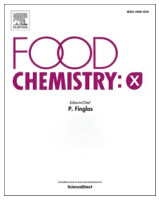
Food Chemistry: X 24 (2024) 101889

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



TMT-based quantitative proteomics reveals the effects of electromagnetic field and freezing preservation techniques on mutton quality

Xing Lei [a,b], Wei Su [a,b,*], Rongmei Zhou [a,b], Yingchun Mu [a,b]

[a] School of Wine and Food Engineering, Guizhou University, Guiyang 550025, China
[b] Key Laboratory of Agricultural and Livestock Product Storage and Processing Technology of Guizhou Province, Guiyang 550025, China

ARTICLE INFO

Keywords:
Mutton
Preservation
Proteomics
Differentially expressed proteins:
Bioinformatics

ABSTRACT

This study investigated the effects of electromagnetic field preservation (EP) and freezing storage (FS) on the quality of northern Qianbei Ma mutton. Using tandem mass tagging (TMT)-labeled quantitative proteomics and bioinformatics, it was observed that EP more effectively inhibited pH increase and maintained a* and b* values compared to FS. Furthermore, the EP group was able to better maintain the water-holding capacity and tenderness of the mutton under prolonged storage. Proteomics analysis identified 397 differentially expressed proteins (DEPs) between the two storage methods at the same storage duration. GO and KEGG enrichment analyses indicated that proteins such as A0A452DSW4, A0A452E8M7, and D3JYV6 were involved in energy metabolism and redox processes, while A0A452EJ66, A0A452DSW4, and A0A452FJE8 played significant roles in protein binding. Overall, EP technology demonstrated superior benefits for maintaining mutton quality, suggesting a novel approach for mutton preservation.

1. Introduction

The Qianbei Ma sheep, native to the Guizhou Plateau region, are renowned for their impressive size, robust physique, and exquisite flavor. This dual-purpose breed provides both meat and skin and is notable for its adaptability, high fertility, genetic stability, and disease resistance. However, fresh mutton has a relatively short shelf life, making it prone to spoilage and pathogenic microorganisms. The most common preservation method for fresh mutton is low-temperature freezing, which effectively inhibits microbial proliferation and slows down chemical and enzymatic reactions. Low-temperature preservation techniques are classified based on storage temperature and include refrigeration, ice temperature, micro-frozen, and freezing storage (Chen et al., 2020). While these methods suppress microbial activity and mitigate meat drying to some extent, their limited storage periods lead to rapid quality deterioration. This short shelf life diminishes the commercial value of the product and fails to fully satisfy consumer demand for longer-lasting meat products.

In recent years, electromagnetic field (EMF) preservation technology has emerged as a novel method for food preservation. The fundamental principle involves the generation of low-frequency (50–60 Hz) electrostatic waves through the EMF preservation machine, causing the water molecules in the food to resonate at a smaller scale, influencing the freezing of water and the formation of fine ice crystals. Consequently, a subcooling state of −3 °C can be achieved, which prevents freezing and the formation of larger ice crystals (Kaur & Kumar, 2020). Additionally, the EMF provides space for electronic energy to inhibit the redox reaction in food, extending the food preservation period. EMF preservation is safe and efficient, potentially addressing the shortcomings of existing technologies, and holds broad application prospects. Recent studies have demonstrated that electric field-based preservation techniques yield positive results across various food products. For example, high-voltage electric field treatment of mackerel inhibits bacterial growth and reduces total volatile salt nitrogen (Bai et al., 2015). Additionally, high-voltage electrostatic field treatment of chilled pork improves solubility and decreases the hardness of myofibrillar proteins (Xu et al., 2023). Furthermore, very low-frequency pulsed electric field treatment of frozen tilapia slows down color changes, reduces protein oxidation and denaturation, and results in a tighter texture of the samples (Wang et al., 2022).

Protein is a fundamental component of meat quality and plays a pivotal role in regulating physiological and biochemical processes within cells and tissues. Alterations in protein content, transformation, degradation, and the peptide substances produced following

* Corresponding author at: School of Wine and Food Engineering, Guizhou University, Guiyang 550025, China.
E-mail address: suwei1886@163.com (W. Su).

https://doi.org/10.1016/j.fochx.2024.101889
Received 11 July 2024; Received in revised form 7 October 2024; Accepted 10 October 2024
Available online 11 October 2024

degradation, in addition to the post-translational modification processes that occur in post-slaughter sheep carcasses, all affect a series of physiological and biochemical reactions. These include muscle signal transduction, glycolytic reactions, and apoptosis. These reaction pathways lead to changes in protein composition, protein-related properties, cellular localization, stability, and activity, ultimately affecting meat quality. Nowadays, proteomics is widely used as a powerful genomic tool in meat science research, greatly contributing to the understanding of complex biological mechanisms and quality-related biomarkers of meat (Kiyimba et al., 2021). The in-depth study of proteomics allows us to comprehend muscle physiological and biochemical processes from the perspective of protein molecules. With the advancement of omics technologies, traditional gel proteomics methods, which often suffer from low sensitivity and high throughput limitations, are increasingly insufficient for analyzing modern complex biological samples. In recent years, TMT-based quantitative proteomics had attracted much attention, and Ma et al. (2020) found that TMT quantitative proteomics was more suitable for analyzing thousands of proteins in complex biological samples because of its high technical reproducibility, high proteome coverage, and more reliable peptide identification and quantitative analysis. Leveraging these advantages, TMT quantitative proteomics technology has been widely employed to explore differentially expressed proteins in Beijing black pigs with varying meat quality (Hou et al., 2020); to compare protein profile differences between Tibetan and Yorkshire pig tenderloins (Wang et al., 2021); and to characterize muscle-specific proteomic changes in beef during the early post-mortem period (Zhai et al., 2020). However, these studies have mainly focused on the differences in protein expression between different meat varieties and qualities, and relatively few studies have investigated the mechanisms of quality changes during meat storage.

This study investigates the impact of electromagnetic field (EP) and freezing storage (FS) on the quality of mutton from the Longissimus thoracis et lumborum muscle of Qianbei Ma sheep. It explores the physical indices and protein mechanisms affecting mutton quality under these two preservation and storage processes. Changes in mutton quality over storage periods are analyzed through a protein-centric approach, aiming to identify novel technologies for mutton and other meat product preservation.

2. Materials and methods

2.1. Sample preparation

Three Qianbei Ma sheep with similar physical conditions and healthy development were selected from Xishui Shunlong Livestock Slaughtering Co., Ltd. in Guizhou Province, China. To ensure consistency, samples were obtained from the Longissimus thoracis et lumborum muscle of each sheep. After slaughter, the muscle samples were transported to the laboratory in insulated, refrigerated containers. Excess fat and connective tissues were carefully removed under aseptic conditions. Following the experimental design, the mutton was randomly divided into 12 portions (approximately 100 g each) and vacuum-packed in sterile nylon/EVOH/PE composite bags. For the EP (EMF preservation) and FS (freezing storage) experiments, the samples of the EP group were placed in the EMF preservation device. The parameters of the equipment were: power supply: 220 V, power: <0.5 W/m^3, output current: ≤ 10 mA, output voltage: ≤ 3000 V, EMF BX3000, EMF voltage values of 2000 and 3000 V, space voltage values of 50–200 V, receiving space of 25–30 m^2, and a temperature of -3 °C to 0 °C (cold storage provided by Shanghai Taste Foods Co., Ltd. in China). For the FS group, the samples were placed in a refrigerator at -20 °C, and for determination, samples were taken out and placed at 4 °C for 12 h. Two groups of samples from the 0th, 15th, 30th, and 45th days of storage were taken for physicochemical indicators and proteomics analysis using a random sampling method. Three samples from each group were used for each measurement, and the average value was recorded.

2.2. Physicochemical indicators of mutton

To assess the quality characteristics of mutton based on pH value, meat color, water-holding capacity (WHC), and shear force (SF) analysis, the following procedures were employed:

pH analysis: 2 g of churned mutton sample was weighed, added with 18 mL of 0.9 % sodium chloride solution, stirred on a magnetic stirrer for 30 min, and then determined using a pH meter (PHS-3C, Shanghai Yue Ping Scientific Instrument Co). The pH meter was calibrated with buffers of pH 4.00 and 6.86 before measurement. Three parallels were measured simultaneously, and the results were averaged.

Meat color analysis: Mutton samples were placed under a light source, exposed for 20 min, and color values (Lab*) were measured using a colorimeter (NH300, Shenzhen Senses Time Technology) with D65 light source, 10° observation angle, 8 mm measurement area diameter, and 50 mm illumination area diameter. The colorimeter was calibrated with a white standard.

WHC: Determined using a modified pressure method as described by by Zhang et al. (2021). Meat samples were cut and weighed using an analytical balance (OHAUS Instrument) with a sensitivity of 0.0001 g. After applying a 35 kg pressure and shaking the samples for 5 min, they were reweighed. The WHC was calculated by eq. (1).

$$\text{WHC}\ (\%) = \frac{m_1}{m_0} \times 100 \tag{1}$$

Where: m_0 is the initial weight of the sample (g), m_1 is the weight of the sample after pressure release (g).

