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Changes in meat quality, metabolites and microorganisms of mutton during cold chain storage

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ABSTRACT

During the cold chain storage process, changes in metabolites and microorganisms are highly likely to lead to changes in meat quality. To elucidate the changes in the composition of metabolites and microbiota during cold chain storage of mutton, this study utilized untargeted metabolome and 5R 16S rRNA sequencing analyses to investigate the changes in the *longissimus dorsi* under different cold chain temperatures (4 °C and −20 °C). With the extension of cold chain storage time, the meat color darkened and the content of C18:2n-6, C20:3n-6, and C23:0 were significantly increased in mutton. In this study, nine metabolites, including 1,2-Dioleoyl-*sn*-glycero-3-phosphoethanolamine, alanylphenylala-nine, indole-3-acrylic acid and the others, were significantly altered during cold chain storage. The abundance of the dominant microorganisms, including *Brachymonas*, *Aeromonas*, *Corynebacterium* and *Steroidobacter*, was significantly altered. Furthermore, a high correlation was observed between the different metabolites and microorganisms. These findings provide an in-depth understanding of the effects of different cold chain storage temperatures and times on the quality of mutton.

1. Introduction

Cold chain storage, which is critical to maintaining the quality and extending the shelf life of mutton products, is a continuous temperature-controlled storage and transportation system for refrigerated products between consumers and upstream suppliers (Ndraha, Hsiao, Vlajic, Yang, & Lin, 2018). There are two main types of common cold chain storage: frozen storage and refrigerated storage (Wang, He, Gan, & Li, 2018). Frozen storage involves rapidly freezing of meat after slaughter and storing it in an environment below −18 °C (Wang, He, Gan, & Li, 2018). Refrigerated storage refers to chilling meat after slaughter and quarantine, maintaining the core temperature of the carcass at 0 ~ 6 °C during the subsequent storage and transportation (Santos, Castro, Delgadillo, & Saraiva, 2020; Yu et al., 2022).

The cold chain storage extends the shelf life of chicken breast meat by inhibiting microbial growth and slowing down the chemical and enzymatic reactions that cause spoilage (Rinwi, Sun, Ma, & Wang, 2023). Nevertheless, meat products still undergo certain changes during the cold chain process (Wang, He, Gan, & Li, 2018). Factors such as fatty acids, moisture, temperature, water holding capacity, and oxidation could affect the meat quality and even lead to meat rancidity (Balan et al., 2019; Muzolf-Panek, & Kaczmarek, 2021). Additionally, myoglobin, lipids, and proteins in meat may be oxidized simultaneously during storage, with each oxidation process potentially influencing its quality (Wang, He, Gan, & Li, 2018). Moreover, fresh meat are susceptible to microbial contamination. The increased abundance of *Pseudomonas*, *Acinetobacter*, *Brochothrix*, and *Lactobacillales* could lead to meat spoilage and shortened its storage time (Zhang, Jiang, Guo, Bai, & Zhao, 2020). Inappropriate transport conditions could significantly affect the physical and chemical quality of meat products and human health (Brasil et al., 2014). Therefore, it is essential to optimize the transport conditions for meat products.

Metabolomics has been widely applied to quantitate the endogenous and exogenous metabolite composition of small molecules in cells, tissues, and biological fluids (Alonso, Marsal, & Julià, 2015; Christodoulou et al., 2020). Nowadays, metabolomics also provided a powerful tool for

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assessing meat quality and nutrition (Harlina et al., 2022; Zhang et al., 2023). Metabolites were associated with meat quality traits and could be denoted as metabolite biomarkers for intramuscular fat content (IMF), shear force, and cooking loss (Chen et al., 2024). Aldehydes and ketones were the important meat flavor precursors in meat products (Yang et al., 2022), which were easily modified by diets (Zhang et al., 2022). In addition, metabolomics could also be used to analyze the effects of packaging systems (Barahona, Hachemi, Olleta, González, & Campo, 2020), processing methods (Li et al., 2022), storage (Jung et al., 2022; Li et al., 2022), and ages (Li et al., 2022) on the flavor metabolite content of meat products.

An increasing number of studies have found that the meat spoilage microbiota or microbial communities in the food production environments are strongly associated with meat product quality and food safety (Ashaolu, Khalifa, Mesak, Lorenzo, & Farag, 2023; Thung et al., 2016; Wang et al., 2021). Harmful microorganisms in meat proliferated rapidly following the extension of storage time (Mohammed et al., 2021). *Acinetobacter*, *Enterobacter*, *Lactic acid bacteria*, *Escherichia coli O157: H7*, *Bacillus cereus*, *Clostridium botulinum spores* and *methicillin-resistance Staphylococcus aureus* could cause the deterioration of meat and meat products (Liu et al., 2022; Liu et al., 2023; Pateiro et al., 2021). It is worth noting that several microbiological markers have been considered as indicators of the microbial quality of food and the hygienic conditions of the production process (Mladenović et al., 2021). Several microorganisms, such as *Firmicutes*, *Bacteroidetes* and *Prevotella copri*, have bend found to alter multiple metabolic pathways and influence fat deposition in pork (Chen et al., 2022). The proliferation of harmful microorganisms lead to severe acidification, the emission of off-odor compounds, and the formation of ropy slime (Pothakos, Devlieghere, Villani, Björkroth, & Ercolini, 2015).

Numerous studies have been conducted on the assessment of meat quality in chicken (Deng, Liu, Li, Xu, & Zhou, 2022), beef (Kerth, Wall, Hicks, & Miller, 2023), and pork metabolism (Nevrkla et al., 2023). As one of the top four consumed meats in China, mutton has shown a stable increase, reaching up to 5.31 million tons in 2023. However, the changes in mutton quality, metabolites, and microorganisms during cold chain transportation are still unknown. Consequently, this study was performed to profile the meat color, pH, shear force and fatty acid content of mutton under simulated cold chain conditions. Metabolites and microbial changes were analyzed using ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS) and 16S rRNA sequencing.

2. Materials and methods

2.1. Sample collection and treatment

The *longissimus dorsi* muscles were sampled from nine Chengdu Brown goats (6 months old), which reared at the National Conservation Farm of Chengdu Brown goat (Dayi County, Chengdu, China) and fed an identical diet (Zhong et al., 2022). All the sampling procedures were reviewed and approved by the Animal Care and Use Committee of Sichuan Agricultural University (Permit number, Dky-2021202036). After removing the superficial fascia, each *longissimus dorsi* was divided into four equal parts, placed separately into self-sealing polyethylene bags in a foam box filled with ice packs, and immediately transported to laboratory. The following four treatments were implemented: initial condition (Con), refrigerated storage (RS, 4 °C for 48 h), and frozen storage (FS1, −20 °C for 7 d; FS2, −20 °C for 28 d). Each group consisted of nine muscles from the nine goats. Following the completion of appropriate cold chain simulation storage, frozen samples were thawed at 4 °C for 3 h. Each group of cold-chain treatment samples was partially used for meat quality testing and partially stored at −80 °C for subsequent metabolomic and 5R 16S rRNA sequencing analyses.

2.2. Analysis of meat color, pH, and shear force

Quality analysis of meat samples after different cold chain treatments, including meat color, shear force and pH, was performed. Meat color was evaluated using a precision colorimeter (3nh, Shenzhen, China). The lightness, redness, and yellowness of the samples were expressed as CIE L*, a*, and b*, respectively. To ensure accurate representation of the meat color, three locations on each muscle were analyzed and measured within one hour of processing. The pH value of mutton was measured by a pH meter (Tenovo, Beijing, China). In addition, meat samples were heated in a water bath at 80 °C until the core temperature reached 70 °C, and then the shear force parallel to the direction of muscle fibers was measured using a digital display muscle tenderness meter (Tenovo, Beijing, China). Each sample was tested five times to mitigate experimental errors, and the mean values were calculated using the least squares method. The tabular results were expressed as the mean value and the standard error of the mean value. $P < 0.05$ was considered significant.

2.3. Fatty acid profiles measurement

Lyophilized powder samples (200 mg) were mixed with 3 mL extract mixture (chloroform: methanol: water = 8: 4: 3), vortexed for 1 min, and ultrasound for 3 min under an ice bath. The mixture was placed for 2 h and then centrifuged at 1500 rpm for 5 min. The supernatant was collected, and the aforementioned steps were repeated twice. The entire supernatant obtained was transferred to a centrifuge tube and dried with a vacuum drying box to obtain the fatty acid glyceride mixture. The extracts were redissolved into 2 mL KOH-CH_3OH solution (0.5 mol/L), sealed, and swirled for 1 min. The mixture was then heated at 50 °C for 10 min to obtain the free fatty acid mixture. After cooling, the reaction continued by adding 2 mL BF3-CH_3OH solution, vortexed for 10 s, and then placed in a water bath at 80 °C for 2 min. After cooling once more, 1 mL n-hexane and 2 mL saturated NaCl solution were added for extraction followed by vortexing. The mixture was centrifuged at 1000 rpm for 5 min to facilitate layer separation. The upper n-hexane layer was transferred into a 2 mL glass gas chromatography (GC) vial and sealed with teflon-lined screw-caps for further analysis. Subsequently, the individual fatty acid content was determined using a GC2010 gas chromatograph fitted with a flame ionisation detector (FID) according to the machine instructions. Briefly, lipids were extracted from a 3 g sample, saponified, derivatized, and then analyzed using a gas chromatograph to determine the specific fatty acid contained in the sample. Each sample was tested five times. The mean values were calculated using the least squares method, with significance set at $P < 0.05$.

