sup>b</sup>	0.00 ± 0.00 <sup>b</sup>	0.00 ± 0.00 <sup>b</sup>	
	1-Octen-3-ol	0.00 ± 0.00 <sup>b</sup>	0.00 ± 0.00 <sup>b</sup>	3.37 ± 1.03 <sup>a</sup>	1.17 ± 0.61 <sup>b</sup>	
Furans	2-Pentylfuran	0.00 ± 0.00 <sup>b</sup>	0.00 ± 0.00 <sup>b</sup>	2.07 ± 0.68 <sup>a</sup>	0.78 ± 0.42 <sup>ab</sup>	
Carboxylic Acids	Methyl diethylthiocarbamate	0.00 ± 0.00 <sup>b</sup>	0.00 ± 0.00 <sup>b</sup>	1.18 ± 0.27 <sup>a</sup>	0.95 ± 0.48 <sup>a</sup>	
Siloxanes	Dodecamethylcyclohexasiloxane	2.77 ± 1.72	4.45 ± 3.84	1.45 ± 1.09	11.08 ± 6.7	
Others	5-Methyltetradecane	0.25 ± 0.02 <sup>a</sup>	0.00 ± 0.00 <sup>b</sup>	0.00 ± 0.00 <sup>b</sup>	0.00 ± 0.00 <sup>b</sup>	
	Cyclopentadecane	0.29 ± 0.02 <sup>a</sup>	0.00 ± 0.00 <sup>b</sup>	0.00 ± 0.00 <sup>b</sup>	0.00 ± 0.00 <sup>b</sup>	
	4-Methylhexadecane	1.48 ± 0.03 <sup>a</sup>	1.02 ± 0.51 <sup>a</sup>	0.00 ± 0.00 <sup>b</sup>	0.00 ± 0.00 <sup>b</sup>	
	1,3-Hexadiene, 3-ethyl-2-methyl-	0.00 ± 0.00 <sup>b</sup>	0.00 ± 0.00 <sup>b</sup>	1.05 ± 0.31 <sup>a</sup>	0.00 ± 0.00 <sup>b</sup>	



**Fig. 1.** Distribution profiles of VOCs among six classes in goose breast muscles during chilled storage.

hexadecane, 2-methylhexadecane, heptadecane, octadecane), terpenes (bute hydrocarbon, phytane) and others (5-methyltetradecane, cyclopentadecane, 4-methylhexadecane).

### 3.2. Changes in amino acid profile and fatty acid composition

In addition, we measured changes in the amino acid profile and fatty acid composition of Yangzhou goose breast muscles at different chilling times (0, 3, and 7 d). The amino acid composition of the chilled breast muscle is shown in Table 2. A total of 17 total amino acids were detected. As chilled storage was prolonged, the total amino acid content gradually decreased ( $P > 0.05$ ). The essential amino acid content did not change significantly ( $P > 0.05$ ). Breast muscles stored at +4 °C were characterized by significantly ( $P < 0.05$ ) higher Lys loss on 7 d compared to before chilled, whereas the level of His increased on the contrary on 7 d. During the 7 d chilled period, the Gly content reached its highest on 3 d and then began to decrease.

The content of the 15 fatty acids detected in the breast muscle in this study is presented in Fig. 2. Under chilled storage, fatty acids (including SFA, MUFA, and PUFA) showed slight fluctuations within 7 d, but no significant changes were observed ( $P > 0.05$ ).

### 3.3. Microbiological analysis

#### 3.3.1. Richness in microbiota profiles of goose breast muscles at different chilled times

In the present study, we compared the differential microbiota profiles of breast muscles during the chilled period using 16S rRNA analysis. The Good's coverage of all samples had a coverage rate of over 99%, as is shown in Fig. 3A. It was concluded that the data examined were sufficient to reveal the existence and relative abundances of most bacterial communities.  $\alpha$ -diversity is a measure of how many microbial species are present within a single sample and the proportion of each species. The observed species and the Chao1, Shannon, Pielou, and Simpson indices are commonly used to measure  $\alpha$ -diversity. The Chao1, Shannon, and Pielou indices gradually decreased with increasing chilling time and significantly decreased between 3 and 5 d of chilled storage ( $P < 0.05$ ). The same applies to the observed species and Faith\_pd. There was not much difference in the Simpson index between 0 d and 3 d of chilling time and between 5 d and 7 d. However, compared to day 3, the Simpson index on day 5 was significantly decreased ( $P < 0.05$ ).