SF determination (Schmidt et al., 2013): Mutton samples were placed in a high-temperature cooking bag with a 75 °C water bath for 25 min, then removed and cooled to room temperature. The samples were cut into 10 mm × 10 mm × 10 mm pieces along the parallel direction of the muscle fibers. They were then sheared along the perpendicular direction of the muscle fibers using a digital muscle tenderness tester (C-LM3B, Tianxiang Feidu Instrumentation Co., Ltd.), and the SF values of the samples were recorded (kg*f).

2.3. TMT-labeled quantitative proteomic technology

2.3.1. Protein extraction

The mutton samples of FS and EP were crushed and homogenized in 0.5 mL of radioimmunoprecipitation assay buffer (25 mmolLexp.-1 Tris-HCl, 1 % SDS, 1 % sodium deoxycholate, 15 mmolLexp.-1 NaCl, pH 7.6) for 2 min. The lysates were centrifuged at 16,061*g* for 15 min, and the supernatants were collected. Quantitative analysis of protein was performed by the BCA method.

2.3.2. Digestion and TMT labeling

In 100 μL protein solution, added 200 μL pre-cooled 80 % acetone for precipitation twice, and centrifuged at 16,061*g* for 10 min. Added 100 μL protein resuspension solution, and sonicated in a water bath for 3 min. Added Dithiothreitol (DTT) to a final concentration of 5 mmolLexp.-1, and incubated at 55 °C with shaking for 20 min. The sample was cooled to room temperature, iodoacetamide (IAA) was added to a final concentration of 15 mmolLexp.-1, and reacted in the dark for 30 min to alkylate the reduced disulfide bonds. Trypsin (Promega) was mixed with the sample at a ratio of 1:50 (Trypsin: Protein). After a brief centrifugation, the mixture was shaken and incubated at 37 °C and 71.5 g overnight. The sample was centrifuged at high speed for 10 min, and an equal amount of protein was transferred to a new Eppendorf tube. The TMT labeling kit (Rockford, Thermo) was used for labeling according to the instructions.

2.3.3. High pH-HPLC classification and Nanolc-MS/MS analysis

Freeze-dried peptide samples were reconstituted in 50 μL of 10 mmolLexp.-1 ammonium acetate solution. RPUPLC was employed for separation under alkaline conditions using a 150 mm × 2.1 mm column

(XBridge BEH C18 XP Column, Waters). The mobile phase consisted of 10 mmolLexp.-1 ammonium acetate for phase A and 10 mmolLexp.-1 ammonium acetate, 10 % H2O, and 90 % ACN for phase B. Twelve fractions were collected at 1-min intervals, combined, and freeze-dried for storage at −80 °C.

For nanoLC-MS/MS analysis, the method of Zheng et al. (2022) was followed with minor modifications. 2 μg of total peptides were separated and analyzed using a nano-UPLC (Thermo) coupled to a Q Exactive HFX Orbitrap instrument (Thermo) with a nano-electrospray ion source. A reversed-phase column (100 μm ID × 15 cm) and mobile phases: H2O (LCMS, ANPEL Laboratory Technologies (Shanghai) Inc) with 0.1 % FA (formic acid, Sigma-Aldrich), 2 % ACN (Acetonitrile, ANPEL Laboratory Technologies (Shanghai) Inc) (phase A), and 80 % ACN, 0.1 % FA (phase B) were utilized. A 90-min gradient was executed at a 300 nL/min flow rate. Data-dependent acquisition (DDA) was conducted in profile and positive mode with an Orbitrap analyzer. MS1 resolution was set to 120,000 (@200 *m/z*) and m/z range of 350–1600; for MS2, resolution was set to 45 k, NCE of 32 %, and an isolation window of 0.7 m/z. The automatic gain control target for MS1 was set to 3E6 with a max IT of 30 ms, and 1E5 for MS2 with a max IT of 96 ms. The top 20 most intense ions were fragmented by HCD with a normalized collision energy of 32 % and an isolation window of 0.7 m/z. The dynamic exclusion time window was 45 s, single-charged peaks and peaks with a charge exceeding 6 were excluded from the DDA procedure.

2.3.4. Database search & quantification

The vendor's raw MS files were processed using Proteome Discoverer (PD) software (Version 2.4.0.305) and the built-in Sequest HT search engine. MS spectra lists were searched against their species-level UniProt FASTA databases (uniprot-Caprinae-9963-2022-10. fasta), with Carbamidomethyl [C], TMT 6 plex (K), and TMT 6 plex (N-term) as fixed modifications, and Oxidation (M) and Acetyl (Protein Nterm) as variable modifications. Trypsin was used as the protease. A maximum of 2 missed cleavages was allowed. The false discovery rate (FDR) was set to 0.01 for both PSM and peptide levels. Peptide identification was performed with an initial precursor mass deviation of up to 10 ppm and a fragment mass deviation of 0.02 Da. Unique peptides and Razor peptides were used for protein quantification, and total peptide amount was used for normalization. All other parameters were kept as default. DEPs were screened based on a significance level of $P < 0.05$ and a fold change (FC) threshold of ≥ 1.4 (up-regulation) or ≤ 0.72 (down-regulation). Relevant data have been saved to Mendeley Data, V1, doi: 10.17632/jrjzny3bzd.1.

2.3.5. Bioinformatics analysis

The UniProt database (https://www.uniprot.org/) and NCBI database (https://www.ncbi.nlm.nih.gov/) were employed for protein searching and functional annotation. The Gene Ontology (GO) database and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database were utilized for gene ontology and pathway annotation.

2.4. Statistical analysis

Results were expressed as mean ± standard error. Each experiment was replicated three times. One-way analysis of variance (ANOVA) and Duncan's statistical test were conducted using SPSS 26 software, with significance set at $P < 0.05$. Principal component analysis (PCA) was performed using SIMCA 14.1 software. The remaining graphs were plotted using Origin 2022 software.

3. Results and discussion

3.1. Changes in the pH of mutton during storage

The pH value is a critical factor affecting the quality and shelf life of meat under various storage methods. As illustrated in Fig. 1, the pH of mutton exhibited a gradual increase during storage for both the EP and FS groups. This increase can be attributed to the decomposition of proteins and nitrogenous compounds by microorganisms and endogenous enzymes, which generate alkaline substances, thus elevating the pH (Toomik et al., 2023). However, the pH increase in the FS.

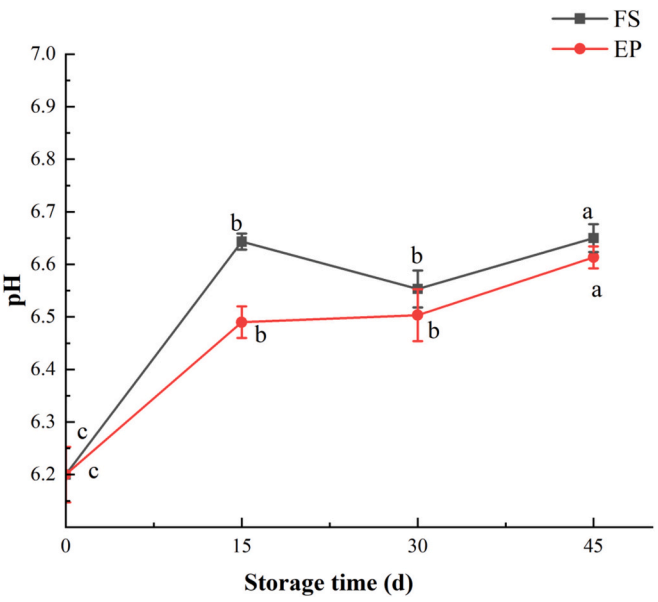


Fig. 1. The changes in pH values of mutton during storage.
Note: a-e indicates that the difference between different storage times of the same storage method is significant ($P < 0.05$), the same as below.

group was significantly higher than that in the EP group during the early storage period (0–15 days). This difference is likely due to the inhibitory effect of EMF on microorganisms (Seyfali et al., 2024). The EP group was effective in inhibiting microbial and endogenous enzyme activity in mutton, whereas freezing at −20 °C could completely inhibit cellular metabolic activity. Freezing is known to be an effective method for microbial inactivation (Mohammed et al., 2021). However, the higher pH in the FS group during the pre-storage period may be attributed to the time required for the samples to freeze completely. Overall, the EP group demonstrated superior capability in suppressing the pH increase compared to the FS group.

3.2. Changes in the color of mutton during storage

Meat color is a key indicator of meat quality and often influences consumers' willingness to buy. Fig. 2 clearly shows that the storage method significantly impacts the color difference value of mutton. The initial L* value of mutton was 44.16 ± 1.61. There was an overall decreasing trend in the L* value in both the FS and EP groups, indicating that the surface brightness of the mutton gradually decreased during storage. This decline is likely due to the oxidation of surface tissues, resulting in a darkening of the meat color and a loss of luster, consistent with the observations made by Vieira et al. (2022). The L* values decreased to varying degrees during storage under both conditions. However, the EP group exhibited a more pronounced decrease in the initial stages, potentially due to the impact of EMF on the surface pigmentation of the mutton (Bocker & Silva, 2022), resulting in darkening of the meat color. This could also reflect the loss due to dripping, with the EP group showing a more consistent color, likely because the ice crystals formed by the mutton were smaller under EMF conditions, leading to lower drip loss compared to freezing conditions.