2.4. Untargeted metabolomics profiling

2.4.1. Extraction of metabolites

The *longissimus dorsi* muscle samples (70 mg) were mixed with 1 mL tissue extract (methanol: acetonitrile: water = 2:2:1, v/v/v) and three steel balls in an EP tube. The mixture was ground for the 60 s, sonicated for 1 h at low temperature, and then placed at −20 °C for 2 h. After centrifugation at 16,000*g* at 4 °C for 30 min, the supernatant was dried using a high-speed vacuum enrichment centrifuge (Eppendorf, Hamburg, Germany). Subsequently, the resulting powder was redissolved with 140 μL methanol aqueous solution (methano: water = 1:1, v/v), vortexed, and centrifuged at 20,000*g* for 20 min at 4 °C. The resulting supernatant was used for analysis.

2.4.2. UHPLC-QTOF-MS analysis

The SHIMADZU-LC30 was utilized as an ultrahigh-performance liquid chromatography system equipped with an ACQUITY UPLC HSS T3 column (Waters, MA, USA) maintained at 40 °C. Samples were injected into an autosampler at 4 °C, with flow rate of 0.3 mL/min, and injection volume of 2 μL throughout the analysis. The mobile phase

comprised a 0.1 % aqueous formic acid solution and acetonitrile. The liquid phase gradient was set as follows: the gradient was 0 % acetonitrile for 2 min; 2 ~ 6 min, the acetonitrile content increased linearly from 0 % to 48 %; then over the next 4 min, the acetonitrile content was linearly increased to 100 % and maintained for 2 min, followed by a linear reduction to 0 % over 0.1 min, and finally maintained for 2.9 min.

After the samples were separated by UPLC, the QE Plus mass spectrometer (Thermo Scientific, Shanghai, China) was used for mass spectrometry analysis. The electron spray ionization (ESI) source conditions were set as follows: the spray voltage was set at 3.8 kV for positive model and −3.2 kV for the negative model, with a sheath gas set at 30 arbitrary units. The capillary temperature was 320 °C. For full scans, the analyzer covered a mass range of *m/z* 70 ~ 1,050, achieving a mass resolution of 70 000. The HCD scan was employed for data-dependent MS/MS acquisition, with normalized collision energy set at 20, 30, and 40 eV. Dynamic exclusion was applied to remove redundant information from the MS/MS spectra.

2.4.3. Data analysis

The original data were processed using MSDIAL software for peak alignment, retention time correction, and peak area extraction. Standardized for retention time, *m/z*, observed value, and peak intensity were performed. Orthogonal partial least squares discriminant analysis (OPLS-DA) was employed to determine the overall metabolic changes between comparable groups. In the OPLS-DA model, metabolites with variable importance in projection score (VIP) > 1 is regarded more important compared to others. Subsequently, differential metabolites (DFMs) were filtered based on the following criteria: $|\log2(\text{fold change})| \geq 0.6$, VIP value ≥ 1. To illustrate the major metabolism changes during the refrigerated and frozen storage, Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis was performed based on the annotated DFMs. In addition, by comparing the differential metabolites of three storage conditions, the unique and common metabolites of the refrigerated and frozen storage were identified.

2.5. Microbiological analysis

2.5.1. DNA extraction and 5R 16S rRNA gene amplification

Genomic DNA of day 0 and day 28 was extracted from 0.2 g *longissimus dorsi* muscle samples using the CTAB method (Nejman et al., 2020). The concentration and purity of the extracted DNA was checked by 1 % agarose gel electrophoresis. In addition, several controls were incorporated to account for potential sources of environment contamination and different handling steps. For amplification of the five regions (V2, V3, V5, V6, and V8) on the 16S rDNA, two rounds of amplification were performed using the specific primers (Table S1). The first round involved the use of specific primers, followed by a second round with the specific primers along with the barcodes and Illumina sequencing connectors (Fig. S1). The PCR reaction was as follows: an initial denaturation at 98 °C for 30 s, followed by 30 cycles of denaturation at 98 °C for 10 s, annealing at 62 °C for 10 s, extension at 62 °C for 15 s, and a final extension at 72 °C for5 min. Subsequently, the PCR products were purified by using the E.Z.N.A. Gel Extraction Kit, and then the libraries were sequenced on the Illumina NovaSeq 6000 system (LC-Bio, Hangzhou, China).

2.5.2. Illumina MiSeq sequencing and data processing

To classify bacterial taxonomy in the samples and calculate the relative abundance, the Short Multiple Regions Framework (SMURF) analysis was employed to merge the counts of the five regions mentioned above (Lin et al., 2023). Quality control of the raw data involved filtering bacterial data with a total read count of less than 1000 and a relative abundance of less than 10^{-4}. Any bacteria identified in negative control samples were considered as potential contaminants both at the sampling and experimental stages. Taxonomic classification was determined according to the Greengenes database (https://greengenes.lbl.gov).

The richness and alpha diversity indices, including Chao1, Simpson, and Shannon indexes, were computed to analyze the microbial community, and $P < 0.05$ was regarded as the significant level. Beta diversity analysis, including principal coordinates analysis (PCoA) and nonmetric multidimensional scaling (NMDS), was performed to compare species diversity across different samples. The relative abundance of each bacterial species was calculated to evaluate their distributions at each taxonomic level. Species with higher relative abundance were identified and compared. The dominant bacteria profiles were visualized using the ggplot2 package in R. Then, linear discriminant analysis effect size (LEfSe) was utilized to identify microbial biomarkers in high-dimensional data. The dominant bacteria were defined with LDA > 3, $P < 0.05$.

2.6. Statistical analysis

Data statistical analyses were conducted using R (v 4.2.3) for Windows. T-test was employed to assess significant differences, with the detection limit of 0.05. Spearman's correlation coefficient was calculated to identify associations between metabolite and microorganism with the threshold of $|r| > 0.6$ and $P < 0.05$.

3. Results

3.1. Changes in meat quality under refrigerated and frozen storage

The three meat color parameters, pH values, and shear force are listed in Table S2. Significant differences were observed in the L* value and b* value between the refrigerated and frozen muscle samples (Fig. 1A-C). To be specific, the L* value of frozen storage after 28 days was significantly lower than that of the refrigerated and fresh storage. Similarly, the L* value of frozen storage after 7 days was statistically significantly lower than that of the refrigerated storage. The b* value of frozen storage was also significantly lower than that of fresh storage, and the b* value of frozen storage after 28 days was statistically significantly lower than that of refrigerated storage. Whereas, the a* value had no significant difference between different storage times. The goat muscle darkened during storage. Additionally, pH and shear force did not exhibit significant change under different storage conditions.

3.2. Differences in fatty acid composition under refrigerated and frozen storage

As shown in Table S3, the majority of fatty acids did not exhibit significant changes in content under different storage conditions. Notably, FS1 had higher proportions of certain polyunsaturated fatty acids compared to the Con (Fig. 1D-F). Specifically, statistically significant increase were observed in the proportions of C18:2n-6 and C20:3n-6 between the fresh and frozen storage conditions. Furthermore, the frozen storage exhibited a relatively higher proportions of C23:0 compared to the initial condition.