#### 3.3.2. Changes in the relative abundance of microbial communities in chilled goose breast muscles

The top 20 relative abundances of the phyla are shown in Fig. 3B, and the top 20 relative abundances of the genera are shown in Fig. 3C. The majority of components of the microbiota belong to two major phyla (relative abundances were  $> 1\%$ ) (Table S1), including *Proteobacteria* (22.0–98.3%) and *Firmicutes* (1.4–37.6%). The relative abundance of *Proteobacteria* gradually increased during chilled storage. However, the results of *Firmicutes* had the opposite effect. In addition, the relative abundances of *Actinobacteria* (5.5–9.7%) and *Bacteroidetes* (5.0%) were also abundant when stored in the early period, but decreased ( $< 1\%$ ) in the late period. Meanwhile, the proportions of *Pseudomonas* (3.4–79.0%) and *Acinetobacter* (1.7–14.2%) were more abundant in the genus (Table S2). Except for these two genera, the genera that also presented the greatest abundances (relative abundances were  $> 1\%$ ) on 0 d were *Psychrobacter* (5.5%), *Macrocooccus* (5.3%), *Faecalibacterium* (3.2%), *Roseburia* (3.6%), *Corynebacterium* (2.6%), *Limnobacter* (2.4%), *Bacteroides* (1.9%), *Streptococcus* (1.4%), *Arthrobacter* (3.3%), *Blautia* (1.7%),

**Table 2**

Amino acid composition of breast muscles at different chilled time (unit: g/100 g meat sample).

Amino acid	0d	3d	5d	7d
Asparagic acid(Asp)	1.26 ± 0.03	1.25 ± 0.04	1.24 ± 0.02	1.20 ± 0.05
Threonine(Thr)	0.65 ± 0.65	0.65 ± 0.65	0.65 ± 0.65	0.62 ± 0.62
Serine(Ser)	0.54 ± 0.01	0.55 ± 0.01	0.52 ± 0.01	0.51 ± 0.03
Glutamic acid(Glu)	1.99 ± 0.04	1.95 ± 0.06	1.94 ± 0.04	1.86 ± 0.09
Glycine(Gly)	0.66 ± 0.06 <sup>ab</sup>	0.72 ± 0.03 <sup>a</sup>	0.57 ± 0.01 <sup>b</sup>	0.57 ± 0.01 <sup>ab</sup>
Alanine(Ala)	0.82 ± 0.03	0.84 ± 0.02	0.79 ± 0.01	0.78 ± 0.02
Cysteine(Cys)	0.10 ± 0.01	0.09 ± 0.00	0.11 ± 0.00	0.10 ± 0.01
Valine(Val)	0.64 ± 0.02	0.62 ± 0.01	0.64 ± 0.01	0.62 ± 0.02
Methionine(Met)	0.09 ± 0.01	0.06 ± 0.01	0.09 ± 0.01	0.06 ± 0.01
Isoleucine(Ile)	0.55 ± 0.01	0.53 ± 0.02	0.57 ± 0.02	0.53 ± 0.02
Leucine(Leu)	1.13 ± 0.03	1.10 ± 0.03	1.12 ± 0.02	1.08 ± 0.04
Tyrosine(Tyr)	0.40 ± 0.01	0.40 ± 0.01	0.42 ± 0.01	0.39 ± 0.02
Phenylalanine(Phe)	0.52 ± 0.01	0.51 ± 0.01	0.52 ± 0.01	0.49 ± 0.02
Lysine(Lys)	1.22 ± 0.00 <sup>a</sup>	1.19 ± 0.03 <sup>ab</sup>	1.15 ± 0.02 <sup>ab</sup>	1.10 ± 0.04 <sup>b</sup>
Histidine(His)	0.26 ± 0.01 <sup>b</sup>	0.26 ± 0.00 <sup>b</sup>	0.33 ± 0.01 <sup>ab</sup>	0.34 ± 0.02 <sup>a</sup>
Arginine(Arg)	0.87 ± 0.02	0.87 ± 0.03	0.78 ± 0.00	0.78 ± 0.03
Proline(Pro)	0.52 ± 0.04	0.61 ± 0.05	0.50 ± 0.01	0.54 ± 0.02
Essential amino acids	6.07 ± 0.12	5.91 ± 0.15	5.97 ± 0.13	5.7 ± 0.23
Total amino acids	12.22 ± 0.29	12.20 ± 0.31	11.94 ± 0.19	11.58 ± 0.45
Total umami amino acids	5.6 ± 0.15	5.63 ± 0.16	5.32 ± 0.07	5.19 ± 0.19
Total sweet amino acids	3.27 ± 0.15	3.43 ± 0.09	3.13 ± 0.04	3.09 ± 0.11
Total bitter amino acids	4.07 ± 0.07	3.98 ± 0.1	3.99 ± 0.07	3.86 ± 0.15