The present study revealed an overall trend in a* values, which initially decreased, subsequently increased, and then exhibited a gradual decline. The initial decrease in a* observed during the initial storage period may be attributed to myoglobin oxidation, resulting in

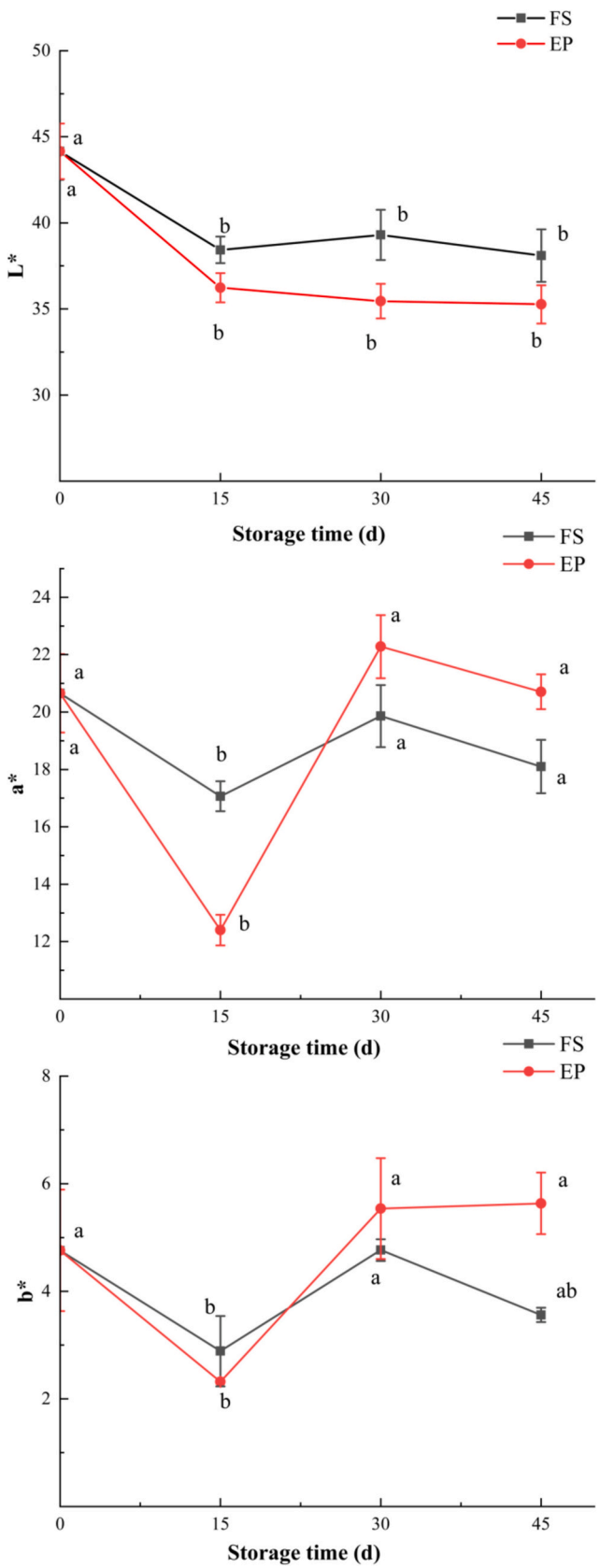


Fig. 2. The changes of color difference value (L*, a*, b*) of mutton during storage.

reduced a* values for both FS and EP, to 17.07 ± 0.53 and 12.40 ± 0.53, respectively. In the initial stages of storage, EMF may have hindered the formation of high-iron myoglobin by influencing the oxidation state of the iron atoms within the hemoglobin moiety, thereby reducing the oxidation of oxymyoglobin to high-iron myoglobin. This resulted in a more pronounced decline in the a* value. The generation of ozone on the surface of mutton (Wei et al., 2016) may have affected myoglobin through oxidation, resulting in the formation of oxygenated myoglobin, which enhanced meat color. At the later stage of storage (30–45 days), the a* value decreased, indicating that the meat color began to brown and the quality of the mutton began to deteriorate. The b* values exhibited a trend similar to that of the a* values, consistent with the findings of Esmer et al. (2011). Overall, the EP group demonstrated a superior ability to maintain the redness and yellowness values of the mutton.

3.3. Changes in the WHC of mutton during storage

The trend of WHC change in mutton is illustrated in Fig. 3. Generally, from 0 to 45 days, irrespective of the storage method, the WHC of mutton exhibited a notable decline ($P < 0.05$) compared to the initial value. At day 45, the WHC decreased by 12.89 % in the FS group and 8.94 % in the EP group. While the decrease in the EP group was less than that in the FS group, there was no significant difference in the change in WHC between the two groups during the storage process. It can be concluded that, irrespective of the storage method, the proteins in mutton underwent denaturation over time, resulting in less organized muscle tissue and a reduced water retention capacity. This led to a significant decrease in WHC during thawing. However, EMF reduced the average ice crystal size in freezing meat (Mahato et al., 2023), mitigating the decrease in WHC during thawing and resulting in the final WHC of the EP group being higher than that of the FS group.

3.4. Changes in shear force of mutton during storage

In evaluating the palatability of mutton, tenderness is a crucial quality indicator. The degree of tenderness is typically reflected by changes in SF, with a reduction in SF indicating greater tenderness in the meat. As illustrated in Fig. 4, the FS group exhibited a notable elevation in SF throughout the storage period, reaching a 298.6 % increase ($P <$ 0.05). This finding indicates that the freezing storage method had a

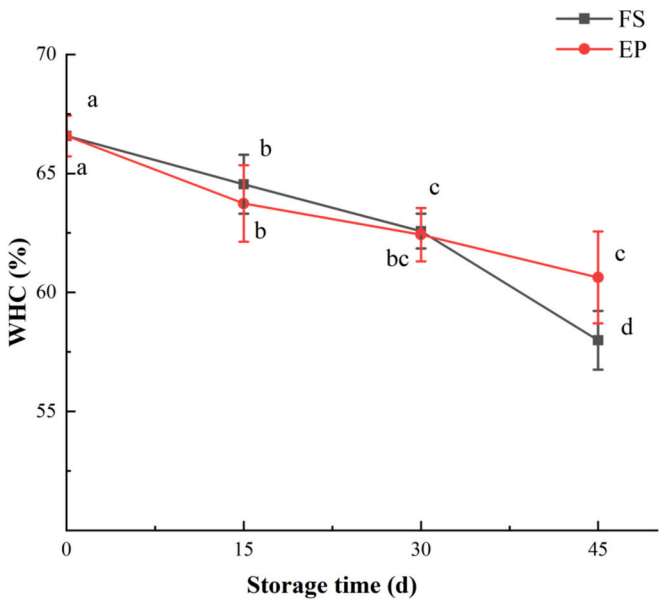


Fig. 3. The changes in WHC of mutton during storage.

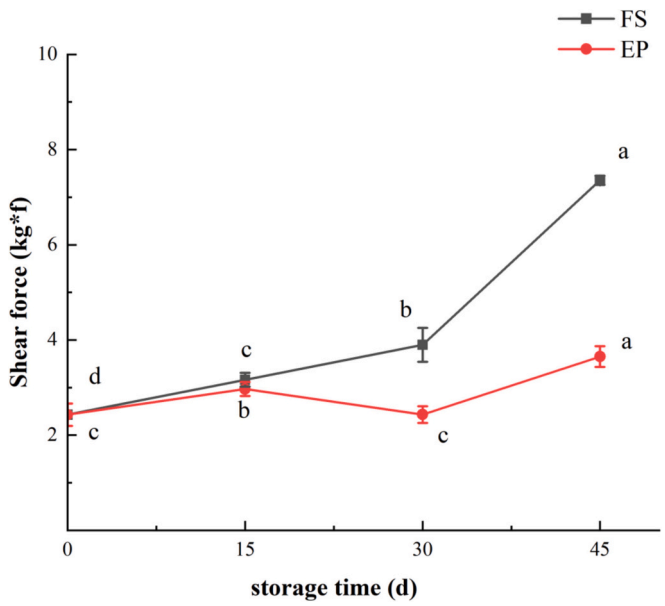


Fig. 4. The changes in the shearing force of mutton during storage.

notable adverse effect on the SF of mutton. The SF of the EP group also showed a significant increase, but its value was lower than that of the FS group. This suggests that the EMF treatment positively affected the tenderness of mutton to a certain extent, which might have helped maintain the structural integrity of the muscle tissues and slowed down the rate of SF increase, thereby improving the tenderness of mutton.

3.5. Proteomic changes in mutton

3.5.1. Principal component analysis and intergroup analysis of differentially expressed proteins

PCA was employed to observe intergroup separation and outliers, reflecting variability in the raw data. The PCA scatter plot for mutton proteins (Fig. 5) demonstrated that PC1 accounted for 92.1 % and PC2 for 1.9 % of the variability, with a total contribution rate of 94.0 %, indicating a satisfactory representation of the raw data. Fig. 5 illustrated the formation of discrete clusters for the day 0, EP group (15, 30, 45 d), and FS group (15, 30, 45 d) samples. Notably, the EP and FS samples displayed a greater distance from the day 0 samples, signifying a significant impact of the storage treatments on the composition of mutton proteins.