3.3. Metabolic changes under refrigerated and frozen storage

The OPLS-DA score plots demonstrated the apparent separation of the meat metabolites among Con, RS, FS1, and FS2 (Fig. 2A), suggesting significant changes in the metabolite content of refrigerated and frozen mutton following extension of cold chain storage. The volcano plot illustrated the variations in different cold chain conditions by displaying the amount of variation in the differential metabolites. Specifically, with the extension of cold chain time (from day 0 to day 28), refrigerated storage produced significant alterations, with 60 DFMs in the RS group compared to the CON, including 36 upregulated and 24 downregulated metabolites (Fig. 2B). In frozen storage, 300 DFMs were markedly altered in the FS group compared to metabolites in the CON group, with

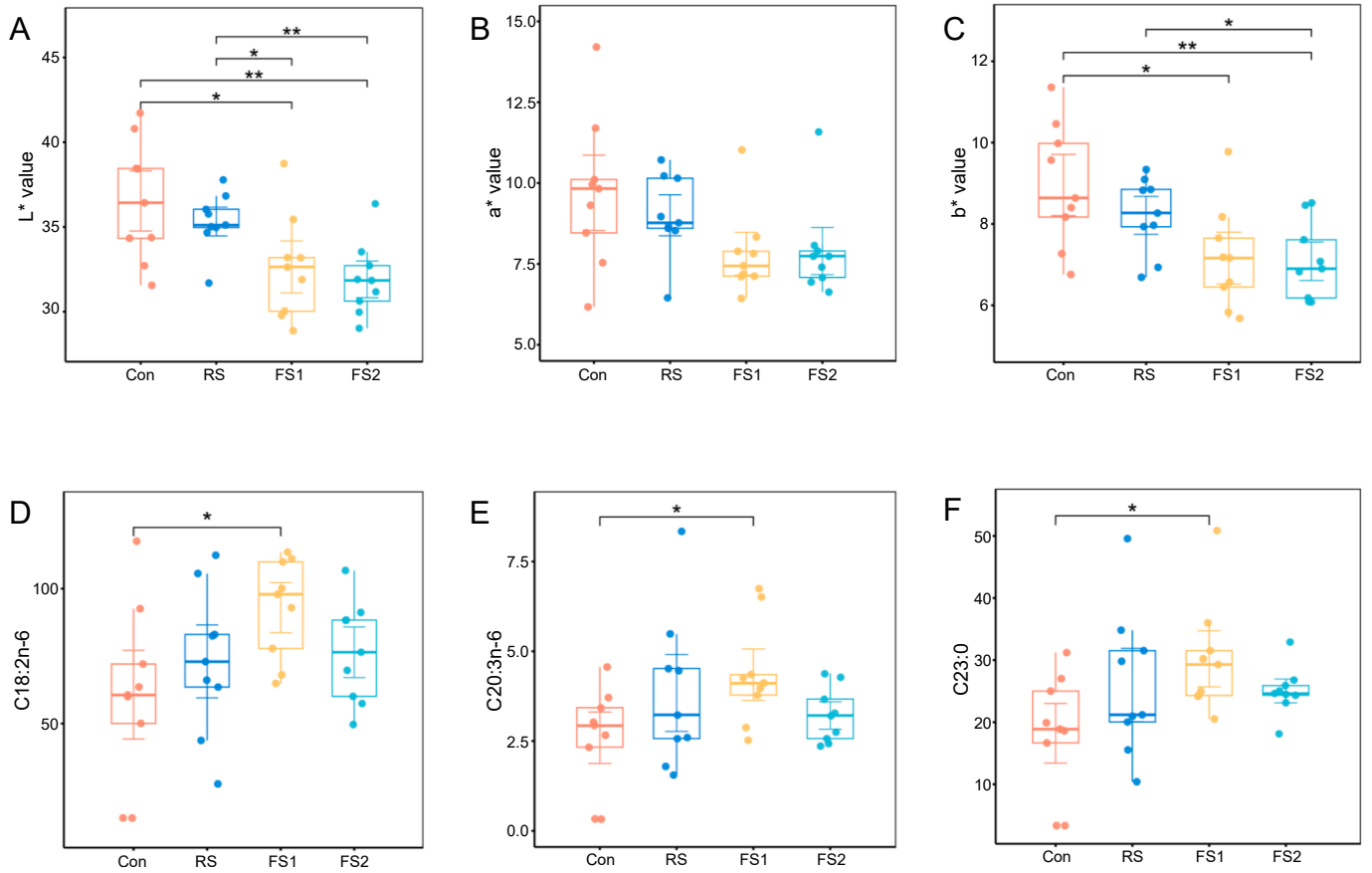

Fig. 1. Analysis of different meat quality indexes and variation of different fatty acid content in meat samples. (A, B, and C) Comparison of L* value, a* value and b* value during the Con, RS and FS. (D, E, and F) Comparison of C18:2n-6, C20:3n-6 and C23:0 content during the refrigerated and frozen storage. * indicates $P < 0.05$, ** indicates $P < 0.01$.

93 upregulated and 207 downregulated metabolites in the FS1 group, and 99 upregulated and 201 downregulated metabolites in the FS2 group (Fig. 2C and 2D). Subsequently, KEGG pathway enrichment analysis was performed to facilitate the understanding of the biological mechanisms associated with different preservation conditions (Fig. 2E-G). The differential metabolites were primarily enriched in various pathways, including substance dependence, pyrimidine metabolism, purine metabolism, signaling molecules and interaction, energy metabolism, nucleotide metabolism, nervous system, membrane transport, cell growth and death, metabolism of other amino acids.

3.4. Special metabolic characteristics under refrigerated and frozen storage

Comparative analysis of metabolites revealed shared and condition-associated DFMs in three preservation conditions (Fig. 3A). Among them, nine DFMs were shared, 47 metabolites were RS condition-associated DFMs, 29 metabolites were FS1 condition-associated DFMs, and 29 metabolites were FS2 condition-associated DFMs. The nine metabolites exhibiting significant changes in common under different storage conditions were selected as the biomarkers. The heatmap visualized the trend of changes in these common differential metabolites (Fig. 3B). Briefly, the RS had relatively higher proportions of diaminopropane, diallyl sulfide, alanylphenylalanine, 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine, indole-3-acrylic acid, acetoacetic acid and relatively lower proportions of PC(15:0/20:4(5Z,8Z,11Z,14Z)), methionine sulfoxide and carbon number 8 compared to the Con. In addition, 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine, indole-3-acrylic acid and acetoacetic acid were upregulated in the FS compared to the Con, whereas PC(15:0/20:4(5Z,8Z,11Z,14Z)), methionine sulfoxide and four other metabolites were downregulated. Subsequently, KEGG enrichment analysis was performed to gain insight into the biological mechanisms that are specifically associated with different preservation conditions (Fig. 3C-E). The results showed significantly enrichment in various metabolic pathways, including tryptophan metabolism, pyrimidine metabolism, purine metabolism, glutathione metabolism, histidine metabolism, arginine biosynthesis, lysine degradation, alanine, aspartate and glutamate metabolism, valine, leucine and isoleucine degradation, pentose and glucuronate interconversions, fatty acid biosynthesis.

3.5. Changes in meat microbiota composition after frozen storage

To investigate the changes in microbial composition during the cold chain process, 5R 16S rRNA sequence analysis was used to analyze the microbial composition of meat samples. The alpha diversity (Shannon, Simpson, and Chao1) of the meat microbiota during storage indicated similar bacterial diversity indices between frozen meat samples stored for 0 and 28 days (Fig. 4A-C). The PCoA plot and NMDS analysis revealed a clear separation between Con and FS2 (Fig. 4D and 4E), resulting from the changes in microbes composition after frozen storage. The primary enrichment of microorganisms of frozen meat samples after frozen storage at the family and genus level is shown in Fig. 4F. Based on relative abundance, the top 10 most abundant genus are shown in Fig. 4G. At the genus level, compared to the Con, *Achromobacter*, *Bergeyella*, *Bosea*, *Brachymonas*, *Citrobacter* and *Steroidobacter* were more abundant, while *Aeromonas* and *Corynebacterium* were less abundant in FS2 (Fig. 4H). Significant differences in bacteria between Con and FS2