*Prevotella* (1.5%), *Planomicrobium* (2.4%), *Aquabacterium* (1.2%), *Acidovorax* (1.2%), *Gemmiger* (1.6%) and *Rothia* (1.3%). However, only two genera were relatively abundant (relative abundances were > 1%) at 7 d, including *Pseudomonas* (79.0%) and *Acinetobacter* (14.2%).

### 3.3.3. Microbial diversity changes during chilled storage

β-diversity refers to the diversity of species composition or the rate of species replacement along environmental gradients among different

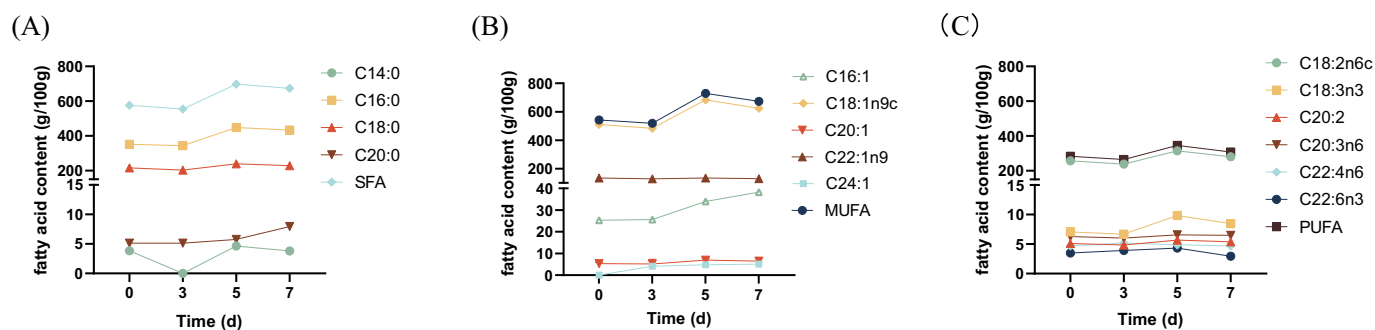
communities. For the NMDS analysis in Fig. 4A, it can be seen that the microbial profiles at 0 and 3 d, as well as at 5 and 7 d, were more similar. The microbial profiles of samples stored for 0 d could be clearly distinguished from those of samples stored for 7 d. To study which species were common and unique among the different groups, the Venn diagram in Fig. 4B was used for community analysis. OTUs refer to effective representative sequences of microorganisms. Interestingly, 389 OTUs were shared among the four groups, accounting for approximately 1.97% of the total OTUs. In addition, 6500 OTUs were unique to the 0d chilled storage group, and then decreased as chilled storage was extended. The 3, 5, and 7 d groups harbored 6368, 1919 and 1062 unique OTUs, respectively.

To more intuitively display the microbial community changes at different chilled times, a heat map of β-diversity is used in Fig. 4C. Differences in microbial community diversity among different chilling times were revealed, indicating that the number of microbial communities found in the breast muscles decreased with prolonged storage. Similar to the above results from Fig. 4, the dominant bacterial species of the group of 7 d mainly included *Pseudomonas* and *Acinetobacter*. In addition, we also conducted functional annotation using KEGG pathway analysis (Fig. 4D) to further investigate the main metabolic reactions that occurred during the chilled storage period. KEGG pathway analysis showed that expressed genes were mainly annotated to metabolic pathways, including amino acid metabolism, carbohydrate metabolism, and metabolism of cofactors and vitamins. To establish the relationship between VOCs and bacteria among storage period, we further carried out correlation analysis using the Pearson method. *Pseudomonas* and *Acinetobacter* was positively correlated with nonanal and methyl diethylthiocarbamate (Fig. 4D).

