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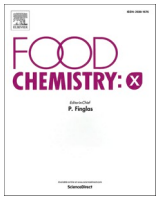

# Integrated lipidomics and microbiomics reveal the quality changes of fresh yak tenderloin during storage

Feiyan Yang [a,1], Xudong He [a,1], Xin Wen [a], Guangfan Qu [a], Hanzhi Zhang [a], Zhang Luo [b], Shuguo Sun [a,*]

[a] National Engineering Laboratory for Deep Process of Rice and Byproducts, Hunan Key Laboratory of Grain-Oil Deep Process and Quality Control, Hunan Key Laboratory of Forestry Edible Resources Safety and Processing, College of Food Science and Engineering, Central South University of Forestry and Technology, Changsha 410004, Hunan, China

[b] College of Food Science, Tibet Agriculture & Animal Husbandry University, Nyingchi 860000, Tibet, China

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ABSTRACT

The changes in lipid and microbial during beef storage exert a substantial impact on the overall quality of beef. In this study, lipidomics and microbiomics were used to evaluate the effects of chilled storage (at 4 °C, CS) and superchilled storage (at −2 °C, SS) on the quality of yak tenderloin. The data revealed that TG, PS, PI, PE, and Car are the key factors contributing to the generation of undesirable odor during the storage of tenderloin. *Macrococcus, Lactobacillus, Myroides,* and *Proteobacteria* directly affect the storage quality of yak tenderloin. Integrated analysis revealed that microbial metabolites interact with lipids, resulting in a deterioration of meat quality. These changes are mediated by *Myroides, Pseudomonas*, and *Lactobacillus*, which regulate fatty oxidation and metabolism of PE, PI, PS, Cer, and SM. These findings have important implications for understanding the changes in quality and microbial activity of refrigerated meat and meat products.

## 1. Introduction

Yaks are the oldest, multifunctional, and dominant livestock species in the Qinghai-Tibet Plateau and adjacent highlands (Xiong et al., 2022). Yak meat is favored by consumers due to its delicious taste and high content of lipids, proteins, vitamins and minerals (Yang et al., 2021). Unfortunately, the high nutritional quality of fresh yak tenderloin leads to its easy spoilage during storage (Sun et al., 2020). The chilled storage and super-chilled storage methods are currently the most widely used methods for preserving freshness (Ramanathan et al., 2023). Although there is a minimal temperature difference between them, the former exhibits significantly superior fresh-keeping efficacy compared to the latter. However, the biological disparities and mechanisms underlying these two storage methods have not been comprehensively elucidated thus far.

The storage of fresh meat can result in the oxidation and degradation of bioactive compounds, such as lipids, proteins, and amino acids, which leads to a decline in nutritional value and sensory attributes (Wang et al., 2022). Different storage temperatures may lead to varying changes in the complex lipids of yak meat, inevitably resulting in different degrees of deterioration in meat quality. The presence of studies also indicates that lipid oxidation can generate free radicals, thereby initiating a cascade of reactions that further exacerbates the degradation of meat quality (Catalá, 2009). Meanwhile, lipids provide a rich source of nutrients for microorganisms, promoting their growth and reproduction. This not only accelerates the spoilage process of yak meat but also may generate harmful substances, posing a threat to human health (O'Keefe, 2016). Therefore, controlling lipid oxidation is key to prolonging the storage period of yak meat. Furthermore, when compared to lipids, proteins demonstrate greater stability in yak meat. Although proteins also undergo degradation and denaturation during storage, this process occurs at a slower rate and has a lesser impact on the quality of the meat. The resistance of proteins to environmental factors is determined by their structure and function (Bhatnagar et al., 2007; Kolhe & Badkar, 2011). Driven by continuous innovation in mass spectrometry and related analysis technology (Zhou et al., 2019), more advanced lipidomics technologies remain the optimal choice for investigating complex lipid changes in yak meat during storage (Xiong et al.,

* Corresponding author at: College of Food Science and Engineering, Central South University of Forestry and Technology, No. 498, Shaoshan Road, Changsha 410004, Hunan, China.
*E-mail address:* sshuguo@163.com (S. Sun).
[1] These authors contributed equally to this work

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2022). Currently, 596 lipids have been identified in fresh yak meat through untargeted lipidomics (Xiong et al., 2022). However, the lipids composition and microorganisms in meat may undergo different changes due to variations in storage temperatures, inevitably resulting in varying degrees of deterioration in meat quality (Sun et al., 2020).

The interaction between spoilage microorganisms and storage temperature is a significant factor contributing to the deterioration of meat lipid quality (Zhang, Duan, et al., 2021). Previous studies have confirmed that different storage temperatures can lead to variations in the types and quantities of spoilage microorganisms on the surface of meat, which leads to varying degrees of decomposition and deterioration of meat lipids (Zhang, Chen, et al., 2021). However, the precise driving mechanism behind meat lipid deterioration resulting from the interaction between spoilage microorganisms and storage temperature remains unclear. The microbial diversity and variation in beef can be evaluated through microbiomics based on high throughput sequencing of 16S rRNA genes, thereby revealing the overall changes in species composition and abundance of spoilage microorganisms during storage at different temperature levels and stages (Li et al., 2019). The impact of spoilage microorganisms on beef lipid metabolism and other macromolecular structure and function necessitates the investigation of microbial diversity and levels on the meat surface at different temperatures and stages of beef storage through microbiomics analysis. As a result, the degradation metabolism of beef lipids and the impact of microorganisms on lipid deterioration in beef during storage at different temperature levels and stages can be better understood through the integration of lipidomics and microbiomics.

The yak tenderloin, being situated within the spinal column of the yak, exhibits relatively limited physical activity, delicate muscle fibers, and enhanced tenderness. Consequently, it serves as an ideal place for investigating variations in meat quality due to its heightened sensitivity towards different storage conditions. Furthermore, tenderloin is abundant in high-quality protein and lipid as well as essential nutrients such as the calcium, iron, zinc. These nutrients may change during storage, thus affecting the storage of tenderloin. Conducting research on yak tenderloin storage technology is beneficial for prolonging its shelf life and enhancing the added value of meat products, thereby increasing economic benefits. In previous studies in our lab, we have determined that the freezing temperature range of yak tenderloin falls between −2.4 °C and − 2.6 °C. Within this specific temperature range, yak meat enters a sub-freezing state which significantly enhances its storage quality and prolongs its shelf life. However, the factors influencing the storage characteristics of yak meat remain unknown (Sun et al., 2020). Additionally, refrigeration is a gentler storage method compared to freezing, which makes it suitable for short-term consumption plans. In this study, an integrated analysis of lipidomics and microbiomics was conducted to comprehensively understand the lipid metabolism of yak tenderloin and the impact of microorganisms on the deterioration of meat during chilled and super-chilled storage. The research also aims to identify the key regulatory pathways of lipid deterioration and discover the critical spoilage microbes involved in this process.

## 2. Materials and methods

### 2.1. Yak tenderloin sample collection

The fresh tenderloin of Tibetan yak after 24 h of the butcher was purchased from six local nature pasture of the species of Sasha Township (93.25 $^{0}$N, 29.89°E) in the Tibet Autonomous Region, China. The details of the a male yak are 400–500 kg, are a normal, healthy, and disease-free individual who is 4 years old. The 200 g samples of tenderloin from the yaks were packaged in sterile bags, placed on ice, and transported to the laboratory within 3 h. For this work, the packaged samples were stored at were stored in the incubator (Compressor-Cooled Incubator ICP260, Memmert, Germany) at −2.0 ± 0.5 °C for 0 d (Sample A), 6 d (Sample B), and 12 d (Sample D); and at 4.0 ± 0.5 °C for 0 d (Sample A), 6 d (Sample C), and 12 d (Sample E), and perform 6 parallel replicates for each group of samples. The lipid and microorganism parameters were analyzed at predetermined time intervals ($n$ = 6 for each time per treatment).

### 2.2. Physical and chemical quality analysis

A sample of 5 g of minced yak meat was mixed with 50 mL of deionize water and homogenized by a homogenizer (XHF-D,Ningbo Scientz Biotechnology Inc., Zhejiang, China) for1 min at 6000 xg. A digital pH meter (pHS—3C, Shanghai Meitele-Tolido Instrument Co. Ltd., China) was utilized to measure the pH of sample homogenate. The TVB-N content of the yak tenderloin samples was measured using semimicro steam distillation with slight modifications (Katsanidis & Zampouni, 2023). POV analysis was determined by International Dairy Federation (IDF) method (Tirani et al., 2022). MDA was measured using the 2-thiobarbituric acid extraction (TBA) method (Katsanidis & Zampouni, 2023). The absorbance at 532 nm was measured spectrophotometrically for each treatment, and the resulting values were quantified using a standard curve generated with MDA concentrations ranging from 8 to 50 nmol. The MDA content was expressed as mg/kg of yak tenderloin.