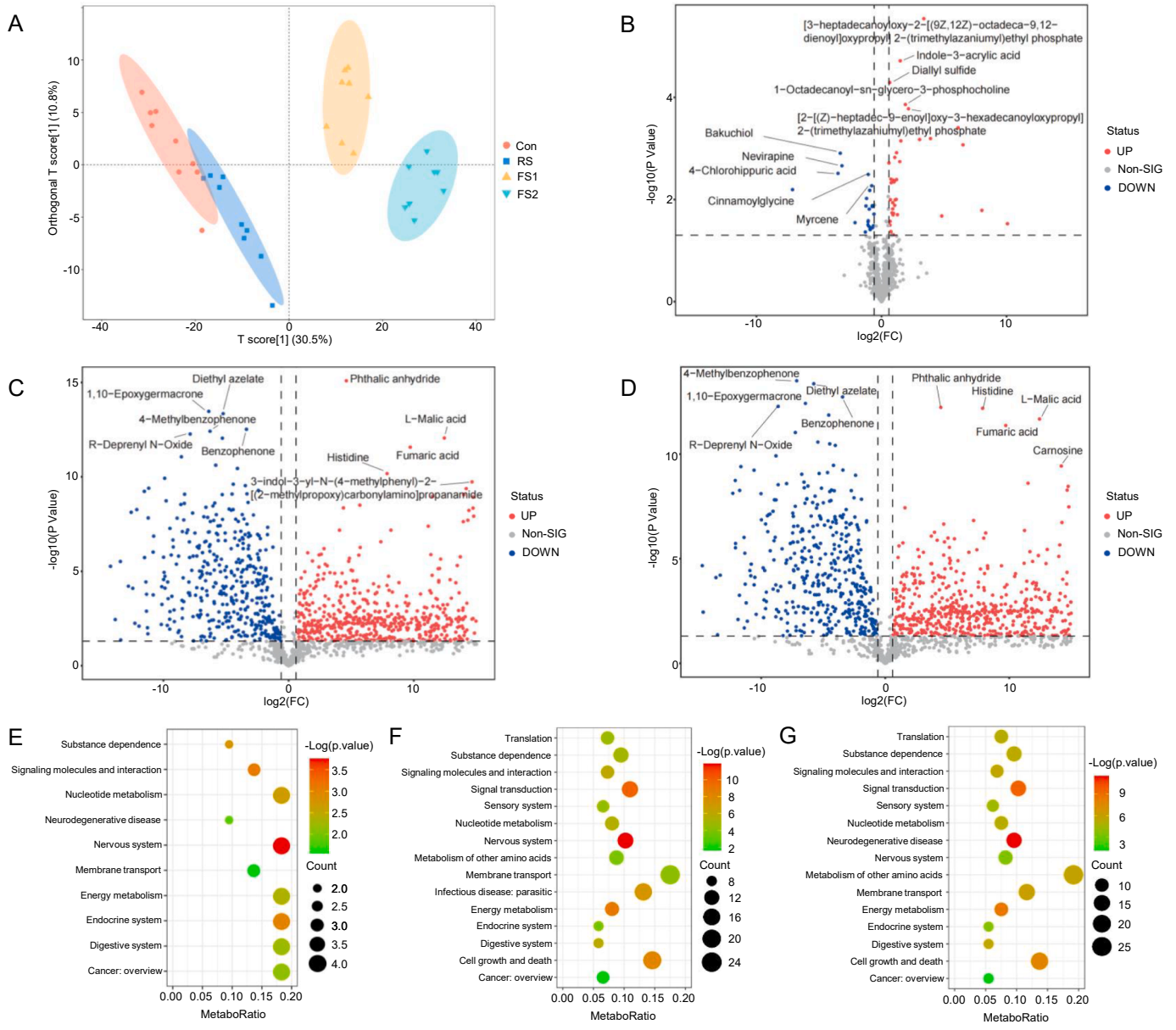

Fig. 2. Preliminary analysis of metabolomic data in different preservation conditions. (A) OPLS-DA score plots. (B, C and D) Volcano plot for differential metabolites of RS and Con, FS1 and Con, FS2 and Con. (E, F, and G) KEGG pathway analyses of RS and Con, FS1 and Con, FS2 and Con.

were confirmed by LEfSe analysis (Fig. 4I), illustrating that *Steroidobacter*, *Bergeyella*, *Achromobacter*, *Bosea* and the others were key microorganisms in frozen treatment.

3.6. Correlations between metabolites and microorganisms

Metabolomic and microbiome data was utilized to evaluate the correlation between metabolites and microorganisms of the *longissimus dorsi* muscle samples stored at the Con and FS condition. A significant association between metabolites and microorganisms was observed (Fig. 5A and 5B). In detail, *Aeromonas* was positively correlated with diallyl sulfide, methionine sulfoxide, PC(15:0/20:4(5Z,8Z,11Z,14Z)), diaminopropane. Conversely, *Aeromonas* was negatively correlated with 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine and Indole-3-acrylic acid. *Corynebacterium* exhibited a positive correlation with diallyl sulfide, PC(15:0/20:4(5Z,8Z,11Z,14Z)), diaminopropane and a negative correlation with 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine. *Brachymonas* showed a positive correlation with acetoacetic acid and indole–3–acrylic acid. Conversely, *Brachymonas* was negatively correlated with diaminopropane, diallyl sulfide, carbon number 8 and alanylphenylalanine.

4. Discussion

Meat quality were prone to deteriorate during storage, such as water-holding capacity (Liu, Hu, Liu, Zheng, & Ma, 2023), drip loss (Tao et al., 2023), shear force (Hayat, Kaka, & Sazili, 2021) and metabolism (Xu et al., 2022). Cold chain storage is a widely utilized preservation method to ensure high-quality transportation of meat products (Chen, Qian, Yang, & Wu, 2022). Nevertheless, it has been found that there are still numerous alterations in meat color during this process (Zhou et al., 2021). In this study, the L* value and the b* value exhibited a significant decline as an extension of the storage. Previous studies have indicated that the color value (L* and b*) of Mexican mutton and pork continued to decrease significantly after cold storage treatment, attributed to the surface moisture loss, surface drying, metmyoglobin formation and

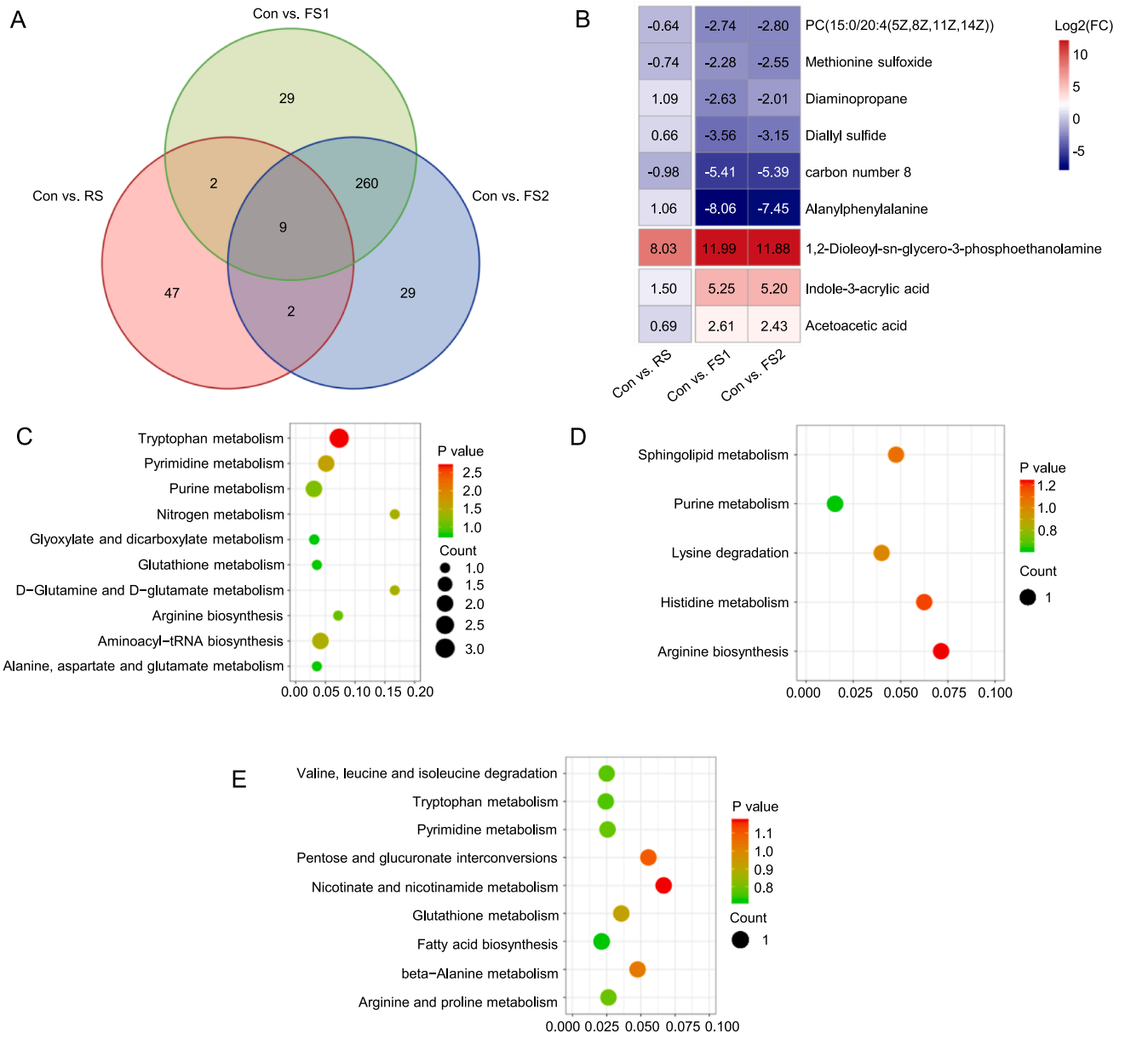

Fig. 3. Further analysis of metabolomic data in different preservation conditions. (A)Venn diagram illustrating the overlap of differential metabolites among the three comparisons (RS vs. Con; FS1 vs. Con; FS2 vs. Con) in the *longissimus dorsi* of Chengdu Brown goat. (B) Heat map of the common differential metabolite clustering of the three comparisons. (C, D, and E) KEGG pathway analysis of particular differential metabolite of the three comparisons.

disruption of muscle cell integrity (Estrada-Solís, Figueroa-Rodríguez, Figueroa-Sandoval, Hernández-Rosas, & Hernández-Cazares, 2016; Fan et al., 2019).