To understand the biological processes influenced by differentially expressed proteins (DEPs), we screened proteins with a significance threshold of $P < 0.05$, using fold change (FC) criteria of $\geq$1.4 for up-regulation and $\leq$ 0.72 for down-regulation. As shown in Fig. 6, the variation in the quantity of DEPs revealed significant differences between FS and EP methods at different time points. Additionally, correlation analysis of DEPs within the same storage method (Figs. 6-**C and 6-D**) revealed significant differences between FS and EP samples across different time points. This finding indicates that the omics data collected from the samples exhibited strong repeatability, and that the samples from various storage periods were sufficiently representative, meeting the requirements for further in-depth analysis.

3.5.2. Effects of differentially expressed proteins on mutton quality during storage

In FS15 vs 0, 14 proteins were screened for upregulation, including titin, nebulin, and galectin. Titin and nebulin are essential for maintaining the stability of myofibrils. Their disruption resulted in the fragmentation of myofibrils and a loss of muscle cell integrity, leading to a softening of the muscle (Huff Lonergan et al., 2010). This effect was particularly evident in meat with a high pH level, where these structural proteins were subject to accelerated degradation (Farouk et al., 2012). Gagaoua et al. (2018) found titin correlated with four color traits: negatively with L* and positively with a* and b*. Galectin, linked to apoptosis, showed decreased abundance in tender meat samples (Bjarnadóttir et al., 2012). A total of 712 proteins were found to be down-regulated, including glyceraldehyde-3-phosphate dehydrogenase, pyruvate kinase, HSP27, creatine kinase, α-actinin, triosephosphate isomerase, GSTM3, and troponin-T. The color of meat is primarily determined by the myoglobin content and the final pH. During this stage, the pH of the mutton showed an upward trend, consistent with the findings of Damon et al. (2013). Glyceraldehyde-3-phosphate dehydrogenase and pyruvate kinase exhibited lower redness values and color stability when highly active (Canto et al., 2015). Furthermore, the reduction in creatine kinase levels was associated with a decline in redness value (Choi et al., 2008). Heat shock protein family B (HSP β-1, also known as HSP27), acting as a chaperone protein, prevented protein denaturation. Hwang et al. (2005) found that higher HSP27 abundance corresponded to lower a* and L* values, while Hsp90 was negatively correlated with L* and b* (Zhang et al., 2014). Additionally, α-actinin,

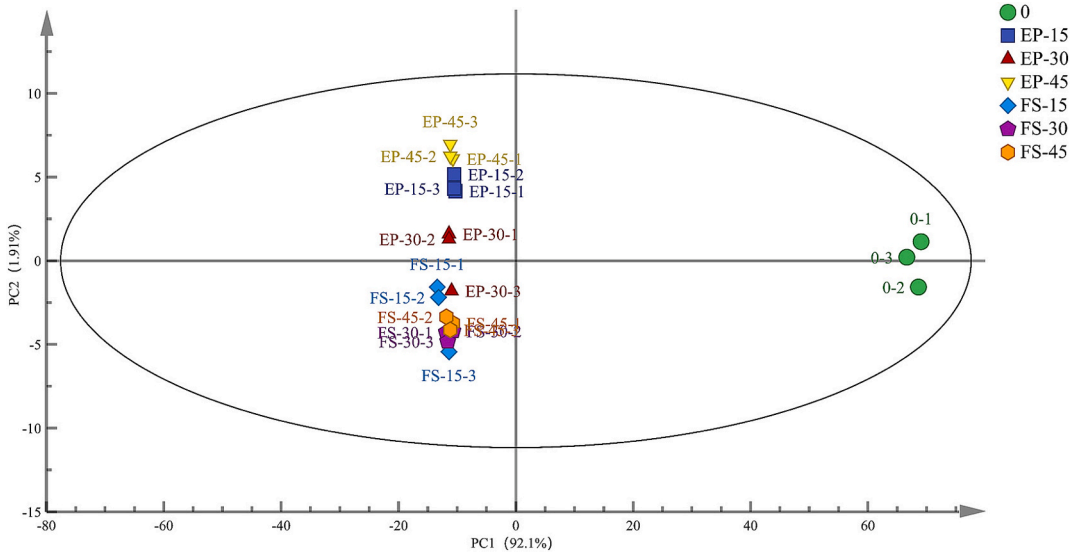


Fig. 5. PCA of proteins.

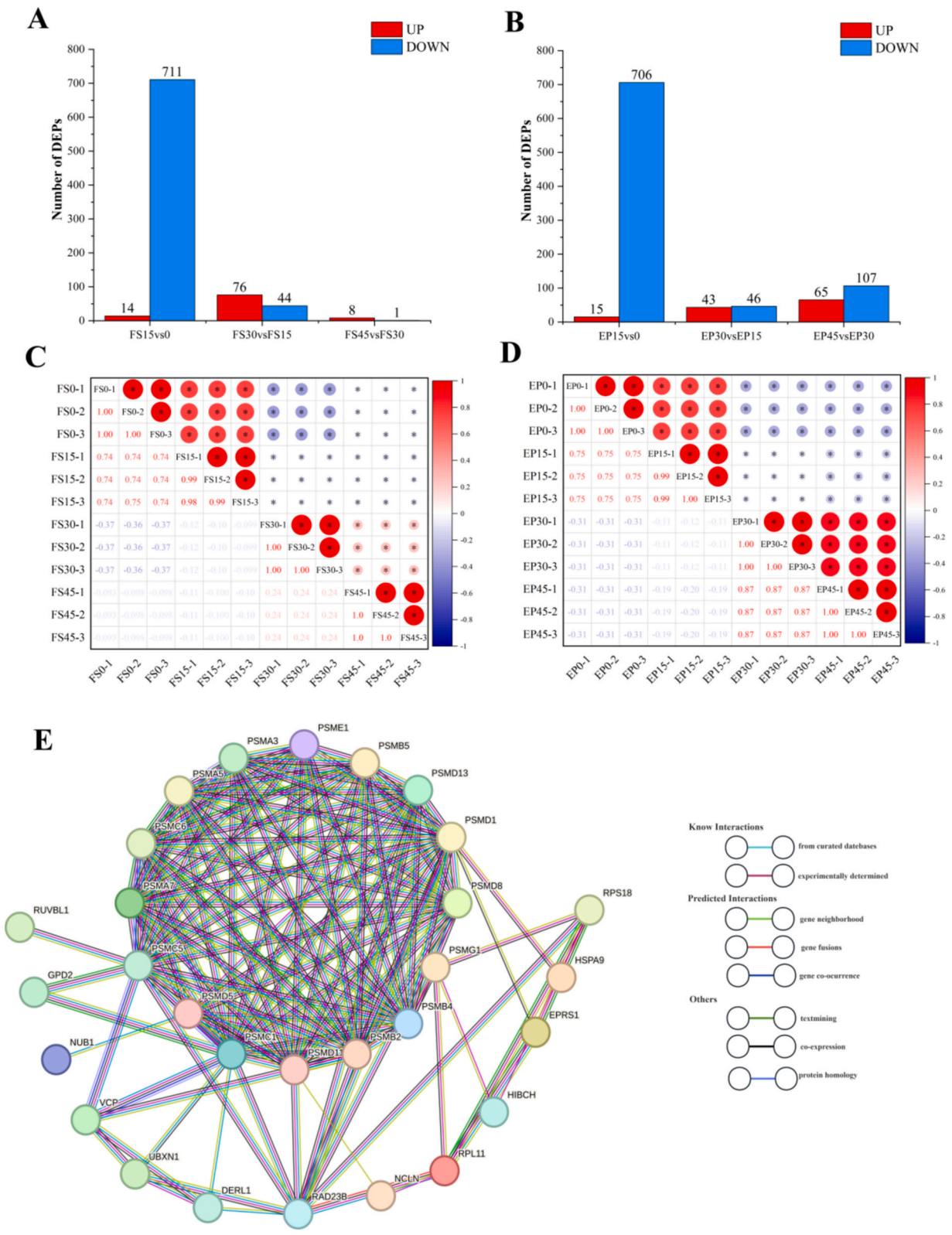


Fig. 6. Statistics of DEPs (differentially expressed proteins), correlation analysis of DEPs between groups under two storage methods, and protein interaction network of differentially expressed proteins.

Statistics of DEPs in the same storage method and different storage time ($P < 0.05$).

Where: A- Statistics on the number of DEPs up- and down-regulated during FS (freezing storage), B- Statistics on the number of DEPs up- and down-regulated during EP (electromagnetic field preservation), C- Correlation analysis of DEPs between groups during FS, D- Correlation analysis of DEPs between groups during EP, *E*-Proteasome-associated differential expressed proteins interaction network.

identified as a structural protein related to meat color, showed a positive correlation with L* and negative correlations with a* and b* (Gagaoua et al., 2017). Triosephosphate isomerase, a glycolytic enzyme, stabilized color when abundant (Nair et al., 2016). During this stage, L*, a*, and b* values decreased, probably due to the combined effects of titin, creatine kinase, heat shock proteins, actin, and triosephosphate isomerase. Most of the water in muscle is located within or between myofibrils, which consisted mainly of myosin, actin, and other structural proteins (Huff-Lonergan & Lonergan, 2005). The decrease in WHC during this stage might have resulted from the down-regulation of myofibrillar proteins. GSTM3, involved in xenobiotic metabolism and catalytic reactions with potentially toxic compounds, was more expressed in tender mutton (Zheng et al., 2022). The degradation of troponin complex proteins, which is associated with tenderness, plays a crucial role in the meat tenderization process (Marino et al., 2015). Jia et al. (2021) identified a positive correlation between the abundance of troponin-T and meat tenderness.