### 2.3. Lipid extraction

A 20 mg sample of yak tenderloin was placed into an Eppendorf tube and mixed with 120 mL of pre-chilled isopropanol (IPA) buffer. The mixture was thoroughly vortex-mixed for 1 min, followed by a 10-min incubation at room temperature. Subsequently, the sample was stored in an EP tube and placed in a − 20 °C refrigerator overnight to induce precipitation. Afterwards, the sample was centrifuged at 4000 rpm for 20 min at a temperature of 4 °C. The resulting supernatant was transferred into a new 96-well plate and diluted to a ratio of 1:10 with a mixture of isopropyl alcohol (IPA), acetonitrile, and water in the proportion of 2:1:1. Subsequently, equal volumes of each sample were mixed in a new tube to create the quality control sample (QC). The corresponding new EP tube was used to transfer 100 μL of each sample (including QC), followed by adding 300 μL of methanol (pre-cooled at −20 °C for 0.5 h or more). The tubes were finally placed in a refrigerator at −20 °C for 20 min and then centrifuged at 14000 rpm for 20 min under a temperature of 4 °C. The lipid metabolites extraction procedure was performed six times for each sample. All samples were stored at −20 °C before being used.

### 2.4. Lipid analysis

HPLC/MS parameter (UPLC-MS system, 2777C, ACQUITY UPLC CSH C18 column, 100 mm × 2.1 mm internal diameter and 1.7 μm in particle size, Waters Technologies, USA) was performed according to the published methods (Mi et al., 2018). The column temperature was maintained at 55 °C. *mobile* phase A consisted of a mixture of CAN/water (60:40, *v*/v) with 0.1 % formic acid and 10 mM ammonium formate, while mobile phase B comprised IPA: CAN (90:10, v/v) with 0.1 % formic acid and 10 mM ammonium formate. The gradient elution program was executed with the following parameters: 0–0.5 min, 2 % B; 0.5–8.0 min, a linear increase from 2 % to 98 % B; 8.0–9.0 min, hold at 98 % B; 9.0–9.1 min, a linear decrease from 98 % to 2 % B; and finally held at 2 % B for the remaining time of the run (10 min total).

The detection of eluted metabolites from the column was performed using a high-resolution tandem mass spectrometer Triple TOF 5600 plus (SCIEX, UK). Q-TOF/MS analysis was conducted in both positive and negative ion modes under the following conditions: For positive ion mode, the capillary voltage was set at 2000 V, cone voltage at 30 V, collision energy ranged from 19 to 45 eV. The recorded mass range was from 100 to 2000 Da with a dwell time of 50 ms for each analysis. For negative ion mode, the capillary voltage was set at 1500 V, cone voltage

at 30 V, collision energy ranged from 19 to 45 eV. The recorded mass range was from 50 to 2000 Da with a dwell time of also being set as 50 ms for each analysis by IDA for data collection. The process involves implementing real-time quality correction for LE signals every 3 s during data acquisition.

### 2.5. Bioinformatics analysis

Student's *t*-tests, variance analyses, and ROC curve analyses were used for SDL screening according to the published methods with slight modifications (Li et al., 2019). The screening criteria for differential lipids were $p < 0.05$, variable importance in the projection (VIP) score > 1.2, fold change (FC) >2 or < 0.5, and area under the ROC curve (AUC) >0.9. Online databases such as the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database (https://www.kegg.jp/kegg/), comprehensive livestock metabolome database (LMDB, available at http://www.lmdb.ca), LIPID MAPS database (http://www.lipidmaps.org), and PubChem database (https://pubchem.ncbi.nlm.nih.gov) were utilized for metabolite annotation and exploration of lipid biosynthesis and biodegradation pathways. All the experiments were replicated at six times.

### 2.6. DNA extraction, polymerase chain reaction (PCR) and sequencing

To assess the microbial diversity, total DNA was extracted from each individual sample. At the same time, DNA was quantified using NanoDrop, and the quality of DNA extraction was assessed through 1.2 % agarose gel electrophoresis. The PCR amplification utilizes PFU high-fidelity DNA polymerase and carefully controls the number of amplification cycles to minimize them while ensuring consistent amplification conditions for each batch of samples. The reaction product was purified using Vazyme VAHTSTM DNA Clean Beads and quantified with the Quant-iT PicoGreen dsDNA Assay Kit using a Microplate reader (BioTek, Flx800). The TruSeq Nano DNA LT Library Prep Kit from Illumina was utilized to prepare the sequencing library, which was subsequently subjected to double-terminal sequencing on the Illumina MiSeq/NovaSeq platform.

### 2.7. Microbiological analysis

The raw sequencing data was merged and statistically screened for the length distribution of high-quality sequences contained in all samples using QIIME2 (2019.4) and R language scripts to obtain valid sequences. The remaining valid sequences are clustered with 100 % similarity using the DADA2 method after chimera detection. The species annotation is performed using the Naive Bayes classifier with default parameters in the QIIME2 software for each ASV's feature sequence or each OTU's representative sequence. To minimize differences in sequencing depth among different samples, the leveling depth is set to 95 % of the minimum sample sequence volume using the Qiime feature-table rarefy function. The sparse curve was calculated using QIIME2 (2019.4) and the R ggplot2 package, including Chao1 and Good's coverage for characterizing coverage as well as other α diversity measures, reflecting the complexity of samples at different storage time points. The OTU-based system clustering utilizes the hidden state prediction algorithm in Castor, employs PICRUSt2, HUMAnN2, R scripts, ggplot2 packages, and other statistical packages. The functional genes of the community were predicted using the reference genomic data included in the software, and their biological functions were annotated based on MetaCyc and KEGG pathways.

### 2.8. Statistical analysis

The SIMCA 15 software package (Umetrics, Umea, Sweden) was used for the identification and analysis of lipid metabolism through principal component analysis (PCA), orthogonal partial least squares-discriminant analysis (OPLS-DA), VIP value calculation, permutation tests of OPLS-DA, and volcano plots. Cytoscape 3.7.0 (Seattle, WA, USA) was used for the analysis of correlation networks. The web-based tool MetaboAnalyst (http://www.metaboanalyst.ca) was utilized to analyze ROC curves, hierarchical clustering, and lipid metabolism pathways. Quality data was analyzed using one-way ANOVA with SPSS19 software (IBM, United States). The least significant difference (LSD) test was used to assess the significance of differences between the means at a significance level of $p < 0.05$.

## 3. Results

### 3.1. Changes of TVB-N, pH and lipid oxidation index during CS and SS

The POV, MDA, pH, and TVB-N values of meat is closely related to its freshness. The lipid oxidation characteristics of yak tenderloin during SS and CS storage, including POV, MDA, pH, and TVB-N levels, are summarized in Table 1. The levels of POV and MDA of yak tenderloin gradually increased with the prolongation of storage time, and CS exhibited a significantly higher level than SS ($p < 0.05$). These findings suggested that CS can expedite lipid oxidation of yak tenderloin and lead to the formation of rancidity products. The TVB-N content demonstrated a gradual increase with the extension of storage time, and the CS at 12 d exhibited a significantly higher value compared to that of SS ($p < 0.05$). Compared to the slow increase in pH during CS, the rapid and significant increase in pH during SS may be attributed to the inhibition effects of microorganisms and enzymes that decompose proteins into basic substances such as ammonia, amines, and other nitrogen-containing compounds (Balamatsia et al., 2007). Therefore, CS is more likely to accelerate meat deterioration.