Myoglobin is one of the major pigments responsible for maintaining meat color (Zhu et al., 2024). The concentration and state of myoglobin in the muscle and the redox state of myoglobin co-influenced the meat color, causing different shades and depths (Ramanathan, Suman, & Faustman, 2020). Our results revealed a significant increase in the content of C18:0 and polyunsaturated fatty acids, including linoleic acid (C18:2n6) and C20:3n6, when comparing frozen storage to refrigerated storage. Linoleic acid is utilized by muscle tissue for energy purposes or stored in adipose tissue (Marangoni et al., 2020). In the modern Western diet, linoleic acid has become the most abundant polyunsaturated fatty acid (Hamilton, & Klett, 2021). The high concentration of unsaturated fatty acids in meat makes it susceptible to oxidative spoilage, resulting in decreased product quality and shelf life (Tian et al., 2022). The change in meat color is primarily due to the oxidation of myoglobin contained in meat, with the degree of oxidation directly proportional to the darkness of the color (Hernández Salueña, Sáenz Gamasa, Diñeiro Rubial, & Alberdi Odriozola, 2019). These findings also explain the darkening of the flesh after storage.

The complex flavor of the meat is closely related to its metabolite composition (Liu et al., 2021; Wang et al., 2020; Yang, Dai, Ayed, & Liu, 2019). In this study, we observed that the content of Phosphatidylcholine (PC) decreased significantly under refrigeration conditions. PC serves as essential flavor compounds in meat, facilitating the formation of Maillard reaction products during heating, while their oxidative degradation can also catalyze the synthesis of fatty aldehydes (Zhang et al., 2022). A notable decline in PC content under refrigeration conditions suggests phospholipid hydrolysis, likely attributed to the presence of phospholipase (Zhou et al., 2019). However, the PC content increased significantly under frozen condition, indicating a possible reaction between alcohol and phosphoric acid to form phosphate ester, which further converts to phospholipids. Diallyl sulfide, an organic

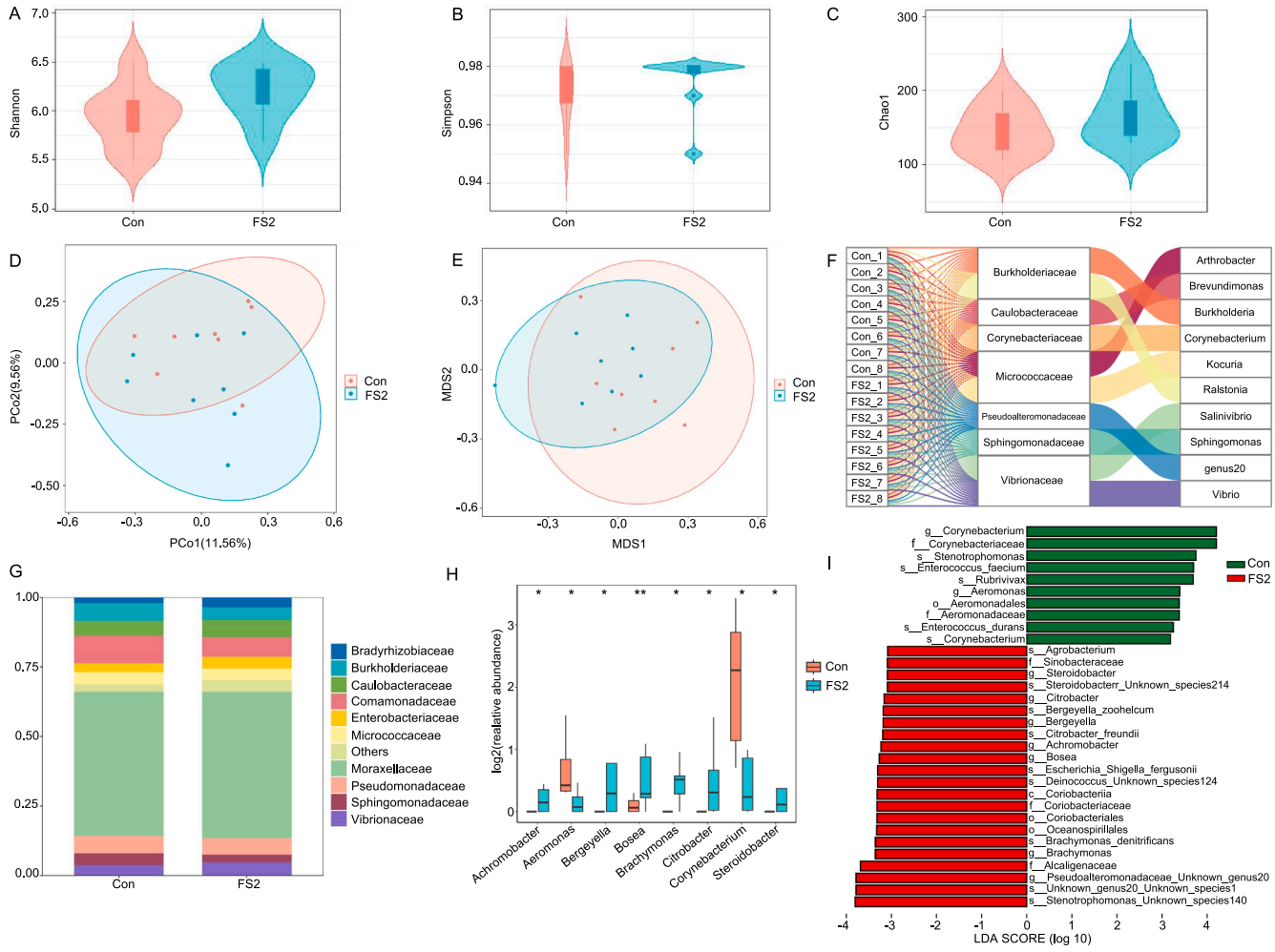

Fig. 4. Analysis of microbial diversity after frozen storage for 28 days. (A, B, and C) The alpha diversity of the microbiota after 28 days of frozen storage (Shannon, Simpson, and Chao1 bacterial diversity indexes). (D and E) Weighted UniFrac PCoA and NMDS plots. (F) Sankey diagram of species composition at family and genus level. (G) Bar chart of microbial community distribution under different conditions at the genus level. (H) Changes in the relative abundances of the eight spoilage bacteria after frozen storage. (I) The LEfSe analysis of Con and FS treatments.

sulfur compound derived from allium plants like garlic and onions, emerges as a potential enhancer of lipid stability (Li, Tang, Li, Chen, & Ma, 2022).

Nucleotide metabolism, pyrimidine metabolism, purine metabolism, pentose and glucuronate interconversions, fatty acid biosynthesis and various amino acid metabolic pathways were significantly enriched in our study. Previous research has highlighted the association of carbohydrate metabolism, amino acid metabolism, nucleotide pathway, lipid metabolism, and histidine metabolism with meat spoilage microorganisms (Stellato et al., 2016; Fang, Feng, Lu, & Zhu, 2022). Carbohydrates and certain amino acids serve as precursors of metabolites associated with spoilage, and spoilage bacteria can synthesize carbohydrates and amino acids via specific amino acid metabolism and purine metabolism associated with meat spoilage (Zhang et al., 2021). The accumulation of metabolites resulting from the bacterial utilization of nutrients such as carbohydrates, amino acids, peptides, and nucleotides often initiates meat spoilage, leading to odor and discoloration (Toomik, Rood, Bowman, & Kocharunchitt, 2023). Consequently, meat products are susceptible to contaminate by food-borne microorganisms, posing significant threats to health and causing substantial economic losses during transportation and storage (Liu, et al., 2022).

Appropriate cold chain patterns can inhibit the growth of microorganisms (Wang et al., 2024). We profiled a significant increase in the abundance of *Brachymonas*, *Bosea*, *Achromobacter*, *Bergeyella*, *Citrobacter*, and *Steroidobacter* in the cold chain simulations. *Brachymonas*, which exhibits a co-occurring pattern with some antibiotic resistance genes, is the potential antibiotic resistance gene hosts and a common denitrifying bacterial genus (Yang et al., 2020). In this study, *Brachymonas* was significantly correlated with diaminopropane, PC (15:0/20:4), glyceryl monopalmitate and another metabolites, suggesting its potential to influence the metabolism of meat products and serve as an important microbiome biomarker of spoilage in frozen meat storage. However, the association of the other increased genera with meat quality has yet to be confirmed.