We observed a significant downregulation of several proteasome-related proteins, including PSCM1, PSMC5, and PSMD13. As core components of the ubiquitin-proteasome pathway, proteasomes are essential for the post-mortem tenderization of meat. Liu et al. (2016) investigated the effects of the ubiquitin-proteasome pathway (UPP) on proteolytic activity during lamb post-mortem tenderization by injecting inhibitors of the ubiquitin-activating enzyme E1 or proteasome inhibitors such as MG-1. They found that inhibiting the proteasome could reduce the degradation of post-mortem muscle ultrastructure, thereby promoting tenderness. At this stage, the increase in WHC and the decrease in tenderness may be closely related to the downregulation of GSTM3, troponin-T, and proteasomes.To further explore the roles of these proteins, we conducted a protein-protein interaction network (PPI) analysis (Figs. 6-**E**) on DEPs during this phase under two storage methods and 16 types of proteases. The results showed a PPI enrichment p-value of 1.0×10^{-16} and a clustering coefficient of 0.748, indicating a significant tightness in the interaction network. In this network, we identified 29 nodes and 134 edges, ultimately determining that 13 DEPs had significant interactions with the proteasome. These proteins mainly include RPL11, RPS18, and EPRS1, which are related to protein synthesis; RAD23B, VCP, UBXN1, RUVBL1, HSPA9, DERL1, and NUB1, which are involved in cellular stress and repair; as well as GPD2, HIBCH, and NCLN, which are associated with metabolism and homeostasis. These findings provide important insights into the role of the proteasome in enhancing mutton quality.

In EP15 vs 0, 15 up-regulated proteins were screened, with ATP2A1 being unique compared to FS15 vs 0. ATP2A1 directly regulated calcium homeostasis. Additionally, a total of 706 down-regulated proteins were found, which had a similar composition to those in FS15 vs 0. Notably, glyceraldehyde-3-phosphate dehydrogenase was down-regulated in FS15 vs 0, potentially contributing to the higher a* values observed in the EP group. At day 15, FS exhibited a higher pH than EP, and the down-regulated carbonic anhydrase exhibited a higher FC in FS than EP, which likely explained the lower pH observed in EP. FS exhibited higher Lab* values than EP, and the down-regulated actinin alpha 1 and actinin alpha 2 exhibited higher FC in EP than FS, while HSPB1 and HSP90B1 exhibited higher FC in FS than EP, which may have contributed to the lower L* observed in EP. Moreover, down-regulated creatine kinase had a higher FC in FS than in EP, while pyruvate kinase exhibited a higher FC in EP than in FS. This discrepancy may result in lower a* values in EP due to the combined effects of actinin alpha 1, actinin alpha 2, HSPB1, and creatine kinase. Down-regulated ACTN2 had a higher FC in FS than EP, and the combined effect of ACTN2 and HSP90B1 may have caused the lower a* in EP. The majority of myosin and actin proteins had higher FC in EP than FS, except ACTN2 in FS, possibly leading to lower WHC in EP. Down-regulated TNNT3, TTLL3, GSTM3, and proteasomes had higher FC in EP than in FS, potentially leading to lower tenderness in EP.

In FS30 vs FS15, 76 up-regulated proteins were screened, including carbonic anhydrase, titin, and myosin light chain 2. Meanwhile, 44 down-regulated proteins were screened, such as α-actinin, myosin binding protein C2, actinin alpha 1, myosin binding protein H, myosin light chain 3, myosin heavy chain 10, troponin-T, and glutathione-S-transferase. The up-regulation of carbonic anhydrase might have been responsible for the pH decrease. The up-regulation of pyruvate kinase and titin, along with the down-regulation of α-actinin, could have resulted in increased a* and b* values and decreased L*. The down-regulation of myosin binding protein C2, actinin alpha 1, myosin binding protein H, myosin light chain 3, and myosin heavy chain 10, together with the up-regulation of myosin-7, myosin light chain 2, and myosin light chain 6B, likely contributed to a decrease in WHC. The down-regulation of troponin-T and GSTM3 also indicated decreased tenderness and increased SF.

In EP30 vs EP15, 44 up-regulated proteins, including titin and myosin binding protein C1, and 46 down-regulated proteins, including Q9MZ05, pyruvate kinase, fructose-bisphosphate aldolase, ENO3, myosin light chain 3, myosin binding protein C2, LDHA, and troponin-T, were identified. ENO3, an important enzyme in glycolytic metabolism, has been found to have a negative correlation with a* value and a positive correlation with pH (Yu et al., 2017). The down-regulation of carbonic anhydrase and ENO3 might have explained the rising pH trend. Canto et al. (2015) found that fructose-bisphosphate aldolase, with high activity, exhibited a lighter red color and color stability. The down-regulation of Q9MZ05, pyruvate kinase, fructose-bisphosphate aldolase, and ENO3, along with the up-regulation of titin, indicated increased a* and b* values compared to the FS group and a decrease in L*. The down-regulation of myosin light chain 3 and myosin binding protein C2, and the up-regulation of myosin binding protein C1, suggested that the combined action of actin and myosin proteins had led to a decrease in WHC. LDHA, mainly present in muscle tissue and part of the lactate dehydrogenase family, has a negative correlation with SF (López-Pedrouso et al., 2021). The down-regulation of LDHA and troponin T had resulted in decreased SF.

In FS45 vs FS30, 8 up-regulated proteins, including A0A8C2XSD9, A0A452E4U5, and A0A452DZU0, and 1 down-regulated protein (A0A836APP1) were identified. These proteins were mostly related to coagulation, immune response, and cellular energy metabolism.

In EP45 vs EP30, 65 up-regulated proteins were screened, including titin, myosin heavy chain 7B, myosin light chain 3, myosin binding protein H, LDHA, and troponin-T. Additionally, 107 down-regulated proteins were identified, including ENO3, Q9MZ05, pyruvate kinase, and triosephosphate isomerase, myosin binding protein C1, sarcalumenin, and myosin light chain kinase 2. The up-regulation of carbonic anhydrase and ENO3 likely caused the pH to decrease. The down-regulation of Q9MZ05, pyruvate kinase, and triosephosphate isomerase, combined with the up-regulation of titin, suggested that the interplay of these proteins, along with ENO3, had resulted in decreased L* and a* values, while b* showed a slight increase. The up-regulation of myosin binding protein C2, myosin heavy chain 7B, myosin light chain 3, and myosin binding protein H, along with the down-regulation of myosin binding protein C1, sarcalumenin, and myosin light chain kinase 2, likely led to a decrease in WHC. The up-regulation of LDHA and troponin-T indicated an increase in SF.

3.5.3. Functional enrichment analysis of DEPs between the two storage methods at 15 days

On the 15th day, the 117 DEPs screened in the EP15 vs FS15 group were subjected to analysis for GO and KEGG pathway enrichment. The GO enrichment analysis results, as shown in Fig. 7-**A**, revealed enrichment in biological processes (BP) such as single-organism processes, organonitrogen compound metabolic processes, and cellular component assembly, potentially impacting mutton freshness, WHC, and tenderness through muscle tissue construction and maintenance. Molecular function (MF) enrichment included metal ion binding, cation binding, cytoskeletal protein binding, and oxidoreductase activity, which may have affected meat color, muscle structure, and oxidative stability.