### 3.2. The changes in lipid profiles of yak tenderloin during CS and SS

A total of 388 lipids were identified and classified into 9 subclasses in yak tenderloin samples during CS and SS, including 18 carnitines (Car), 33 SMs, 4 Cers, 84 TGs, 2 MGs, 203 PCs, 38 PEs, as well as 3 PIs and PSs each (Fig. 1A). The sum of all lipids in the same subclass was considered as the evaluation of the relative abundance of each subclass. Although the number of TG (84) was lower than the number of PC (203), TG accounted for the highest proportion due to its content (43.33 %) among all lipids in fresh yak tenderloin sample (Fig. 1A and B). The changes in lipid profiles of yak tenderloin during SS were significantly lower than

**Table 1**
Lipid oxidation characteristics and TVB-N value of fresh yak tenderloin during CS and SS.

| Traits | storage (°C) | Storage(day) 6d | 12d | SEM 0d |
|---|---|---|---|---|
| POV (g/100 g) | -2 | $0.32 \pm 0.03^{Bbc}$ | $0.36 \pm 0.03^{Bbc}$ | $0.30 \pm 0.03^{c}$ |
| | 4 | $0.38 \pm 0.02^{Aab}$ | $0.51 \pm 0.03^{Aa}$ | |
| MDA (nmol/mg prot) | -2 | $0.58 \pm 0.03^{Bbc}$ | $0.60 \pm 0.03^{Bbc}$ | $0.53 \pm 0.03^{c}$ |
| | 4 | $0.66 \pm 0.03^{Aab}$ | $0.74 \pm 0.03^{Aa}$ | |
| pH | -2 | $5.71 \pm 0.02^{a}$ | $5.84 \pm 0.10^{a}$ | $5.71 \pm 0.07^{a}$ |
| | 4 | $5.75 \pm 0.09^{a}$ | $5.98 \pm 0.08^{a}$ | |
| TVB-N (mg/100 g) | -2 | $8.27 \pm 0.31^{Bb}$ | $9.60 \pm 0.30^{Bb}$ | $6.30 \pm 0.44^{c}$ |
| | 4 | $9.20 \pm 0.56^{Ab}$ | $12.83 \pm 0.48^{Aa}$ | |

A–B Means within a column with different superscript differ significantly at $p < 0.05$. $^{a-c}$ Means within a row with different superscript differ significantly at $p < 0.05$.

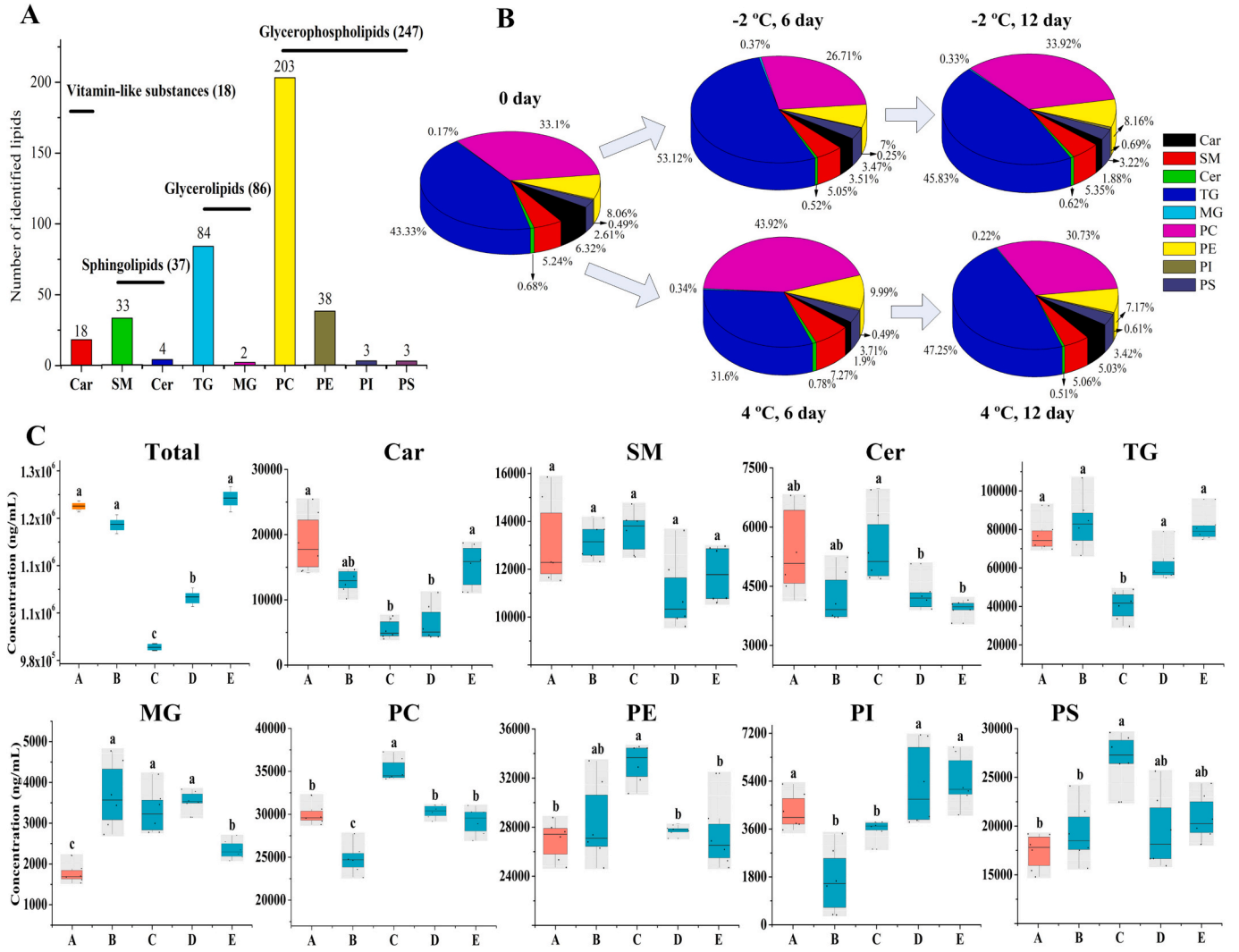



**Fig. 1.** Changes in lipid profiles of yak tenderloin during CS and SS. (**A:** total lipid content of each subclass, **B:** proportion of lipids of each subclass during CS and SS, C: change of lipid content of each subclass during CS and SS).

those during CS ($P < 0.05$) (Fig. 1B). The concentrations of total lipids in yak tenderloin showed a decreasing trend during SS, while they exhibited a pattern of drastic decrease followed by a sharp increase during CS (Fig. 1C). The changes in total lipid concentrations of yak tenderloin during cold storage were primarily attributed to variations content of TG, Car, SM, PE and PC.

### 3.3. Identification of SDLs in yak tenderloin during CS and SS

The supervised PLS-DA approach was employed to discriminate the lipidomic patterns of yak tenderloin during CS and SS, with a few parameters indicating the validity of the method. The results indicated that $R^2Y(cum) > 0.98$ and $Q^2(cum) > 0.84$, suggesting that a more comprehensive analysis of the variables significantly contributes to sample classification and confirms the reliability, effectiveness, and absence of overfitting in the model (Fig. 2A). The lipid profile of yak tenderloin underwent changes during SS, with a total of 20 lipids showing alterations, including 8 Cars, 9 PCs, 2 SMs, and 1 MG. During CS, a significant change was observed in the lipid composition of yak tenderloin, with a total of 60 lipids exhibiting alterations, including 37 TGs, 14 Cars, 8 PCs, and 1 MG. Notably, there were shared specific differential lipids (SDLs) identified in both CS and SS samples of yak tenderloin, and these SDLs included 8 Cars, 1 PC, and 1 MG (Fig. 2B~2E).

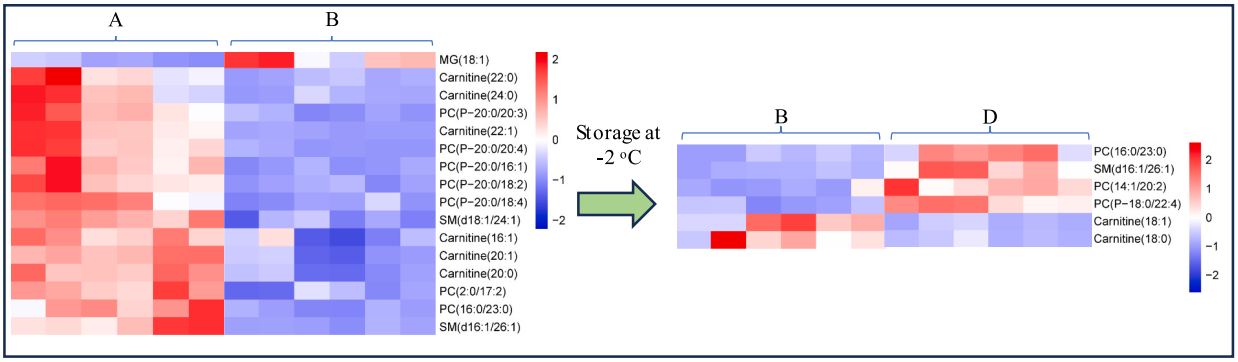
### 3.4. Correlation network analysis of SDLs in yak tenderloin during CS and SS

To evaluate the effects of CS and SS on yak tenderloin lipids, we conducted an unweighted correlation network analysis of 70 SDLs, which could potentially reveal the interactions among different lipid species and classes. The number and intensity of interactions among SDLs, as well as their changes, could reflect the effects of CS and SS on yak tenderloin lipids. Most lipids exhibited a high degree of correlation, with stronger interactions observed among lipids within the same class compared to those between different classes (Fig. 3). During 6 d under SS condition, only 66 significant correlations (B vs. A) ($p < 1e\text{-}3$) were found in yak tenderloin. Cars and PCs formed a closely-knit cluster with other SDLs (Fig. 3A). As storage time increased, a total of 2390 significant correlations (D vs. B) were detected ($p < 1e\text{-}3$) in SS condition, with TGs, Cars, and PCs forming a dense cluster with other SDLs (Fig. 3B). The yak tenderloin stored for 6 days under CS condition exhibited a total of 614 significant correlations (C vs. A) ($p < 1e\text{-}3$), including dense clusters formed by TGs and Cars with other SDLs (Fig. 3C). Additionally, as the storage time increased, a total of 2031 significant correlations (E vs. C) ($p < 1e\text{-}3$) in CS condition, with TGs, Cars, PEs, and PCs forming dense clusters alongside other SDLs (Fig. 3D).