## 4. Discussion

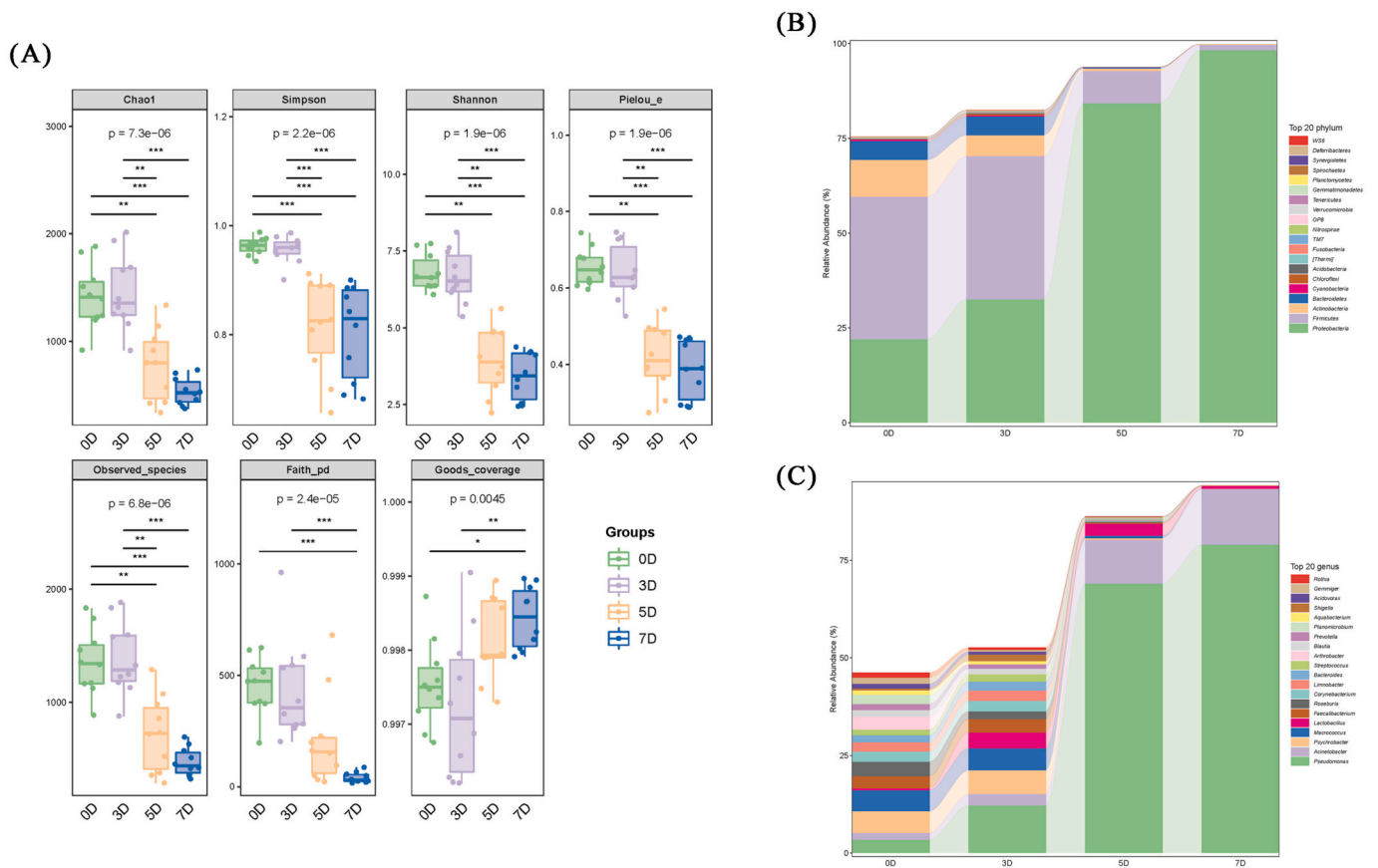
For centuries, meat and meat products have provided the human body with essential dietary components (Pereira & Vicente, 2013). It is rich in amino acids, fatty acids, vitamins, and minerals (Kausar et al., 2019; Pereira & Vicente, 2013; Weng et al., 2021). Goose meat is becoming increasingly popular among consumers because of its high quality. Currently, chilled preservation is the most widely used, economical, and effective method of meat storage. Unfortunately, many unfavorable changes occur in raw meat materials during chilled storage (Dai et al., 2014). However, there are no detailed reports on the effects of low temperatures on the flavor and microbial composition of goose meat. In this study, we investigated the effect of chilled storage on the dynamic composition of VOCs and microbial communities.