5. Conclusion

In this study, we simulated the different cold chain storages and conducted untargeted metabolome and 5R 16S rRNA analysis on different treatments of cold chain stored mutton to investigate the changes in metabolites and microbiota. Cold chain storage can significantly darkens the color of goat muscle and increased the content of C18:2n-6, C20:3n-6, C23:0. Indole-3-acrylic acid, 1,2-Dioleoyl-*sn*-glycero-3-phosphoethanolamine, alanylphenylala-nine, indole-3-acrylic, and the other metabolites were significantly changed, which were mainly involved in nucleotide metabolism, purine metabolism and several amino acid metabolic pathways. Furthermore, the abundance of harmful bacteria, such as *Aeromonas*, *Brachymonas*, *Corynebacterium* and

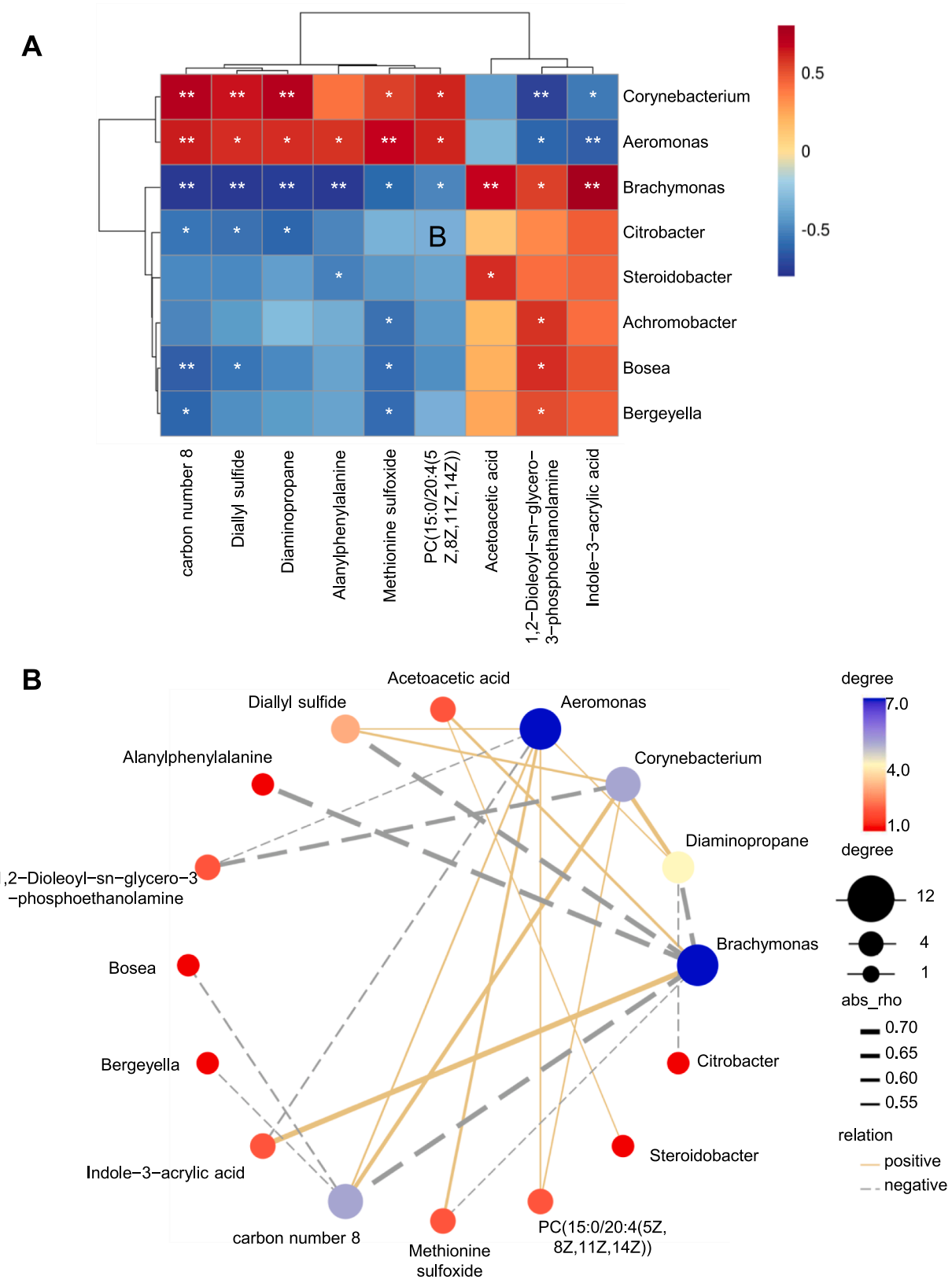

Fig. 5. Correlation analysis between metabolites and microorganisms. (A) The correlation heat map between muscle metabolites and microorganisms. (B) The correlation network diagram between muscle metabolites and microorganisms analysis. Red and blue colors represent positive and negative correlations, respectively. * indicates $P < 0.05$ and ** indicates $P < 0.01$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Steroidobacter, increase significantly. There were significant correlations between these metabolites and microorganisms in mutton. These findings provide a comprehensive insight into the microbial and metabolic profiles in the process of meat storage and transportation.

CRediT authorship contribution statement

Ziwei Guo: Writing – original draft, Methodology, Investigation. **Yibing Chen:** Methodology, Investigation. **Yuqin Wu:** Investigation, Methodology. **Siyuan Zhan:** Visualization, Resources. **Linjie Wang:** Visualization. **Li Li:** Resources. **Hongping Zhang:** Resources. **Zhenying Xu:** Methodology. **Shixiu Qiu:** Investigation. **Jiaxue Cao:** Resources, Methodology. **Jiazhong Guo:** Resources, Methodology. **Lili Niu:** Validation, Supervision, Project administration. **Tao Zhong:** Writing – review & editing, Methodology, Data curation, Conceptualization.

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.

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.foodres.2024.114551.

References

Alonso, A., Marsal, S., & Julià, A. (2015). Analytical methods in untargeted metabolomics: State of the art in 2015. *Frontiers in Bioengineering and Biotechnology, 3*, 23. https://doi.org/10.3389/fbioe.2015.00023

Ashaolu, T. J., Khalifa, I., Mesak, M. A., Lorenzo, J. M., & Farag, M. A. (2023). A comprehensive review of the role of microorganisms on texture change, flavor and biogenic amines formation in fermented meat with their action mechanisms and safety. *Critical Reviews in Food Science and Nutrition, 63*(19), 3538–3555. https://doi.org/10.1080/10408398.2021.1929059

Balan, P., Kim, Y. H. B., Stuart, A. D., Kemp, R., Staincliffe, M., Craigie, C., & Farouk, M. M. (2019). Effect of fast freezing then thaw-aging on meat quality attributes of lamb *M. longissimus* lumborum. *Animal Science Journal, 90*(8), 1060–1069. https://doi.org/10.1111/asj.13216

Barahona, M., Hachemi, M. A., Olleta, J. L., González, M. D. M., & Campo, M. D. M. (2020). Feeding, muscle and packaging effects on meat quality and consumer acceptability of Avileña-Negra Ibérica beef. *Foods, 9*(7), 853. https://doi.org/10.3390/foods9070853

Brasil, L., Queiroz, A., Silva, J., Bezerra, T., Arcanjo, N., Magnani, M., … Madruga, M. (2014). Microbiological and nutritional quality of the goat meat by-product "sarapatel". *Molecules, 19*(1), 1047–1059. https://doi.org/10.3390/molecules19011047

Chen, B., Li, D., Leng, D., Kui, H., Bai, X., & Wang, T. (2022). Gut microbiota and meat quality. *Frontiers in Microbiology, 13*, Article 951726. https://doi.org/10.3389/fmicb.2022.951726

Chen, G., Qi, L., Zhang, S., Peng, H., Lin, Z., Zhang, X., … Luo, W. (2024). Metabolomic, lipidomic, and proteomic profiles provide insights on meat quality differences between Shitou and Wuzong geese. *Food Chemistry, 438*, Article 137967. https://doi.org/10.1016/j.foodchem.2023.137967

Chen, Q., Qian, J., Yang, H., & Wu, W. (2022). Sustainable food cold chain logistics: From microenvironmental monitoring to global impact. *Comprehensive Reviews In Food Science and Food Safety, 21*(5), 4189–4209. https://doi.org/10.1111/1541-4337.13014

Christodoulou, C. C., Zachariou, M., Tomazou, M., Karatzas, E., Demetriou, C. A., Zamba-Papanicolaou, E., & Spyrou, G. M. (2020). Investigating the transition of pre-symptomatic to symptomatic huntington's disease status based on omics data. *International Journal of Molecular Sciences, 21*(19), 7414. https://doi.org/10.3390/ijms21197414

Deng, S., Liu, R., Li, C., Xu, X., & Zhou, G. (2022). Meat quality and flavor compounds of soft-boiled chickens: Effect of Chinese yellow-feathered chicken breed and slaughter age. *Poultry Science, 101*(12), Article 102168. https://doi.org/10.1016/j.psj.2022.102168