I

| Storage temperature | Mode | Comparison | P-value $R^2$ | P-value $Q^2$ | $R^2$ | $Q^2$ |
|---|---|---|---|---|---|---|
| -2 °C | pos | B/A | 0 | 0.005 | 0.996 | 0.935 |
| | pos | D/B | 0 | 0 | 0.988 | 0.842 |
| | neg | B/A | 0 | 0 | 0.995 | 0.897 |
| | neg | D/B | 0 | 0.005 | 0.986 | 0.886 |
| 4 °C | pos | C/A | 0 | 0 | 0.994 | 0.890 |
| | pos | E/C | 0 | 0 | 0.995 | 0.935 |
| | neg | C/A | 0 | 0 | 0.996 | 0.871 |
| | neg | E/C | 0 | 0 | 0.996 | 0.915 |

II




III

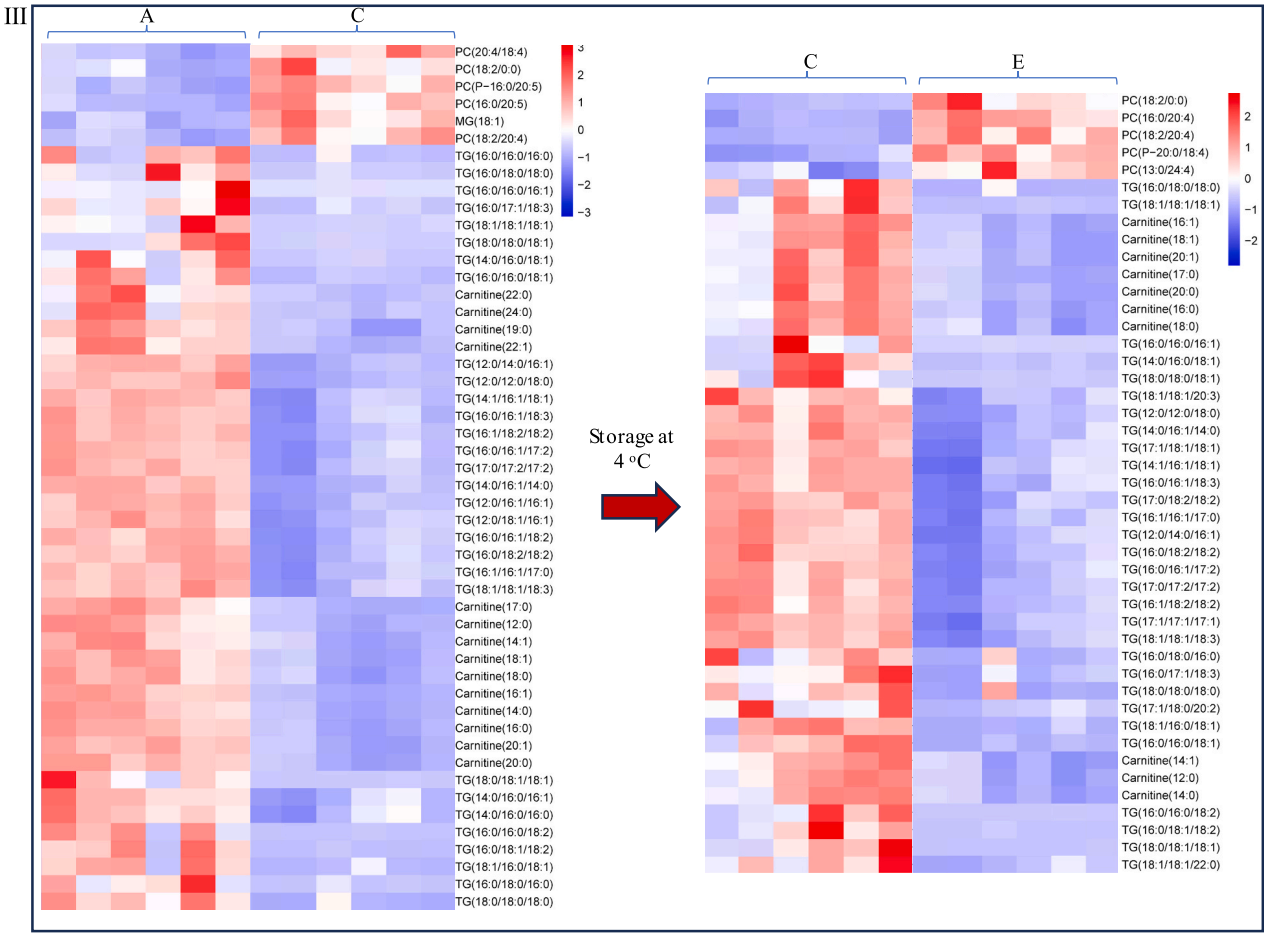



**Fig. 2.** SDLs in yak tenderloin during CS and SS. I: reflect the effectiveness of the PLS-DA method. II and III: represent the relative abundance heat maps of 70 SDLs identified during CS and SS of yak tenderloin).

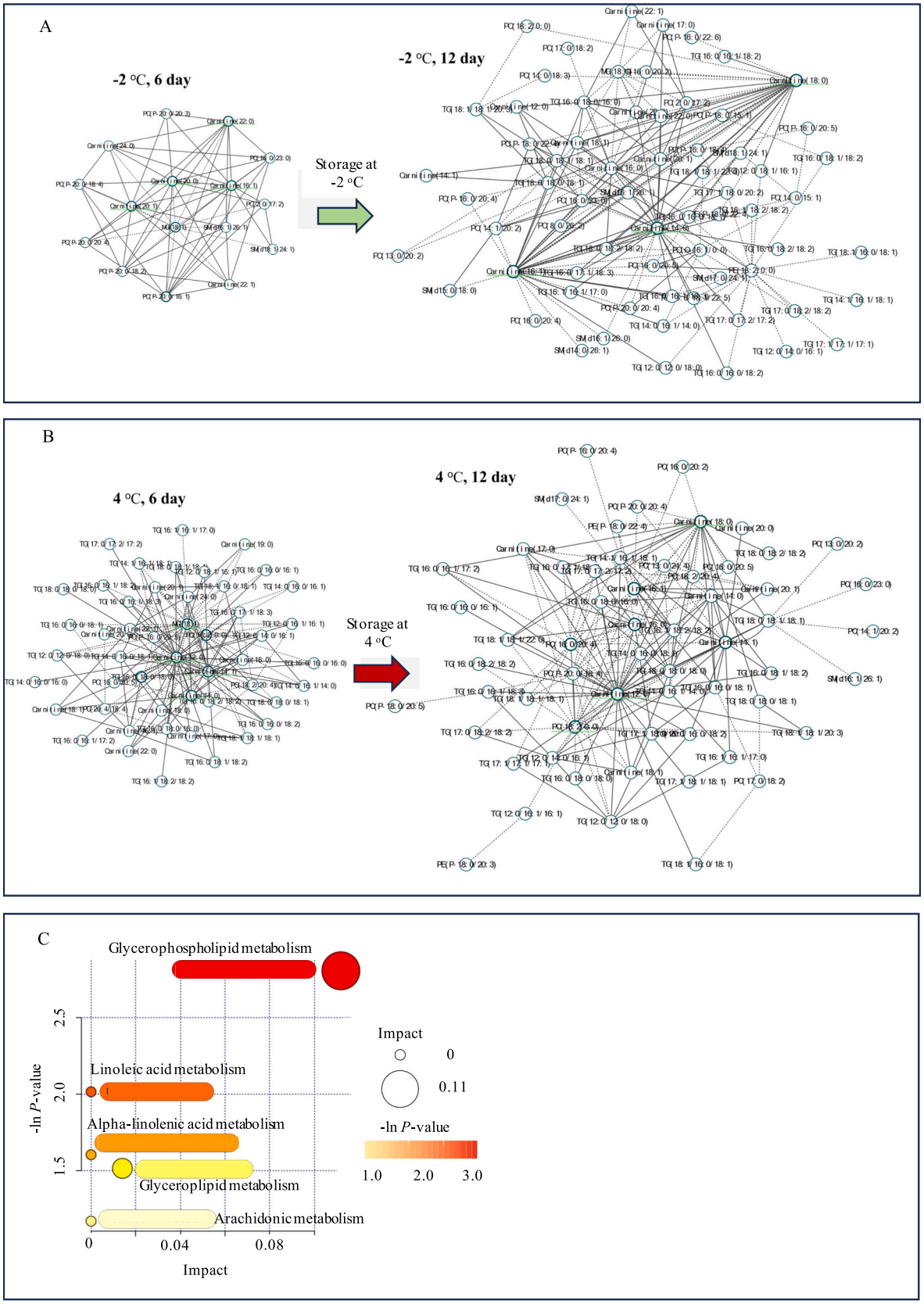



**Fig. 3.** Correlation network analysis of SDLs in yak tenderloin during CS and SS (A and B). Top 5 most relevant SDL pathways (B).