In recent years, VOCs in food have received widespread attention from consumers (Feng et al., 2024). Sensory flavor characteristics of VOCs are important indicators for scientific research. Compared to the instrumental software database, 28 VOCs were identified, most of which were alkanes and aldehydes. In the early period (1–3 d), most VOCs were alkanes, which is similar to the results of a previous study in two



**Fig. 2.** Fatty acid composition of breast muscles at different chilled times (0d, 3d, 5d, 7d). (A) Detected changes in SFA content. (B) Detected changes in MUFA content. (C) Detected changes in PUFA content. (unit: g/100 g meat sample).





**Fig. 3.** (A) Box plot of index difference between groups of Chao1, Simpson, Shannon, and Pielou indices, and observed species at different chilled times. (B) and (C) Bacterial community profiles variation at different chilled times. The top 20 relative abundances of the phyla (B) and the top 20 relative abundances of the genera (C).

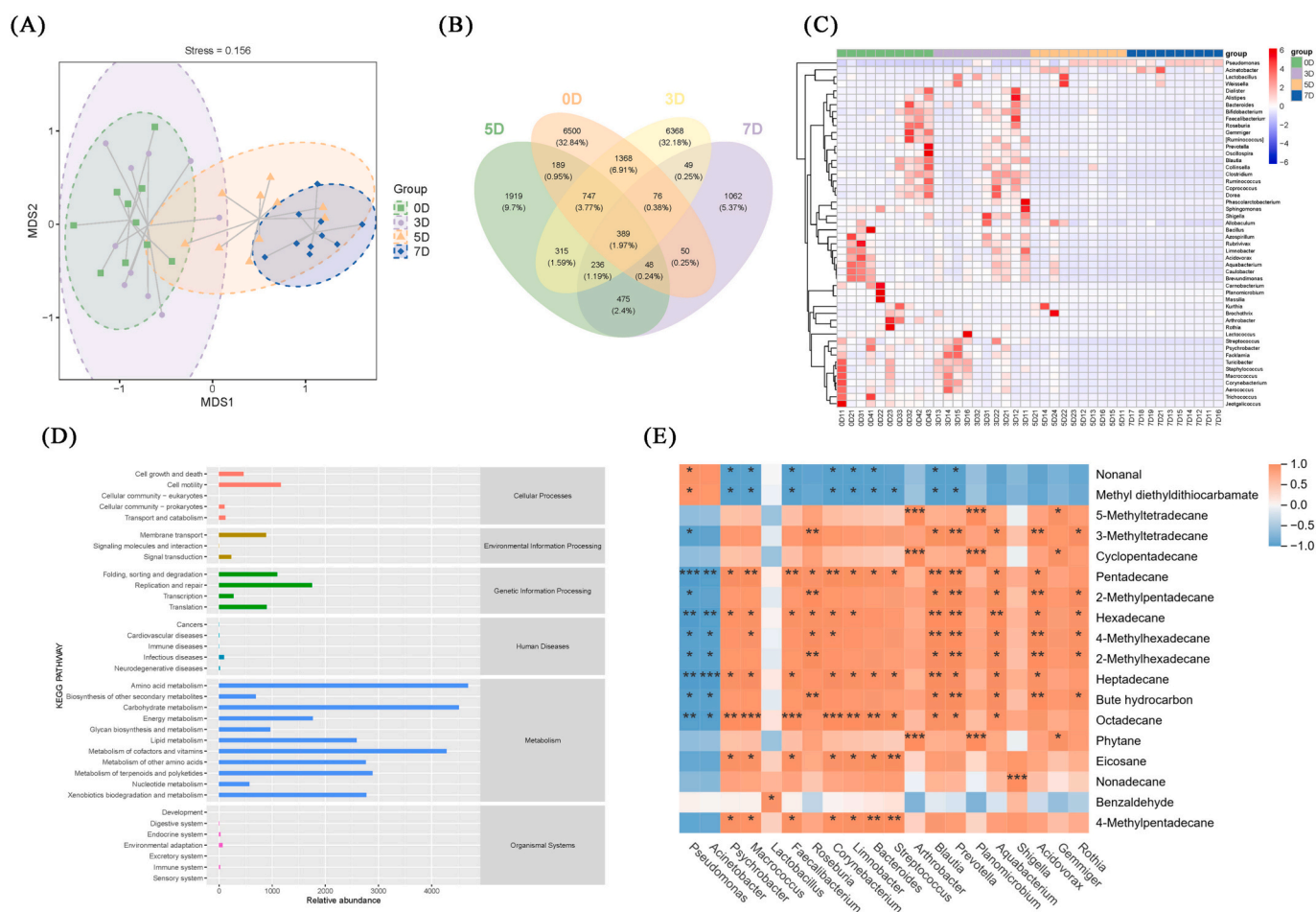
different chicken breeds (Jin et al., 2021). In addition, alkanes have been proven to have an important impact on flavor release and odor switching (Fu et al., 2021; Zhang, Sun, & Zhang, 2019). However, de Brito et al. (2019) reported that alkanes have a low boiling point and poor aroma. From the perspective of changes in alkanes, our findings illustrated that flavor release and olfactory characteristics that are not caused by the volatilization of alkanes themselves may change at the critical point between chilled for 3d and 5d. Remarkably, some VOCs, including