Estrada-Solís, J., Figueroa-Rodríguez, K. A., Figueroa-Sandoval, B., Hernández-Rosas, F., & Hernández-Cazares, A. S. (2016). Microstructure and physical changes in the Mexican cooked lamb meat barbacoa made with chilled and frozen meat. *Meat Science, 118*, 122–128. https://doi.org/10.1016/j.meatsci.2016.04.001

Fan, X. J., Liu, S. Z., Li, H. H., He, J., Feng, J. T., Zhang, X., & Yan, H. (2019). Effects of Portulaca oleracea L. extract on lipid oxidation and color of pork meat during refrigerated storage. *Meat Science, 147*, 82–90. https://doi.org/10.1016/j.meatsci.2018.08.022

Fang, J., Feng, L., Lu, H., & Zhu, J. (2022). Metabolomics reveals spoilage characteristics and interaction of Pseudomonas lundensis and Brochothrix thermosphacta in refrigerated beef. *Food Research International, 156*, Article 111139. https://doi.org/10.1016/j.foodres.2022.111139

Hamilton, J. S., & Klett, E. L. (2021). Linoleic acid and the regulation of glucose homeostasis: A review of the evidence. *Prostaglandins, Leukotrienes, and Essential Fatty Acids, 175*, Article 102366. https://doi.org/10.1016/j.plefa.2021.102366

Harlina, P. W., Maritha, V., Musfiroh, I., Huda, S., Sukri, N., & Muchtaridi, M. (2022). Possibilities of Liquid Chromatography Mass Spectrometry (LC-MS)-Based Metabolomics and Lipidomics in the Authentication of Meat Products: A Mini Review. *Food Science of Animal Resources, 42*(5), 744–761. https://doi.org/10.5851/kosfa.2022.e37

Hayat, M. N., Kaka, U., & Sazili, A. Q. (2021). Assessment of Physicochemical Characteristics and Microbiological Quality in Broiler Chicken Breast Muscle (Pectoralis major) Subjected to Different Temperatures and Lengths of Cold Transportation. *Foods, 10*(4), 874. https://doi.org/10.3390/foods10040874

Hernández Salueña, B., Sáenz Gamasa, C., Diñeiro Rubial, J. M., & Alberdi Odriozola, C. (2019). CIELAB color paths during meat shelf life. *Meat Science, 157*, Article 107889. https://doi.org/10.1016/j.meatsci.2019.107889

Jung, D. Y., Lee, D., Lee, H. J., Kim, H. J., Jung, J. H., Jang, A., & Jo, C. (2022). Comparison of chicken breast quality characteristics and metabolites due to different rearing environments and refrigerated storage. *Poultry Science, 101*(7), Article 101953. https://doi.org/10.1016/j.psj.2022.101953

Kerth, C. R., Wall, K. R., Hicks, Z. M., & Miller, R. K. (2023). Using untargeted metabolomics and volatile aroma compounds to predict expert sensory descriptors and consumer liking of beef loin steaks varying in quality grade, aging time, and degree of doneness. *Meat Science, 204*, Article 109255. https://doi.org/10.1016/j.meatsci.2023.109255

Li, H., Geng, W., Haruna, S. A., Zhou, C., Wang, Y., Ouyang, Q., & Chen, Q. (2022). Identification of characteristic volatiles and metabolomic pathway during pork storage using HS-SPME-GC/MS coupled with multivariate analysis. *Food Chemistry, 373*(Pt A), Article 131431. https://doi.org/10.1016/j.foodchem.2021.131431

Li, J., Zhang, D., Yin, L., Li, Z., Yu, C., Du, H., … Liu, Y. (2022). Integration analysis of metabolome and transcriptome profiles revealed the age-dependent dynamic change in chicken meat. *Food Research International, 156*, Article 111171. https://doi.org/10.1016/j.foodres.2022.111171

Li, S., Tang, S., Li, J., Chen, L., & Ma, Y. (2022). Protective Effects of Four Natural Antioxidants on Hydroxyl-Radical-Induced Lipid and Protein Oxidation in Yak Meat. *Foods, 11*(19), 3062. https://doi.org/10.3390/foods11193062

Li, X., Xie, W., Bai, F., Wang, J., Zhou, X., Gao, R., … Zhao, Y. (2022). Influence of thermal processing on flavor and sensory profile of sturgeon meat. *Food Chemistry, 374*, Article 131689. https://doi.org/10.1016/j.foodchem.2021.131689

Lin, Q., Duan, H., Wang, S., Guo, Z., Wang, S., Chang, Y., … Zhou, C. (2023). Endometrial microbiota in women with and without adenomyosis: A pilot study. *Frontiers in Microbiology, 14*, 1075900. https://doi.org/10.3389/fmicb.2023.1075900

Liu, J., Hu, Z., Liu, D., Zheng, A., & Ma, Q. (2023). Glutathione metabolism-mediated ferroptosis reduces water-holding capacity in beef during cold storage. *Food Chemistry, 398*, Article 133903. https://doi.org/10.1016/j.foodchem.2022.133903

Liu, M., Pan, Y., Feng, M., Guo, W., Fan, X., Feng, L., … Cao, Y. (2022). Garlic essential oil in water nanoemulsion prepared by high-power ultrasound: Properties, stability and its antibacterial mechanism against MRSA isolated from pork. *Ultrasonics Sonochemistry, 90*, Article 106201. https://doi.org/10.1016/j.ultsonch.2022.106201

Liu, T., Mo, Q., Wei, J., Zhao, M., Tang, J., & Feng, F. (2021). Mass spectrometry-based metabolomics to reveal chicken meat improvements by medium-chain monoglycerides supplementation: Taste, fresh meat quality, and composition. *Food Chemistry, 365*, Article 130303. https://doi.org/10.1016/j.foodchem.2021.130303

Liu, Z., Shaposhnikov, M., Zhuang, S., Tu, T., Wang, H., & Wang, L. (2023). Growth and survival of common spoilage and pathogenic bacteria in ground beef and plant-based meat analogues. *Food Research International, 164*, Article 112408. https://doi.org/10.1016/j.foodres.2022.112408

Marangoni, F., Agostoni, C., Borghi, C., Catapano, A. L., Cena, H., Ghiselli, A., … Poli, A. (2020). Dietary linoleic acid and human health: Focus on cardiovascular and cardiometabolic effects. *Atherosclerosis, 292*, 90–98. https://doi.org/10.1016/j.atherosclerosis.2019.11.018

Mladenović, K. G., Grujović, M.Ž., Kiš, M., Furmeg, S., Tkalec, V. J., Stefanović, O. D., & Kocić-Tanackov, S. D. (2021). Enterobacteriaceae in food safety with an emphasis on raw milk and meat. *Applied Microbiology and Biotechnology, 105*(23), 8615–8627. https://doi.org/10.1007/s00253-021-11655-7

Mohammed, H. H. H., He, L., Nawaz, A., Jin, G., Huang, X., Ma, M., … Khalifa, I. (2021). Effect of frozen and refrozen storage of beef and chicken meats on inoculated

microorganisms and meat quality. *Meat Science, 175*, Article 108453. https://doi.org/10.1016/j.meatsci.2021.108453

Muzolf-Panek, M., & Kaczmarek, A. (2021). Chemometric Analysis of Fatty Acid Composition of Raw Chicken, Beef, and Pork Meat with Plant Extract Addition during Refrigerated Storage. *Molecules, 26*(16), 4952. https://doi.org/10.3390/molecules26164952

Ndraha, N., Hsiao, H. I., Vlajic, J., Yang, M. F., & Lin, H. T. V. (2018). Time-temperature abuse in the food cold chain: Review of issues, challenges, and recommendations. *Food Control, 89*, 12–21. https://doi.org/10.1016/j.foodcont.2018.01.027

Nejman, D., Livyatan, I., Fuks, G., Gavert, N., Zwang, Y., Geller, L. T., … Straussman, R. (2020). The human tumor microbiome is composed of tumor type-specific intracellular bacteria. *Science, 368*(6494), 973–980. https://doi.org/10.1126/science.aay9189

Nevrkla, P., Weisbauerová, E., Horký, P., Hadaš, Z., Rozkot, M., & Čtvrtlíková Knitlová, D. (2023). Fatty acid and amino acid profiles in muscle longissimus lumborum et thoracis of the indigenous Prestice Black-Pied pig breed in comparison with a commercial pig hybrid. *Italian Journal of Animal Science, 22*(1), 472–481. https://doi.org/10.1080/1828051X.2023.2206415

Pateiro, M., Munekata, P. E. S., Sant'Ana, A. S., Domínguez, R., Rodríguez-Lázaro, D., & Lorenzo, J. M. (2021). Application of essential oils as antimicrobial agents against spoilage and pathogenic microorganisms in meat products. *International Journal of Food Microbiology, 337*, Article 108966. https://doi.org/10.1016/j.ijfoodmicro.2020.108966