### 3.5. Alterations in metabolic pathways of SDLs

To assess whether the storage temperature affects the metabolism of yak tenderloin lipids, 70 SDLs were mapped to the KEGG, HMDB, and PubChem databases. The search for 70 SDLs against the KEGG pathway database resulted in the identification of a total of 95 metabolic pathways. The effects of these SDLs were further investigated through a pathway analysis conducted using MetaboAnalyst 4.0 software. The findings of this study revealed the identification of the five most pertinent pathways, namely glycerophospholipid metabolism, linoleic acid metabolism, alpha-linolenic acid metabolism, glycerolipid metabolism, and arachidonic acid metabolism (Fig. 3C). Among these, glycerophospholipid and linoleic acid metabolism were found to be the most significantly relevant metabolic pathways, followed by alpha-linolenic acid metabolism.

### 3.6. Microbiomics identification and analysis

The high-throughput sequencing obtained a total of 72,640, 77,424, 76,956, 74,511, 76,993 and 96,201, 85,190, 97,553, 128,115, 125,809 effective sequences. The alpha diversity, read number, and operational taxonomic units (OTUs) were analyzed (Table 2). It is worth noting that the coverage rates of all samples exceeded 99.95 %. Additionally, the Chao1 and Shannon sparse curves exhibited a gradual and consistent trend across all samples, indicating the effective detection of microbial diversity in yak tenderloin during CS and SS. Through analyzing the diversity of individual microbial species (phylum and genus) during CS and SS, a total of 38 microbial phyla, including over 411 microbial genera, were identified. Ascomycota and Firmicutes emerged as the two most predominant phyla. Furthermore, a comparison was made at the genus level between the microbiota composition of yak tenderloin samples during during CS and SS for storage 12 d. The data showed that the main genera are *Candida, Pseudomonas, Macrococcus, Myroides, Lactobacillus, Acinetobacter, Debaryomyce, Psychrobacter, Lactococcus,* and *Kocuria* (Fig. 4). Further analysis found that *Candida, Pseudomonas, Macrococcus, Myroides and Lactobacillus* were the dominant fungal species during both CS and SS. During CS, the bacterial dominance was characterized by the succession from *Macrococcus* (36.46 %) to *Pseudomonas* (97.42 %) (Fig. 4). The relative abundance of these genera exceeded 90 % when stored for 12 d. Under CS conditions, there was a significant increase in the relative abundance of *Candida*, reaching its peak at 96.62 % on day 12 and subsequently stabilizing around 96 %. In contrast, under SS conditions, there was an opposite trend observed in the relative abundance of *Candida*, with the lowest value recorded on day 12 (87.08 %) (Fig. 4). *Pseudomonas* exhibited the same trend under both CS and SS conditions, with its relative abundance increasing over time, and the relative abundance reached a high of 97.42 % at CS for 12 d (Fig. 4).

Our data revealed a diverse microbial community comprising several genera, including *Candida, Pseudomonas, Macrococcus, Myroides, Lactobacillus, Acinetobacter, Debaryomyces, Psychrobacter, Lactococcus,* and *Kocuria* (Fig. 4). Further analysis highlighted that *Candida, Pseudomonas, Macrococcus, Myroides,* and *Lactobacillus* were the dominant fungal species throughout both CS and SS phases. These findings suggest a dynamic shift in microbial dominance from bacterial to fungal populations under the influence of storage conditions. To elucidate the ecological roles and temperature adaptations of these predominant genera, we categorized them based on their optimal growth temperatures: psychrophiles (thriving at <15 °C), mesophiles (optimal growth between 20 and 45 °C), and thermophiles (growing best above 45 °C). Our assessment indicates that among the identified genera, *Psychrobacter* stands out as a prominent psychrophile. Its presence during CS suggests an adaptation to cold environments, potentially playing a role in maintaining microbial activity at low temperatures. The majority of the dominant genera containing mesophiles, such as *Candida, Pseudomonas, Macrococcus, Myroides,* and *Lactobacillus* are classified as mesophiles. Their prevalence across both CS and SS stages implies versatility in temperature adaptation, enabling sustained metabolic activity within the typical temperature range encountered during these storage periods. While none of the primary genera are strict thermophiles based on our current taxonomic classification, it is important to note that some species within these genera may exhibit thermotolerant characteristics, contributing to their resilience under varying thermal conditions. The transition from bacterial dominance during CS to a more balanced or potentially fungal-dominated ecosystem during SS could be attributed to several factors, including changes in temperature, nutrient availability, and competitive interactions among microbial species. Specifically, the initial bacterial proliferation under cold storage conditions might create a niche for subsequent fungal growth as environmental parameters shift towards those favoring fungi, particularly under shelf stability conditions where temperature moderates and other factors like humidity and oxygen levels become more conducive to fungal metabolism. In conclusion, our study not only identifies key microbial players but also highlights the intricate interplay between microbial physiology and environmental factors such as temperature. Future work will delve deeper into the functional roles of these organisms and explore strategies to manage or harness their activities for improved food safety and quality during storage.

### 3.7. Comprehensive analysis reveals the relationship between lipids, microbes, and quality of yak tenderloin

Further analysis the relationship among qualities, microorganisms and lipids of yak tenderloin during CS and SS. 10 genera include *Candida, Pseudomonas, Macrococcus, Myroides, Lactobacillus, Acinetobacter, Lactococcus, Debaryomyce, Psychrobacter, Kocuria* as well as 8 types of differential lipids were selected to explore the relationship with POV, TVB-N, pH and MDA. The correlation coefficient analysis revealed a strong and statistically significant association among these lipids, microorganisms, and the quality of yak tenderloin (Fig. 5 A). Correlation cluster network analysis shows a significant positive correlation between *Pseudomonas, Macrococcus, Myroides*, and *Lactobacillus* with lipid (TG, PS, PC, PI) content and storage quality of yak tenderloin (POV, TVB-N, pH and MDA). On the other hand, *Lactococcus* and *Kocuria* exhibit a significant negative correlation with them (Fig. 5B). A visualization analysis was conducted to investigate the correlation between

**Table 2**
Analysis of microbial diversity of yak tenderloin during CS and SS.

| Sample group | Bacteria | | | | Fungus | | | |
|---|---|---|---|---|---|---|---|---|
| | OTUS | Chao1 | Shannon | Goods_coverage | OTUS | Chao1 | Shannon | Goods_coverage |
| 0d | 532.2 | 534.46 | 3.5545 | 0.9997 | 95 | 98.732 | 2.5436 | 0.9999 |
| 6d_-2 °C | 241.25 | 245.3 | 3.2796 | 0.9996 | 39.1 | 40.727 | 0.5196 | 0.9999 |
| 12d_-2 °C | 180.4 | 183.33 | 2.6543 | 0.9998 | 48.95 | 49.766 | 0.421 | 1 |
| 6d_4°C | 798.1 | 800.07 | 4.4795 | 0.9997 | 39.55 | 40.918 | 0.4752 | 1 |
| 12d_4°C | 103.9 | 106.3 | 2.6191 | 0.9998 | 21.95 | 21.95 | 0.666 | 1 |

Operational taxon (OTUS) is the operating unit of classification; diversity index includes Chao for species richness, Shannon is defined as diversity index, and Goods_coverage is calculated by QIIME software at 97 % similarity level.