aldehydes (nonanal, (E)-2-octenal, hexanal, tetradecanal), alcohol (1-octen-3-ol), furan (2-pentylfuran) and carboxylic acid (methyl diethylthiocarbamate) were generated largely in the late period (5–7 d). Therefore, we hypothesized that these seven substances were potential indicators of spoilage. Our experiments are consistent with previous results showing that hexanal and 1-octen-3-ol could be characteristic biomarkers (Jaaskelainen et al., 2016; Wang et al., 2023). In summary, the VOCs in goose meat changed, and some of them, which were present largely in the late storage period, could be used as potential spoilage indicators.

In addition, we investigated the nutritional profiles (amino acid composition and fatty acid profile) during chilled storage. Our study revealed that chilled storage had no significant effect on the total amino acid content of goose meat, similar to that of essential amino acids. Lys, His, and Gly exhibited the greatest concentration changes. A popular explanation for this is that, during the chilling process, microorganisms decompose proteins in goose meat into various intermediate products, including free amino acids. Jung et al. (2022) also showed that Gly in chicken breast muscle first increased and then decreased within 7 d of chilled storage. As observed in this study, the fatty acid composition of goose muscles varied during storage. Similarly, Chmiel et al. (2019) observed no significant effect of time on the fatty acid composition of

chicken breast meat. These results indicate that chilled storage slightly influences the edible value of meat.