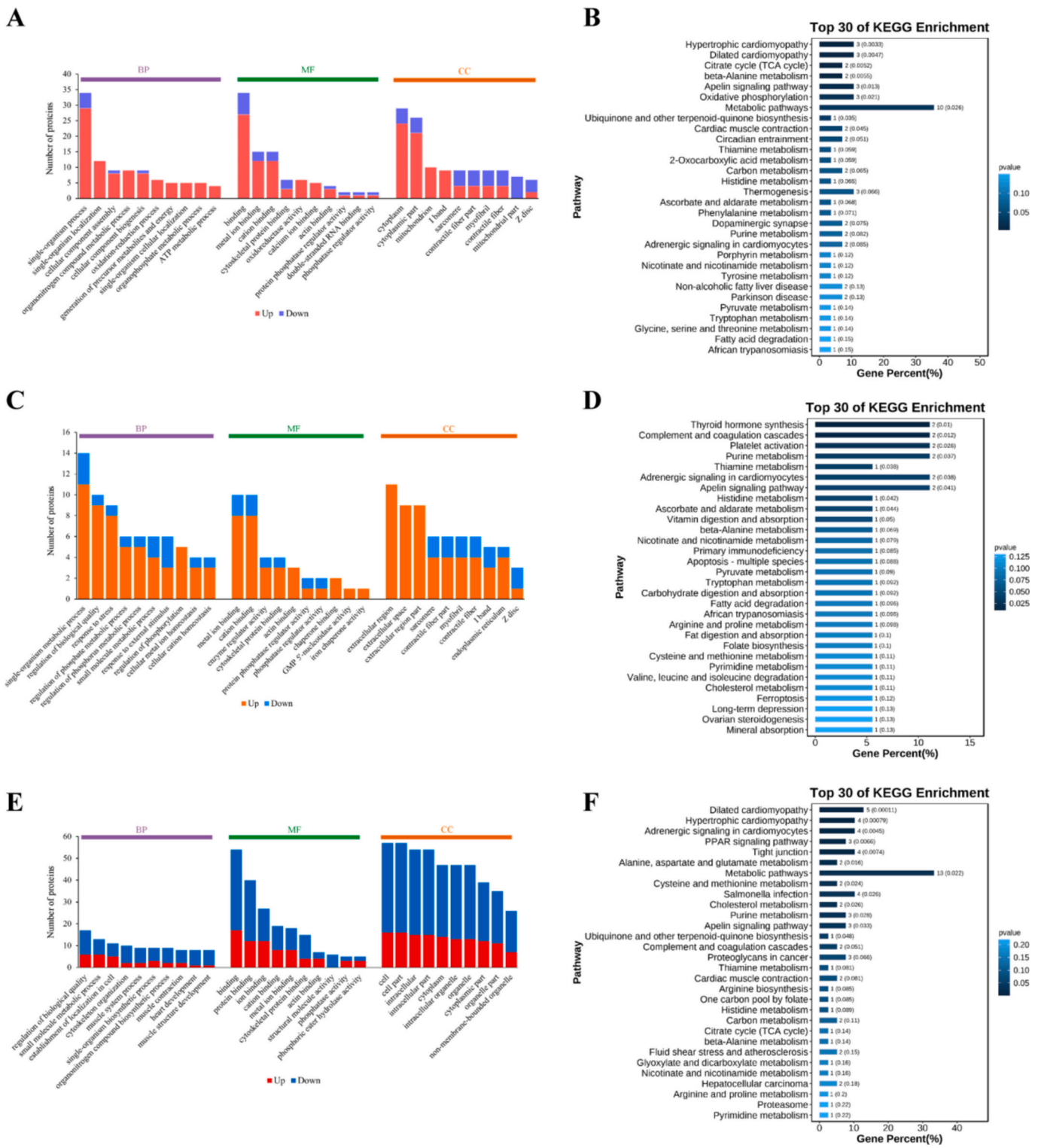


Fig. 7. GO and KEGG enrichment analysis of DEPs under different storage conditions at the same storage time (BP: biological processes, CC: cellular components, MF: molecular functions).
Where: A- GO enrichment analysis of DEPs at 15d for two storage methods, B- KEGG enrichment analysis of DEPs at 15d for two storage methods, C- GO enrichment analysis of DEPs at 30d for two storage methods, D- KEGG enrichment analysis of DEPs at 30d for two storage methods, E- GO enrichment analysis of DEPs at 45d for two storage methods, F- KEGG enrichment analysis of DEPs at 45d for two storage methods.

Cellular component (CC) enrichment encompassed cytoplasmic parts, mitochondria, I band, and contractile fiber parts, related to energy metabolism and muscle structural functions, influencing mutton tenderness and WHC.

Concurrently, KEGG pathway enrichment analysis identified a total of 101 metabolic pathways, with the top 30 significantly enriched pathways ($P < 0.05$) illustrated in Fig. 7-**B**. Significantly enriched pathways included the TCA cycle, β-alanine metabolism, Apelin signaling pathway, oxidative phosphorylation, ubiquinone and other terpenoid-quinone biosynthesis, among others. These metabolic

pathways were consistent with the GO enrichment analysis results, indicating that these DEPs interacted through various BPs, MFs, and CCs to collectively regulate mutton quality changes during storage. In particular, significantly upregulated proteins, such as A0A452E8M7, D3JYV6, A0A452FKR1, and A0A452G6M6, were associated with several metabolic pathways. For instance, the involvement of A0A452E8M7 and D3JYV6 could have influenced the energy supply and metabolism of mutton, subsequently affecting its color, tenderness, and taste. Similarly, the involvement of A0A452FKR1, A0A452G6M6, and D3JYV6 may have enhanced the energy supply and antioxidant capacity of mutton. Furthermore, the involvement of A0A452DSW4 and A0A452FJE8 in protein degradation may have improved the tenderness and flavor of mutton A0A452G4A1 may also have contributed to maintaining the freshness and color of meat. In the Apelin signaling pathway, the significant upregulation of A0A452EJ66, A0A452EP00, and A0A452ERK1 may have impacted cardiac muscle structure and function, thereby enhancing the overall quality of the mutton. In conclusion, the GO function enrichment and KEGG pathway enrichment results indicated that these differentially expressed proteins, through a complex regulatory network of biological processes, molecular functions, and cellular components, collectively influenced mutton freshness, WHC, tenderness, and overall quality during the 15-day storage period.

3.5.4. Functional enrichment analysis of DEPs between the two storage methods at 30 days

On the 30th day, the 93 DEPs screened in the EP30 vs FS30 group were subjected to analysis for GO and KEGG pathway enrichment. The results of the GO enrichment analysis, depicted in Fig. 7**-C**, revealed enrichment in BP, primarily involving single-organism metabolic processes, regulation of biological quality, response to stress, and regulation of phosphate metabolic processes. These processes were associated with energy supply, metabolism, flavor changes, and storage adaptability. MF included metal ion binding, cation binding, enzyme regulator activity, and cytoskeletal protein binding, influencing meat color, tenderness, freshness, and oxidative stability. The CC terms included those relating to the extracellular region, extracellular space, sarcomere, and contractile fiber part, impacting muscle structure and meat characteristics.

KEGG pathway enrichment analysis identified 78 metabolic pathways, with the top 30 pathways illustrated in Fig. 7**-D**. The analysis revealed that several pathways were significantly enriched, including those involved in thyroid hormone synthesis, complement and coagulation cascades, platelet activation, purine metabolism, and thiamine metabolism. These pathways impacted a range of biological processes, including metabolism, immune response, coagulation ability, blood supply, and overall quality. These pathways interacted with one another to regulate the changes in quality that occurred in the mutton during the storage period. At day 30, significant enrichment was observed in thyroid hormone synthesis, purine metabolism, and the Apelin signaling pathway, which were involved in energy supply, metabolism, immune response, and cardiovascular function. The up-regulated proteins A0A452DUP7 and A0A452EUJ3 participated in purine metabolism, while A0A452EJ66 and A0A452EP00 were involved in the Apelin signaling pathway. In accordance with the results obtained on day 15, these proteins and pathways exerted a consistent and pivotal regulatory influence on mutton quality throughout the storage period.

3.5.5. Functional enrichment analysis of DEPs between the two storage methods at 45 days

On the 45th day, the 187 DEPs screened in the EP45 vs FS45 group were subjected to analysis for GO and KEGG pathway enrichment. The GO enrichment analysis, as illustrated in Fig. 7**-E**, revealed that the BP that were most significantly enriched were those involved in regulating biological quality, small molecule metabolism, establishment of cell localization, and cytoskeleton organization. These processes significantly influenced the overall quality and energy metabolism of mutton, thereby affecting characteristics such as color, tenderness, taste, and freshness. MF enrichment included terms such as binding, protein binding, ion binding, and cation binding, which were found to be correlated with oxidative stability, muscle structure, and enzymatic activity. These factors had an additional impact on meat quality characteristics. CC enrichment encompassed terms related to cells, intracellular and extracellular parts, cytoplasm, and organelles, which may have influenced meat texture, freshness, and WHC. The majority of down-regulated proteins were involved across these pathways, particularly in MF, where they affected structural molecular activity.

A total of 76 metabolic pathways were identified as significantly enriched in the KEGG pathway enrichment analysis, with the top 30 pathways illustrated in Fig. 7**-F.** The pathways included the TCA cycle, β-alanine metabolism, apelin signaling pathway, oxidative phosphorylation, ubiquinone and other terpenoid-quinone biosynthesis, and several others. These pathways were closely associated with mutton metabolism, energy supply, muscle structure, antioxidant capacity, and overall quality. For instance, the peroxisome proliferator-activated receptor (PPAR) signaling pathway is associated with fatty acid metabolism, influencing adipose tissue and lipid oxidation during storage. The metabolism of alanine, aspartate, and glutamate plays a crucial role in amino acid metabolism, thereby influencing protein synthesis and degradation. This process significantly affects meat tenderness and flavor. Furthermore, the metabolism of cholesterol and purines directly impacts the energy supply, thereby affecting the overall quality of the meat. The interconnectivity of these pathways collectively regulated mutton taste, freshness, color, and overall quality, reflecting intricate biochemical alterations and regulatory mechanisms that occurred during prolonged storage. In conclusion, the results of the GO functional enrichment and KEGG pathway enrichment analyses indicated that the DEPs collectively influenced the quality characteristics of mutton during the 45-day storage period, including freshness, WHC, tenderness, and overall quality.

4. Correlation analysis between physicochemical indicators and DEPs

The physicochemical indicators of mutton were correlated with the DEPs obtained from the screening, and the top 30 DEPs most correlated with the physicochemical indicators were screened. The correlation heatmap is shown in Fig. 8. The DEPs exhibited significant correlations with various quality traits, suggesting their potential as biomarkers for the comprehensive assessment of mutton quality.