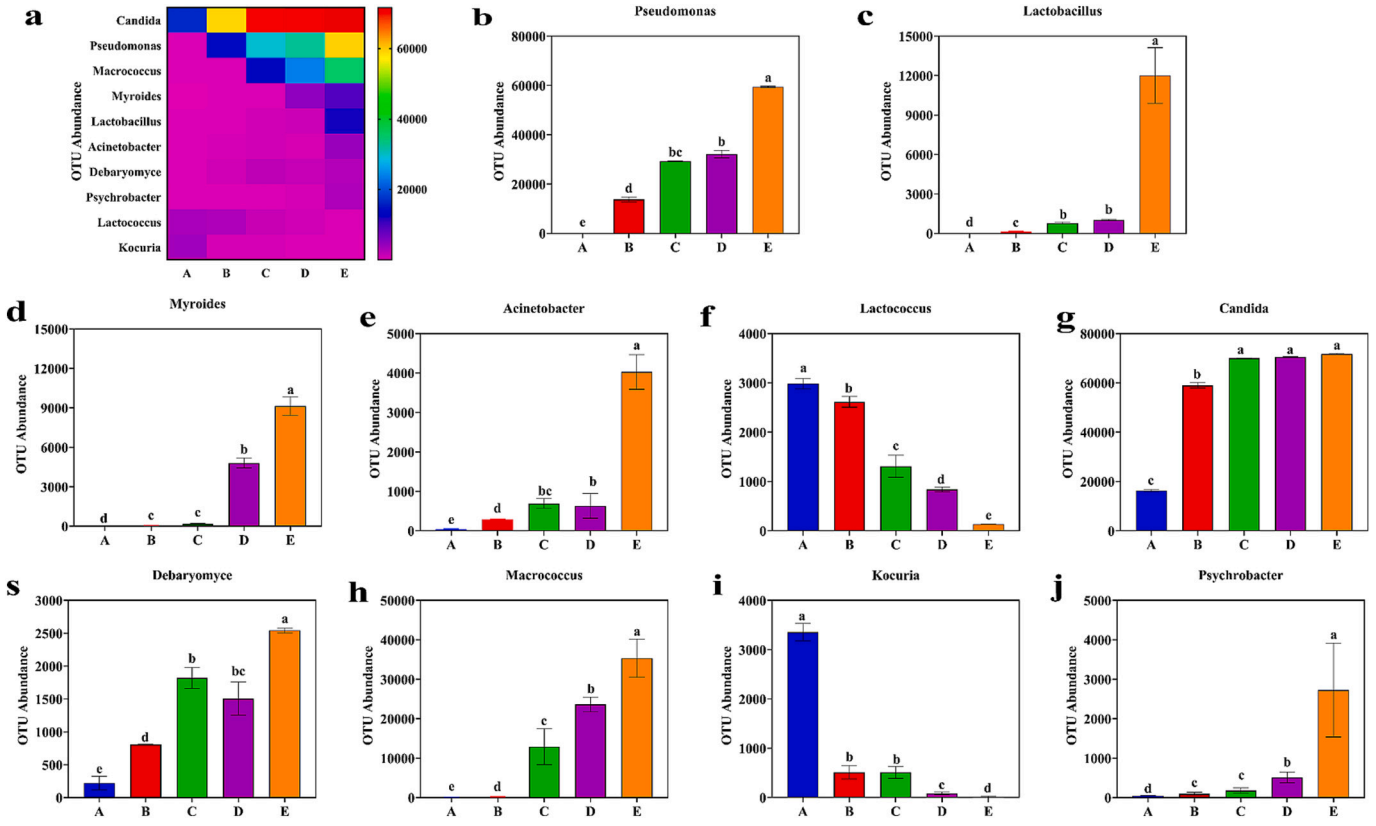



**Fig. 4.** A Relative abundance of the first ten microorganisms (key microbial) in yak tenderloin during CS and SS.

key lipids (Cer, PI, PS, PE) and microorganisms (*Pseudomonas, Macrococcus, Myroides*, and *Lactobacillus*), as well as MDA and TVB-N. Further analysis revealed a significant positive correlation between the genus *Macrococcus* and *Myroides* with PI (correlation coefficients of 0.660 and 0.568 respectively) (Fig. 5C), it indicates that *Macrococcus* and *Myroides* may regulate the decomposition or synthesis of PI in yaks during storage, resulting in the accumulation of MDA and TVB-N contents, thus leading to the deterioration of yak tenderloin. The KEGG pathway was selected as the carrier for comprehensive analysis based on liposome and microbiology data. The results showed that the lipid composition was closely related to microorganisms in yak tenderloin, and those changes were involved in glycerolipid metabolism, linolenic metabolism, arachidonate acid metabolism, sphingolipid metabolism, oxidative phosphorylation and glyceropgospholipid metabolism pathway. Meanwhile, we also observed pronounced alterations in the abundance of *Macrococcus* and *Myroides* genera, as well as changes in lipid PI, MDA, and TVB-N levels during chill storage of yak tenderloin. Therefore, super-chill storage may inhibit the oxidative decomposition of lipid in yak tenderloin by inhibiting the growth of microorganisms, thus maintaining its freshness.

## 4. Discussion

Beef is a complex and heterogeneous meat that contains thousands of lipids. The composition, types, and changes of lipids in meat during storage can reflect its quality characteristics to a certain extent. However, only a limited number of lipids or lipid classes have been identified and investigated during the storage of meat, leaving numerous low-abundance lipids and their alterations unexplored. Our results contribute to a deeper comprehension of the lipid composition, varieties, and alterations in yak tenderloin during storage.

Different lipids found in beef include PC, PE, PS, PI, SM, Car, and TG. Among them, TG, SM, PC, PE and Car are the main lipids that change during in beef storage. Low temperature storage is widely used in meat and meat products to extend their shelf life. However, the occurrence of phospholipid oxidation is highly susceptible during storage periods. The oxidation process is influenced by various factors such as temperature, humidity, storage conditions, and microorganisms. In this study, the levels of POV, TVB-N, pH, and other oxidation indexes in yak tenderloin increased with the extension of storage time, indicating the occurrence of oxidation. The fat content and fatty acid composition have been identified as significant factors that influence the processes of lipolysis and lipid oxidation. A study found that due to the highly oxidative properties of TG, the free fatty acids they break down into under the action of hydrolytic enzymes have a significant impact on the flavor of meat products (Zhou et al., 2021). Hydrolases include lipases that hydrolyze triglycerides into long-chain fatty acids, esterases that hydrolyze triglycerides into short-chain fatty acids, and phospholipases that hydrolyze phospholipids (Toldrá, 2012). The lipolysis of different lipid molecules is independent and primarily catalyzed by acid lipase, neutral lipase, and phospholipase enzymes. These enzymes originate from both endogenous muscle tissue and microorganisms (Feng et al., 2021). Exogenous lipases in meat products are typically derived from environmental and inoculated microorganisms. The lipids also exhibit a high affinity for reactants in condition of environment humidity were higher, thereby increasing the likelihood of their impact on the flavor and nutritional composition of meat products (Zhang et al., 2008; Zhou et al., 2021). The most variable lipid is phospholipid, which undergoes rapid degradation during hydrolysis and serves as the primary source of liberated fatty acids (Jin et al., 2010). Phospholipase is primarily responsible for the hydrolysis of phospholipids, which are classified into A1, A2, C, and D based on the ester bonds they undergo hydrolysis. The relative activities of phospholipase A2 and phospholipase C were highly correlated with the increases in free fatty acids and neutral lipids in meat, respectively (Wang et al., 2014). Some studies have shown that the main cause of muscle lipid oxidation is autoxidation promoted by