Owing to the high content of nutrients in meat, it is a good place for microbial growth and reproduction. Meat undergoes quality changes and spoilage because of the action of microorganisms. To explore changes in the microbial community of geese during chilled storage, we used 16S rRNA amplicon sequencing to reveal the microbial community dynamics of goose meat during the storage period. Even under chilled conditions, which restrict the growth and reproduction of certain microorganisms, there are advantageous microbial communities that are easy to reproduce. The MNDS showed that chilled storage significantly altered the microbial diversity of chilled geese. Changes in the microbiota mainly occurred at the critical point on day 3. In the bacterial community profiles variation, the samples in the early period (1–3 d) showed greater bacterial diversity, whereas the diversity of the bacterial community decreased in the late period (5–7 d). Li, Zhang, and Luo (2018) also reported that fresh and frozen-thawed samples showed greater bacterial diversity and more differences in microbiota on day 0. It is worth noting that *Pseudomonas* (69.0%–79.0%) and *Acinetobacter* (11.3%–14.2%), as the dominant genera in the late period, may be strongly responsible for meat deterioration. It has been proven that the optimal growth temperature of some *Pseudomonas* species is usually close to 0–45 °C (Tribelli & López, 2022), whereas *Acinetobacter* could grow even at 4 °C. Numerous studies have demonstrated that *Pseudomonas* possess the strongest spoilage potential (Arslan et al., 2011; Mohareb et al., 2015; Pellissery et al., 2020; Raposo et al., 2016). Some studies have also indicated that chilled meat can detect the occurrence of *Acinetobacter* during storage (Karanth et al., 2023), and *Acinetobacter* as a pathogen of major public health concern (Doulgeraki et al., 2012).

VOCs are generated by the decomposition of meat components and



**Fig. 4.** (A) Non-metric multidimensional (NMDS) of the bacterial community of breast muscles at different chilled times. (B) Venn diagram shows the unique and shared OTUs of the bacterial communities at different chilled times. The figure was plotted after the homogenisation of all data. (C) Heatmap showing the changes in the microbial communities of goose breast muscles at different chilled times. Bacteria were selected whose relative abundances ranked within the top 50 at the genus level and were specifically indicated. (D) Predicted functional profiles of microbiota using the KEGG Pathway Database. (E) Heatmap showing correlation analysis between top 20 bacteria at genus and characteristic VOCs during storage.

microbial development during meat storage (Li, Zhu, et al., 2018; Pellissery et al., 2020; Shao et al., 2021). Recent studies on VOCs production have shown that microorganisms can release a plethora of volatiles called microbial volatile organic compounds (mVOCs) (Chandrasekaran et al., 2022; Schulz-Bohm et al., 2017). We inferred that key VOCs produced by *Pseudomonas* and *Acinetobacter* were aldehyde (nonanal) and carboxylic acid (methyl diethylthiocarbamate). Li et al. (2020) demonstrated *Pseudomonas versuta* is responsible for the production of alcohol and aldehydes in bream flesh during chilled storage. In addition, numerous studies have focused on the relationship between microbial communities, including *Pseudomonas* and VOCs, and have discussed their possible contribution to spoilage (Casaburi et al., 2015; Yang et al., 2022). Li et al. (2023) reported that VOCs are potentially important for predicting its deterioration of smoked bacon. Exploring the contribution of microorganisms to spoilage and detecting key VOCs capable of predicting meat quality is of great significance in inhibiting spoilage during storage. It may be used to extend the shelf life of meat by inhibiting bacterial growth and reducing VOCs accumulation. Yang et al. (2022) demonstrated the inhibitory effect of CO<sub>2</sub> on VOCs accumulation associated with *B. thermosphacta*, *Pseudomonas* and *Serratia* growth.

Based on the aforementioned results, VOCs and microbial communities in goose breast muscles were altered during short periods of storage, despite storage at low temperatures. We reasoned that changes in the dominant bacteria responsible for driving chilled goose spoilage and causing changes in VOCs mainly occurred between days 3 and 5 of

chilled storage. The optimal chilled storage time for goose meat should not exceed 3 d.

## 5. Conclusions

In this study, it was clear that some potential changes in VOCs and microbial communities occurred during the prolonged storage process of goose meat. Our findings provided microbiome composition in geese over chilled storage and revealed that the bacterial community diversity of goose meat decreases with prolonged storage. *Pseudomonas* and *Acinetobacter* are responsible for the production of aldehyde (nonanal), and carboxylic acid (methyl diethylthiocarbamate) leading to meat-spoilage. Moreover, potential methods could be developed to prolong the shelf-life of goose meat and control microbial growth.

## CRedit authorship contribution statement

Yujiao Guo: Writing – original draft. Zhengfeng Cao: Project administration, Investigation. Kaiqi Weng: Conceptualization. Yang Zhang: Project administration. Yu Zhang: Supervision. Guohong Chen: Supervision. Qi Xu: Writing – review & editing.

## Declaration of competing interest

The authors declare that they have no competing interests.

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fochx.2024.101685>.

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