Pothakos, V., Devlieghere, F., Villani, F., Björkroth, J., & Ercolini, D. (2015). Lactic acid bacteria and their controversial role in fresh meat spoilage. *Meat Science, 109*, 66–74. https://doi.org/10.1016/j.meatsci.2015.04.014

Ramanathan, R., Suman, S. P., & Faustman, C. (2020). Biomolecular Interactions Governing Fresh Meat Color in Post-mortem Skeletal Muscle: A Review. *Journal of Agricultural and Food Chemistry, 68*(46), 12779–12787. https://doi.org/10.1021/acs.jafc.9b08098

Rinwi, T. G., Sun, D. W., Ma, J., & Wang, Q. J. (2023). Effects of isochoric freezing on freezing process and quality attributes of chicken breast meat. *Food Chemistry, 405* (Pt B), Article 134732. https://doi.org/10.1016/j.foodchem.2022.134732

Santos, M. D., Castro, R., Delgadillo, I., & Saraiva, J. A. (2020). Improvement of the refrigerated preservation technology by hyperbaric storage for raw fresh meat. *Journal of the Science of Food and Agriculture, 100*(3), 969–977. https://doi.org/10.1002/jsfa.10083

Stellato, G., La Storia, A., De Filippis, F., Borriello, G., Villani, F., & Ercolini, D. (2016). Overlap of Spoilage-Associated Microbiota between Meat and the Meat Processing Environment in Small-Scale and Large-Scale Retail Distributions. *Applied and Environmental Microbiology, 82*(13), 4045–4054. https://doi.org/10.1128/AEM.00793-16

Tao, Y., Guo, Y., Li, J., Ye, K., Zhang, Y., Zeng, X., & Dou, H. (2023). Effect of temperature fluctuation during superchilling storage on the microstructure and quality of raw pork. *Meat Science, 198*, Article 109096. https://doi.org/10.1016/j.meatsci.2023.109096

Thung, T. Y., Mahyudin, N. A., Basri, D. F., Wan Mohamed Radzi, C. W. J., Nakaguchi, Y., Nishibuchi, M., & Radu, S. (2016). Prevalence and antibiotic resistance of Salmonella Enteritidis and Salmonella Typhimurium in raw chicken meat at retail markets in Malaysia. *Poultry Science, 95*(8), 1888–1893. https://doi.org/10.3382/ps/pew144

Tian, X., Li, J., Luo, Q., Wang, X., Wang, T., Zhou, D., … Lu, Q. (2022). Effects of Purple Corn Anthocyanin on Growth Performance, Meat Quality, Muscle Antioxidant Status, and Fatty Acid Profiles in Goats. *Foods, 11*(9), 1255. https://doi.org/10.3390/foods11091255

Toomik, E., Rood, L., Bowman, J. P., & Kocharunchitt, C. (2023). Microbial spoilage mechanisms of vacuum-packed lamb meat: A review. *International Journal of Food Microbiology, 387*, Article 110056. https://doi.org/10.1016/j.ijfoodmicro.2022.110056

Wang, B., Wang, Y., Zuo, S., Peng, S., Wang, Z., Zhang, Y., & Luo, H. (2021). Untargeted and Targeted Metabolomics Profiling of Muscle Reveals Enhanced Meat Quality in Artificial Pasture Grazing Tan Lambs via Rescheduling the Rumen Bacterial Community. *Journal of Agricultural and Food Chemistry, 69*(2), 846–858. https://doi.org/10.1021/acs.jafc.0c06427

Wang, S., Zhang, D., Yang, Q., Wen, X., Li, X., Yan, T., & Hou, C. (2024). Effects of different cold chain logistics modes on the quality and bacterial community succession of fresh pork. *Meat Science, 213*, Article 109502. https://doi.org/10.1016/j.meatsci.2024.109502

Wang, X., Jiang, G., Kebreab, E., Li, J., Feng, X., Li, C., … Dai, Q. (2020). ^{1}H NMR-based metabolomics study of breast meat from Pekin and Linwu duck of different ages and relation to meat quality. *Food Research International, 133*, Article 109126. https://doi.org/10.1016/j.foodres.2020.109126

Wang, Z., He, Z., Gan, X., & Li, H. (2018). Interrelationship among ferrous myoglobin, lipid and protein oxidations in rabbit meat during refrigerated and superchilled storage. *Meat Science, 146*, 131–139. https://doi.org/10.1016/j.meatsci.2018.08.006

Xu, C., Zang, M., Qiao, X., Wang, S., Zhao, B., Shi, Y., … Wu, J. (2022). Effects of ultrasound-assisted thawing on lamb meat quality and oxidative stability during refrigerated storage using non-targeted metabolomics. *Ultrasonics Sonochemistry, 90*, Article 106211. https://doi.org/10.1016/j.ultsonch.2022.106211

Yang, L., Dai, B., Ayed, C., & Liu, Y. (2019). Comparing the metabolic profiles of raw and cooked pufferfish (Takifugu flavidus) meat by NMR assessment. *Food Chemistry, 290*, 107–113. https://doi.org/10.1016/j.foodchem.2019.03.128

Yang, P., Zhong, G., Yang, J., Zhao, L., Sun, D., Tian, Y., … Rong, L. (2022). Metagenomic and metabolomic profiling reveals the correlation between the microbiota and flavor compounds and nutrients in fermented sausages. *Food Chemistry, 375*, Article 131645. https://doi.org/10.1016/j.foodchem.2021.131645

Yang, Y., Wu, R., Hu, J., Xing, S., Huang, C., Mi, J., & Liao, X. (2020). Dominant denitrifying bacteria are important hosts of antibiotic resistance genes in pig farm anoxic-oxic wastewater treatment processes. *Environment International, 143*, Article 105897. https://doi.org/10.1016/j.envint.2020.105897

Yu, Q., Pan, H., Shao, H., Qian, C., Han, J., Li, Y., & Lou, Y. (2022). UPLC/MS-based untargeted metabolomics reveals the changes in muscle metabolism of electron beam irradiated Solenocera melantho during refrigerated storage. *Food Chemistry, 367*, Article 130713. https://doi.org/10.1016/j.foodchem.2021.130713

Zhang, R., Pavan, E., Ross, A. B., Deb-Choudhury, S., Dixit, Y., Mungure, T. E., … Farouk, M. M. (2023). Molecular insights into quality and authentication of sheep meat from proteomics and metabolomics. *Journal of Proteomics, 276*, Article 104836. https://doi.org/10.1016/j.jprot.2023.104836

Zhang, R., Yang, M., Hou, X., Hou, R., Wang, L., Shi, L., … Zhang, L. (2022). Characterization and difference of lipids and metabolites from Jianhe White Xiang and Large White pork by high-performance liquid chromatography-tandem mass spectrometry. *Food Research International, 162*(Pt A), Article 111946. https://doi.org/10.1016/j.foodres.2022.111946

Zhang, T., Ding, H., Chen, L., Zhang, S., Wu, P., Xie, K., … Wang, J. (2021). Characterization of chilled chicken spoilage using an integrated microbiome and metabolomics analysis. *Food Research International, 144*, Article 110328. https://doi.org/10.1016/j.foodres.2021.110328

Zhang, X., Han, L., Hou, S., Raza, S. H. A., Gui, L., Sun, S., … Aloufi, B. H. (2022). Metabolomics approach reveals high energy diet improves the quality and enhances the flavor of black Tibetan sheep meat by altering the composition of rumen microbiota. *Frontiers in Nutrition, 9*, Article 915558. https://doi.org/10.3389/fnut.2022.915558

Zhang, X. Q., Jiang, T., Guo, N., Bai, L., & Zhao, D. M. (2020). Analysis of Myoglobin Stability and Bacterial Community Diversity in Mutton Chop Rolls During Cold Preservation. *Current Microbiology, 77*(5), 826–835. https://doi.org/10.1007/s00284-020-01873-z

Zhou, X., Zhou, D. Y., Liu, Z. Y., Yin, F. W., Liu, Z. Q., Li, D. Y., & Shahidi, F. (2019). Hydrolysis and oxidation of lipids in mussel Mytilus edulis during cold storage. *Food Chemistry, 272*, 109–116. https://doi.org/10.1016/j.foodchem.2018.08.019

Zhou, X., Zong, X., Zhang, M., Ge, Q., Qi, J., Liang, J., … Xiong, G. (2021). Effect of konjac glucomannan/carrageenan-based edible emulsion coatings with camellia oil on quality and shelf-life of chicken meat. *International Journal of Biological Macromolecules, 183*, 331–339. https://doi.org/10.1016/j.ijbiomac.2021.04.165

Zhu, W., Han, M., Bu, Y., Li, X., Yi, S., Xu, Y., & Li, J. (2024). Plant polyphenols regulating myoglobin oxidation and color stability in red meat and certain fish: A review. *Critical Reviews in Food Science and Nutrition, 64*(8), 2276–2288. https://doi.org/10.1080/10408398.2022.2122922