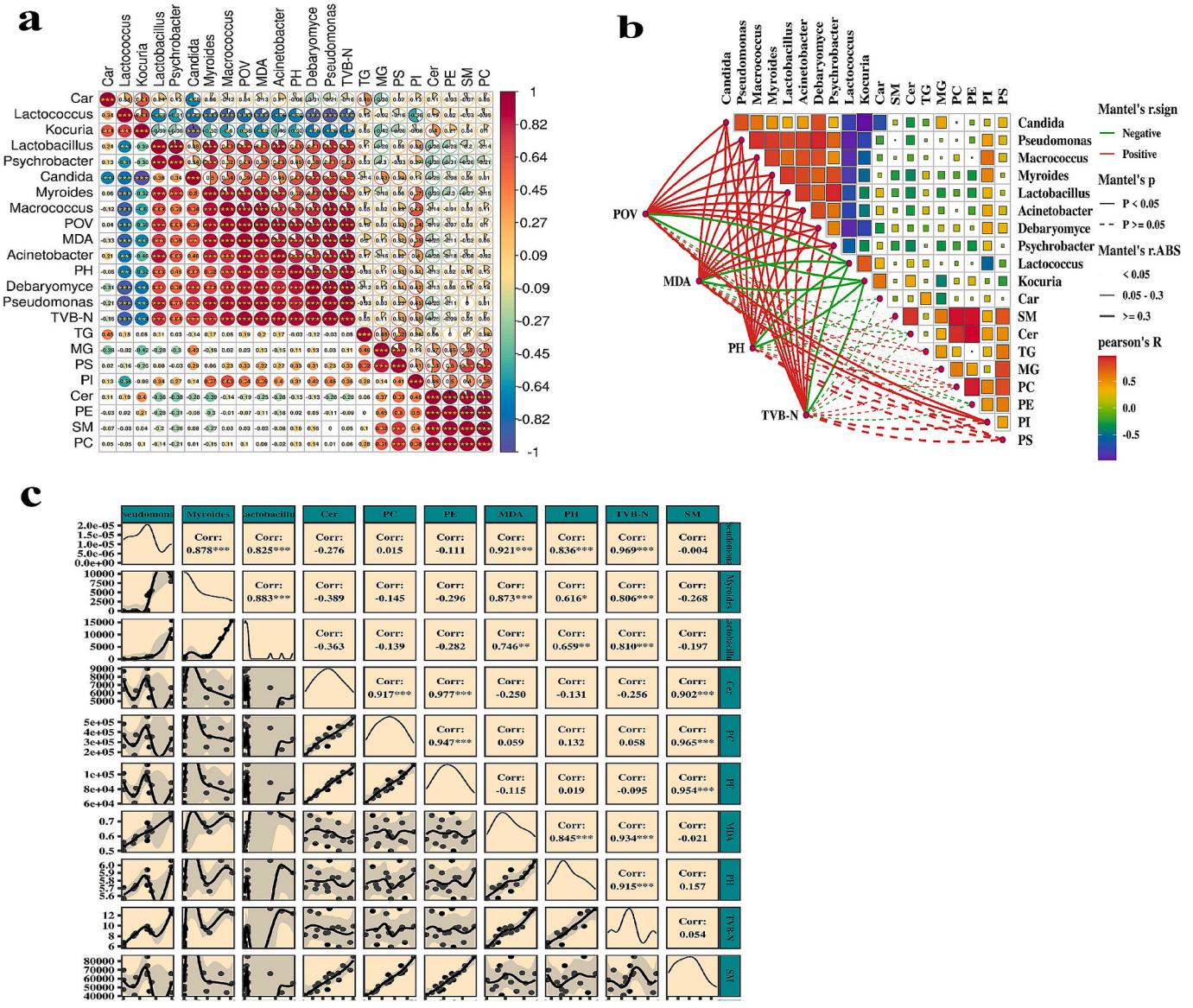



**Fig. 5.** Correlation analysis among differential lipids, microorganisms with quality of yak tenderloin. **a:** The correlation coefficient between lipids, key microorganisms and quality of yak tenderloin (POV, MDA, pH, TVB-N). **b:** The correlation network analysis between lipids, key microorganisms and quality of yak tenderloin (POV, MDA, pH, TVB-N). **c:** The visual analysis of correlation matrices of key lipids (Cer, PC, PE, SM), core microorganisms (*Pseudomonas, Myroides, Lactobacillus*) and quality of yak tenderloin (POV, MDA, pH, TVB-N).

phospholipid hydrolysis, while others have found little relationship between lipolysis and lipid oxidation (Tatiyaborworntham et al., 2022). Phospholipids serve as crucial constituents of cellular membranes also contribute to the lipid content found in meat. Upon oxidation, phospholipids generate a range of secondary byproducts, including aldehydes, ketones, alcohols, and esters (Wang et al., 2013). These secondary products react with proteins and amino acids in meat, forming new compounds (Schiff bases, MDA, ketoimines or ketoimide compounds, esters) that have the potential to alter the flavor of meat products and sometimes produce unpleasant odors (Mahachi et al., 2023). Such as hexaldehyde and octyl aldehyde, exhibit a potent odor and undergo reaction with amino acids to form Schiff bases. This chemical transformation alters the protein structure, potentially leading to changes in meat quality and the development of "rancid" or "sour" odors in meat products (Lorido et al., 2016). The presence of acetone and butanone have the potential to interact with proteins present in meat, leading to the formation of novel protein derivatives that could potentially alter both the texture and taste of the meat (Birks & Kelly, 1997). Furthermore, primarily catalyzed by lipase, the degradation of phospholipids in meat leads to an increase in POV and MDA levels, consequently resulting in the production of undesirable flavor compounds such as rancidity, sourness, greasiness, and other unpleasant flavors (Pikul et al., 1984;). Henceforth, it is imperative to exercise control over TG oxidation in meat and meat products.

According to different reaction conditions, the oxidation pathways of fatty acids can be classified into autoxidation, enzymatic oxidation, and photosensitive oxidation. Autoxidation is an important mechanism of lipid oxidation, including the initiation, transfer, and termination of three stages. In particular, the free radical chain reaction plays a crucial role (Mariutti & Bragagnolo, 2017). At low temperatures, lipid oxidation may occur even though microbial growth and reproduction are inhibited. This is because unsaturated fatty acids in lipids are susceptible to attacks by free radicals, which trigger chain reactions of free radicals. In this process, an initial free radical (such as the superoxide anion radical or hydroxyl radical) attacks the unsaturated bond in a lipid molecule, forming a new free radical and lipid peroxide. This new free radical then

proceeds to attack other lipid molecules, creating more free radicals and peroxides, leading to continued oxidation of lipids (Baron et al., 2007). Previous studies have also shown that the levels of PUFA, EPA, and DHA decrease as lipid oxidation progresses during frozen storage of sardines (Chaijan et al., 2006). A decrease in PUFA may indicate the occurrence of autooxidation, as this spontaneous oxidation process involves unsaturated lipids and oxygen without light and catalyst (Zhou et al., 2019). In addition, there are enzymes in muscle that can catalyze lipid oxidation, such as lipoxygenase (LOX) and myeloperoxidase. The secondary oxidation product of MDA and POV, LOX, still exhibits certain activity at low temperatures and can facilitate the process of lipid oxidation. For example, lipoxygenase can catalyze unsaturated fatty acids to produce primary oxidation products called hydroperoxides, which are then further broken down into compounds such as aldehydes and ketones (Fu et al., 2022). Other studies have also demonstrated that the lipoxygenase activity in mussel meat and tilapia increases with refrigeration time, contributing to oxidation, which aligns with our findings. Therefore, autooxidation and enzyme-catalyzed oxidation can potentially collaborate in facilitating the process of oxidation during refrigeration. At the same time, water is also a necessary condition for microbial growth, and microbial activities may indirectly promote lipid oxidation as well. A similar increase in lipid oxidation has been reported during frozen storage (Refsgaard et al., 2000), and storage at higher freezing temperatures leads to rancidity and an increased release of free fatty acids (Carballo et al., 2019).

The link between microbial reproduction and the chemical changes that occur during meat storage is considered a potential means of identifying indicators for meat quality or freshness (Nychas et al., 2008). We found that *Proteus* and *Firmicutes* are the key microbial taxa at the phylum level under both CS and SS, with the most intense variation in abundance observed in CS. Previous studies have consistently reported *Firmicutes* and *Proteobacteria* as the dominant phyla associated with meat deterioration (Büyüksırıt Bedir & Kuleaşan, 2021). The *Lactobacillus* in *Firmicutes* is capable of fermenting carbohydrates in meat, resulting in the production of lactic acid, which can increase the acidity of the meat and impact its flavor and preservation (Friedrich et al., 2008). Meanwhile, certain members of the *Firmicutes*, specifically *Clostridium* species, exhibit a remarkable ability to thrive in anaerobic environments, leading to the production of malodorous compounds and gases such as carbon dioxide and hydrogen sulfide. These chemical constituents can induce swelling and generate unpleasant odors in vacuum-packaged meat products (Dai et al., 2023). Additionally, certain bacteria belonging to the *Proteobacteria* phylum, such as *Escherichia coli* and *Salmonella*, are prevalent foodborne pathogens that significantly increase the risk of meat contamination. *Proteobacteria* species also can produce pigments and enzymes that may adversely affect the color, texture, appearance, and flavor of meat (Dai et al., 2023). Our results confirmed that changes in *Lactobacillus* and *Proteobacteria* after 12 d of refrigeration were associated with beef spoilage.