In the FS group (Fig. 8-A), pH and SF were found to be significantly positively correlated with eight DEPs, with peroxiredoxin-1 and superoxide dismutase [Cu—Zn] showing highly significant positive correlations with SF ($P \leq 0.001$). This can be attributed to the protective role of peroxiredoxin-1 and superoxide dismutase [Cu—Zn] against reactive oxygen species damage to myofibrillar proteins, as observed in the study by Malheiros et al. (2019). Additionally, pH and SF were significantly negatively correlated with 22 DEPs, with troponin T and myomesin 2 being notably negatively correlated with shear force. Troponin T and myomesin 2 play critical roles in muscle contraction regulation, and their increased expression tends to result in more tender mutton, as highlighted by López-Pedrouso et al. (2021). b* was significantly positively correlated with 1 DEP and negatively correlated with 1 DEP. a* showed significant positive correlations with 4 DEPs and negative correlations with 4 DEPs. L* and WHC were significantly positively correlated with 22 DEPs and negatively correlated with 8 DEPs. Notably, superoxide dismutase was negatively correlated with WHC, consistent with findings by Zhang et al. (2019), while four and a half LIM domains 1 protein exhibited a negative correlation with L*, similar to Gagaoua et al. (2018).

In the EP group (Fig. 8-B), pH and SF were significantly positively correlated with 8 DEPs and negatively correlated with 22 DEPs. L* and WHC were significantly negatively correlated with 22 DEPs and

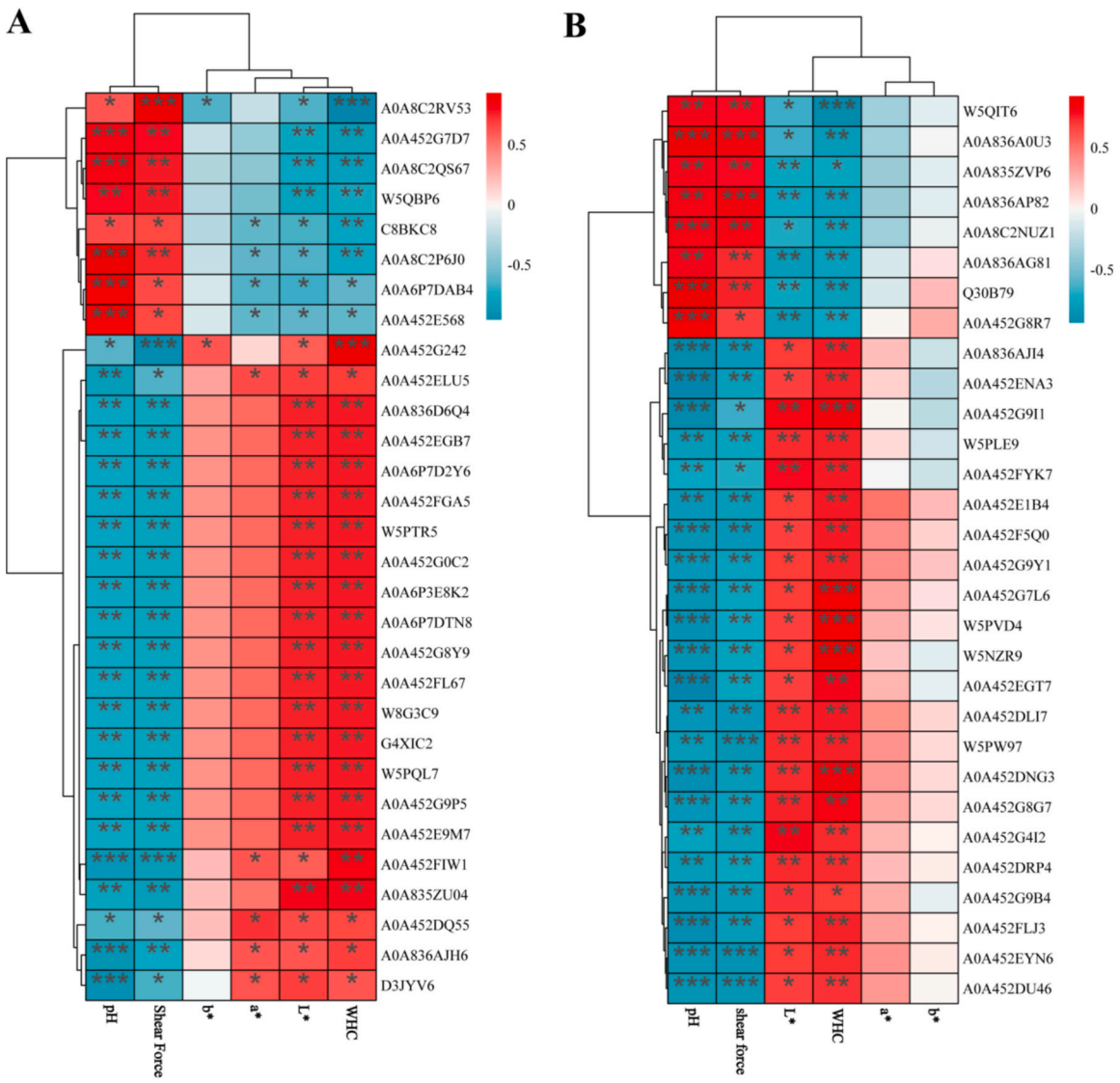


Fig. 8. Heatmap of correlation analysis quality characteristics and DEPs of mutton under FS (A) and EP (B).
Note: red represents positive correlation, green and blue represents negative correlation. Different colors represent different correlation coefficients r, and the right side of the legend is the color range of different r values. The significance values are: * $0.01 < P \leq 0.05$; * * $0.001 < P \leq 0.01$; * * * $P \leq 0.001$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

positively correlated with 8 DEPs. Tubulin alpha chain showed a positive correlation with WHC, reflecting its role as a structural protein that helps preserve water in muscle, as observed by Zhang et al. (2019). No significant correlations were found between these DEPs and a* and b*. Additionally, titin exhibited a negative correlation with shear force and a positive correlation with L*. The degradation of titin can disrupt myofibrillar proteins, affecting mutton tenderness and light scattering, as discussed in Gagaoua et al. (2018).

5. Conclusions

This study presented a comparative analysis of the effects of two storage methods, FS and EP, on the physicochemical indicators of mutton quality in Qianbei Ma sheep. The results indicated that compared to the FS group, the EP group demonstrated a better ability to inhibit pH increase and maintain a* and b* values during the storage of mutton. With prolonged storage time, the EP group exhibited superior performance in controlling mutton tenderness and WHC. The quantitative proteomics technique with tandem mass tagging revealed that mutton quality was closely related to protein expression levels. In particular, changes in pH were associated with carbonic anhydrase expression levels, changes in color were mainly associated with myoglobin, creatine kinase, glycolytic enzymes, chaperonins, and structural proteins, changes in WHC were associated with myofibrillar fibrillar proteins, and changes in SF were associated with troponin-T, proteasome and GSTM3. Furthermore, GO and KEGG enrichment analyses revealed that certain proteins, including A0A452DSW4, A0A452E8M7, and D3JYV6, were involved in energy metabolism and redox processes. In contrast, A0A452EJ66, A0A452DSW4, and A0A452FJE played important roles in protein binding. In conclusion, EP proved more effective in maintaining mutton quality. These findings offered insights into the molecular mechanisms underlying varied storage conditions and suggested potential directions for future advancements in mutton preservation technologies.

CRediT authorship contribution statement

Xing Lei: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Wei Su:** Resources, Funding acquisition. **Rongmei Zhou:** Data curation.

Yingchun Mu: Writing – review & editing.

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.

Acknowledgments

This research was supported by the National Natural Science Foundation of China (32160554), Guizhou Science and Technology Program (QKHPTRC [2020] 4009 (006)), and (QKHPTRC [2020] 5004).

References

Bai, Y., Hu, Y., & Qu, M. (2015). Preliminary studies on Spanish mackerel fresh-keeping method in high-voltage electric field. *Journal of Aquatic Food Product Technology, 24*(8), 732–739. https://doi.org/10.1080/10498850.2013.808724

Bjarnadóttir, S. G., Hollung, K., Høy, M., Bendixen, E., Codrea, M. C., & Veiseth-Kent, E. (2012). Changes in protein abundance between tender and tough meat from bovine longissimus thoracis muscle assessed by isobaric tag for relative and absolute quantitation (iTRAQ) and 2-dimensional gel electrophoresis analysis. *Journal of Animal Science, 90*(6), 2035–2043. https://doi.org/10.2527/jas.2011-4721

Bocker, R., & Silva, E. K. (2022). Pulsed electric field assisted extraction of natural food pigments and colorings from plant matrices. *Food Chemistry: X, 15*, Article 100398. https://doi.org/10.1016/j.fochx.2022.100398

Canto, A. C. V. C., Suman, S. P., Nair, M. N., Li, S., Rentfrow, G., Beach, C. M., … King, D. A. (2015). Differential abundance of sarcoplasmic proteome explains animal effect on beef longissimus lumborum color stability. *Meat Science, 102*, 90–98. https://doi.org/10.1016/j.meatsci.2014.11.011

Chen, X., Zhu, L., Liang, R., Mao, Y., Hopkins, D. L., Li, K., … Luo, X. (2020). Shelf-life and bacterial community dynamics of vacuum packaged beef during long-term super-chilled storage sourced from two Chinese abattoirs. *Food Research International, 130*, Article 108937. https://doi.org/10.1016/j.foodres.2019.108937