The association between temperature and microbial activity is widely recognized, and under refrigerated conditions, cryophilic bacteria such as *Lactobacillus* and *Pseudomonas* have a propensity to dominate meat products (Stanborough et al., 2017). Therefore, it is reasonable to find the dominance of *Pseudomonas* in the microflora during storage. The data we obtained indicated a decrease in *Pseudomonas* levels in SS, and compared to CS, beef storage 12 d at SS demonstrated superior preservation, which is consistent with previous studies (Gupta et al., 2004). As the storage process progresses, changes in the structure of bacterial communities result in the production of metabolites that affect lipids within the same system, leading to a deterioration in meat quality (Ben Akacha et al., 2023). *Lactobacillus* and *Pseudomonas* have been confirmed as the predominant spoilage microorganisms derived from raw meat (Aguilar et al., 2021). In this study, as the storage time increased, *Lactobacillus* exhibited a lower relative abundance in SS compared to CS. This resulted in a reduction of sour taste and an increase in pH. The correlation analysis has confirmed a negative relationship between the pH value and *Lactobacillus*, specifically in the SS process. This suggests that SS hinders the synthesis of organic acids, particularly lactic acid, which provides a fresh perspective on rancidity in beef storage. The metabolites produced by certain microorganisms facilitate the degradation of fats, while *Pseudomonas*, which contains the lipase enzyme, can enhance the process of fat oxidation (Ferrer et al., 2001). The genus *Pseudomonas* is a prominent group of bacteria responsible for the spoilage of fresh food during cold storage conditions. *Pseudomonas* exhibits robust metabolic activity and possesses the ability to secrete diverse protein enzymes and lipases, facilitating the breakdown of proteins and fats in meat. Additionally, they release various primary/secondary metabolites such as ketones, volatile fatty acids, hydrogen sulfide, amines, and acetic acid. These metabolites induce oxidative processes and the development of rancidity in meat, thereby impacting its flavor, color, and texture (Altakhis et al., 2021). Our data suggest a similar phenomenon in beef, suggesting that the inhibition of *Lactobacillus* and *Pseudomonas* are key indicators for beef storage.

The notable reduction in Car concentration observed during SS could potentially be linked to changes in the abundance of *Lactobacillus* and *Acinetobacter*. Previous studies have indicated that *Lactobacillus* strains obtained from meat products exhibit lipase-producing abilities, and their presence can facilitate fat hydrolysis, resulting in the accumulation of fatty acids and subsequent degradation of Car (Suganthi et al., 2013). Conversely, the increase in MDA levels may be associated with the down-regulation of both Car and *Lactobacillus* (Suganthi et al., 2013). Furthermore, the elevated content of PI may be associated with a reduction in *Myroides* abundance. *Myroides* has been reported to produce serine hydrolase enzymes, which may have a detrimental impact on the storage quality of meat, as it enzymatically breaks down proteins in meat, resulting in texture softening and potential mucus formation, thereby affecting the sensory attributes of meat (Suganthi et al., 2013). Microbiomics data also showed that the main spoilage microorganism *Pseudomonas aeruginosa* could grow under aerobic conditions using Car as the sole source of carbon and nitrogen. The bacterium *Pseudomonas aeruginosa* A7244 utilizes an inducible active carrier transport system to absorb Car, which is subsequently degraded into glycine, contributing to the flavorful substance of meat (Strack et al., 1962). The results of our study demonstrated a negative correlation between the duration of storage in CS and the abundance of *Pseudomonas*, leading to a reduction in Car content. Previous studies have demonstrated the presence of a complete Car biosynthesis pathway in *Candida* (Strijbis et al., 2009). Additionally, the presence of Car acetyltransferase has been confirmed in *Candida tropicalis*, while a decrease in the abundance of *Candida* was observed during CS (Jung et al., 1993). The abundance of *Pseudomonas*, a microorganism associated with spoilage, exhibited a significant increase in CS compared to storage at SS. *Pseudomonas* was found to be significantly correlated with PI and PS. Specifically, *Pseudomonas* can produce key enzymes such as PISD, PLA2G, LYPLA1, CKI1, PCYT1, CPT1, LCAT, GPCPD1 and GDE1 to regulate the metabolism of PE, PI and PS (Sun et al., 2020). The implementation of CS, therefore, facilitated the proliferation of Pseudomonas bacteria, resulting in the degradation of PE into ethanolamin, and produce fishy and stench (Altakhis et al., 2021). Another research has shown that *Pseudomonas* can produce high concentrations of proteins and polysaccharides in the biofilm state, enhancing the bacteria's self-aggregation ability and surface hydrophobicity (Cherny & Sauer, 2020). Additionally, it may also promote yak meat spoilage by secreting enzymatic substances to utilize the phospholipids in meat as a nutrient source, thereby further promoting biofilm formation and maintenance. This enzymatic breakdown significantly compromises the nutritional integrity of meat. Our results show that where PE degradation during CS led to an increase in POV and TVB-N, and a positive correlation is observed between ethanolamine levels and indicators such as POV, pH, and TVB-N. This finding was also confirmed during the refrigeration of herrings and black rockfish (Zhao et al., 2020). The comprehensive results showed that the synergistic

action of temperature and spoilage microorganisms was not conducive to the storage of yak tenderloin.

Another result reveals that SM, which serves as the pivotal biosynthetic pathway of Cer, exhibits a decrease during CS. A research indicated that the increase in MDA levels may be associated with the reduction in SM, which is consistent with the findings of this investigation (Soloviev et al., 2005). At the same time, we found that the *Myroides* and *Lactobacillus* could produce enzymes (such as SMaseD, CERK, and SPH) to regulate Cer metabolism (Lew et al., 2013). Interestingly, its abundance initially increased but subsequently decreased during SS, which contradicted the observed change in Cer concentration. Specifically, SMaseD and CERK are enzymes that facilitate the degradation of SM and Cer into Ceramide-P. Additionally, sphingomyelinase (SMase) is a crucial enzyme in sphingomyelin metabolism as it converts SM into Cer and phosphocholine (Ueda et al., 2022). During the initial stage of SS, the accumulation of *Lactobacillus* may enhance Cer degradation, while the reduction in *Lactobacillus* during cold storage results in an increase in pH value. It has been reported that the presence of *Lactobacillus plantarum* leads to the most rapid decrease in pH levels in fermented dough (Gaglio et al., 2018). In summary, this study elucidates the potential correlation between ceramide metabolism regulation and the observed decrease in SM levels and increase in MDA levels during cold storage, while the modulation of ceramide metabolism by *Lactobacillus*-produced enzymes is implicated in this process. These findings have significant implications for comprehending the quality alterations and microbial activity of refrigerated meat and meat products.

## 5. Conclusion

This study elucidated the diversity and dynamic changes of lipids and microbial communities in yak beef during SS and CS, while also analyzing the impact of temperature and microorganisms on lipids for the first time. During the entire storage process, a total of 388 lipid species and 411 microbial genera were identified. The quality of meat is influenced by TG, SM, PI, PS, PE, and Car as the key factors. During the storage of yak tenderloin, these lipids undergo oxidation or hydrolysis, leading to a deterioration in meat quality and the generation of undesirable odor. *Proteus* and *Firmicutes* are the main bacterial communities, in which *Candida, Lactobacillus, Myroides, Macrococcus* and *Pseudomonas* have a direct impact on the storage quality of yak tenderloin. These bacteria dominate under refrigerated conditions and affect the preservation effect of yak tenderloin. The study also found that microbial metabolites interact with lipids, leading to a decline in meat quality. Specific microorganisms such as *Myroides* can promote fatty hydrolysis, while *Pseudomonas* can produce proteases related to regulate the metabolism of PE and PI, PS, and affect the flavor of yak tenderloin. Furthermore, the enzymes produced by *Lactobacillus* can regulate the metabolism of Cer and SM, promoting an increase in MDA levels in yak tenderloin during storage and subsequently affecting the lipid composition and oxidation of yak tenderloin. In conclusion, lipid oxidation, hydrolysis and microbial activity during storage are the main factors leading to the deterioration of yak tenderloin quality. Controlling these biochemical and microbial processes is crucial for improving the quality of meat storage and extending shelf life. These findings have important implications for understanding the changes in quality and microbial activity of refrigerated meat and meat products.

## CRediT authorship contribution statement

**Feiyan Yang:** Writing – original draft, Methodology, Investigation. **Xudong He:** Investigation, Data curation. **Xin Wen:** Validation, Methodology, Investigation. **Guangfan Qu:** Software, Methodology, Investigation. **Hanzhi Zhang:** Methodology, Investigation. **Zhang Luo:** Resources, Project administration. **Shuguo Sun:** Writing – review & editing, Supervision, Funding acquisition.

## Declaration of competing interest

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

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## Data availability

Data will be made available on request.

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