Choi, Y. M., Ryu, Y. C., Lee, S. H., Go, G. W., Shin, H. G., Kim, K. H., … Kim, B. C. (2008). Effects of supercritical carbon dioxide treatment for sterilization purpose on meat quality of porcine longissimus dorsi muscle. *LWT - Food Science and Technology, 41*(2), 317–322. https://doi.org/10.1016/j.lwt.2007.02.020

Damon, M., Denieul, K., Vincent, A., Bonhomme, N., Wyszynska-Koko, J., & Lebret, B. (2013). Associations between muscle gene expression pattern and technological and sensory meat traits highlight new biomarkers for pork quality assessment. *Meat Science, 95*(3), 744–754. https://doi.org/10.1016/j.meatsci.2013.01.016

Esmer, O. K., Irkin, R., Degirmencioglu, N., & Degirmencioglu, A. (2011). The effects of modified atmosphere gas composition on microbiological criteria, color and oxidation values of minced beef meat. *Meat Science, 88*(2), 221–226. https://doi.org/10.1016/j.meatsci.2010.12.021

Farouk, M. M., Mustafa, N. M., Wu, G., & Krsinic, G. (2012). The “sponge effect” hypothesis: An alternative explanation of the improvement in the waterholding capacity of meat with ageing. *Meat Science, 90*(3), 670–677. https://doi.org/10.1016/j.meatsci.2011.10.012

Gagaoua, M., Bonnet, M., de Koning, L., & Picard, B. (2018). Reverse phase protein array for the quantification and validation of protein biomarkers of beef qualities: The case of meat color from charolais breed. *Meat Science, 145*, 308–319. https://doi.org/10.1016/j.meatsci.2018.06.039

Gagaoua, M., Couvreur, S., le Bec, G., Aminot, G., & Picard, B. (2017). Associations among protein biomarkers and pH and color traits Inlongissimus thoracis andrectus abdominis muscles in protected designation of origin maine-anjou cull cows. *Journal of Agricultural and Food Chemistry, 65*(17), 3569–3580. https://doi.org/10.1021/acs.jafc.7b00434

Hou, X., Liu, Q., Meng, Q., Wang, L., Yan, H., Zhang, L., & Wang, L. (2020). TMT-based quantitative proteomic analysis of porcine muscle associated with postmortem meat quality. *Food Chemistry, 328*, Article 127133. https://doi.org/10.1016/j.foodchem.2020.127133

Huff Lonergan, E., Zhang, W., & Lonergan, S. M. (2010). Biochemistry of postmortem muscle — lessons on mechanisms of meat tenderization. *Meat Science, 86*(1), 184–195. https://doi.org/10.1016/j.meatsci.2010.05.004

Huff-Lonergan, E., & Lonergan, S. M. (2005). Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. *Meat Science, 71*(1), 194–204. https://doi.org/10.1016/j.meatsci.2005.04.022

Hwang, I. H., Park, B. Y., Kim, J. H., Cho, S. H., & Lee, J. M. (2005). Assessment of postmortem proteolysis by gel-based proteome analysis and its relationship to meat quality traits in pig longissimus. *Meat Science, 69*(1), 79–91. https://doi.org/10.1016/j.meatsci.2004.06.019

Jia, W., Zhang, R., Liu, L., Zhu, Z., Xu, M., & Shi, L. (2021). Molecular mechanism of protein dynamic change for Hengshan goat meat during freezing storage based on high-throughput proteomics. *Food Resarch International, 143*, 110289. https://doi.org/10.1016/j.foodres.2021.110289

Kaur, M., & Kumar, M. (2020). An innovation in magnetic field assisted freezing of perishable fruits and vegetables: A review. *Food Reviews International, 36*(8), 761–780. https://doi.org/10.1080/87559129.2019.1683746

Kiyimba, F., Hartson, S. D., Rogers, J., VanOverbeke, D. L., Mafi, G. G., & Ramanathan, R. (2021). Changes in glycolytic and mitochondrial protein profiles regulates postmortem muscle acidification and oxygen consumption in dark-cutting beef. *Journal of Proteomics, 232*, Article 104016. https://doi.org/10.1016/j.jprot.2020.104016

Liu, Y., Du, M., Li, X., Chen, L., Shen, Q., Tian, J., & Zhang, D. (2016). Role of the ubiquitin-proteasome pathway on proteolytic activity in postmortem proteolysis and tenderisation of sheep skeletal muscle. *International Journal of Food Science & Technology, 51*(11), 2353–2359. https://doi.org/10.1111/ijfs.13214

López-Pedrouso, M., Lorenzo, J. M., di Stasio, L., Brugiapaglia, A., & Franco, D. (2021). Quantitative proteomic analysis of beef tenderness of piemontese young bulls by SWATH-MS. *Food Chemistry, 356*, Article 129711. https://doi.org/10.1016/j.foodchem.2021.129711

Ma, C., Wang, W., Wang, Y., Sun, Y., Kang, L., Zhang, Q., & Jiang, Y. (2020). TMT-labeled quantitative proteomic analyses on the longissimus dorsi to identify the proteins underlying intramuscular fat content in pigs. *Journal of Proteomics, 213*, Article 103630. https://doi.org/10.1016/j.jprot.2019.103630

Mahato, S., Zhu, Z., & Sun, D. (2023). Effects of frequencies ranging from 100 to 400 Hz on electromagnetic field assisted freezing of grass carp (Ctenopharyngodon idellus). *LWT - Food Science and Technology, 174*. https://doi.org/10.1016/j.lwt.2022.114399

Malheiros, J. M., Braga, C. P., Grove, R. A., Ribeiro, F. A., Calkins, C. R., Adamec, J., & Chardulo, L. A. L. (2019). Influence of oxidative damage to proteins on meat tenderness using a proteomics approach. *Meat Science, 148*, 64–71. https://doi.org/10.1016/j.meatsci.2018.08.016

Marino, R., Della Malva, A., & Albenzio, M. (2015). Proteolytic changes of myofibrillar proteins in podolian meat during aging: Focusing on tenderness. *Journal of Animal Science, 93*(3), 1376–1387. https://doi.org/10.2527/jas.2014-8351

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*. https://doi.org/10.1016/j.meatsci.2021.108453

Nair, M. N., Suman, S. P., Chatli, M. K., Li, S., Joseph, P., Beach, C. M., & Rentfrow, G. (2016). Proteome basis for intramuscular variation in color stability of beef semimembranosus. *Meat Science, 113*, 9–16. https://doi.org/10.1016/j.meatsci.2015.11.003

Schmidt, H., Scheier, R., & Hopkins, D. L. (2013). Preliminary investigation on the relationship of Raman spectra of sheep meat with shear force and cooking loss. *Meat Science, 93*(1), 138–143. https://doi.org/10.1016/j.meatsci.2012.08.019

Seyfali, E., Khoshtaghaza, M. H., Rouhi, M., Sarlak, Z., & Najafi, G. (2024). The potential of pulsed electromagnetic field-generated shock waves for reducing microbial load and improving homogenization in raw milk. *Heliyon, 10*(11), Article e32204. https://doi.org/10.1016/j.heliyon.2024.e32204

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

Vieira, C., Guerra-Rivas, C., Martínez, B., Rubio, B., & Manso, T. (2022). Effects of grape pomace supplementation on the diet of lactating ewes as compared to vitamin E on the meat shelf life of suckling lambs. *Meat Science, 184*, Article 108666. https://doi.org/10.1016/j.meatsci.2021.108666

Wang, J., Wang, Q., Xu, L., & Sun, D. (2022). Effects of extremely low frequency pulsed electric field (ELF-PEF) on the quality and microstructure of tilapia during cold storage. *LWT - Food Science and Technology, 169*, Article 113937. https://doi.org/10.1016/j.lwt.2022.113937

Wang, J., Xiao, J., Liu, X., Gao, Y., Luo, Z., Gu, X., Zhang, J., Wu, D., & Geng, F. (2021). Tandem mass tag-labeled quantitative proteomic analysis of tenderloins between tibetan and yorkshire pigs. *Meat Science, 172*, Article 108343. https://doi.org/10.1016/j.meatsci.2020.108343

Wei, L. S., Peng, B. F., Li, M., & Zhang, Y. F. (2016). A numerical study of species and electric field distributions in pulsed DBD in oxygen for ozone generation. *Vacuum, 125*, 123–132. https://doi.org/10.1016/j.vacuum.2015.12.011

Xu, Y., Zhang, D., Xie, F., Li, X., Schroyen, M., Chen, L., & Hou, C. (2023). Changes in water holding capacity of chilled fresh pork in controlled freezing-point storage assisted by different modes of electrostatic field action. *Meat Science*. , Article 109269. https://doi.org/10.1016/j.meatsci.2023.109269

Yu, Q., Wu, W., Tian, X., Hou, M., Dai, R., & Li, X. (2017). Unraveling proteome changes of holstein beef M. Semitendinosus and its relationship to meat discoloration during post-mortem storage analyzed by label-free mass spectrometry. *Journal of Proteomics, 154*, 85–93. https://doi.org/10.1016/j.jprot.2016.12.012

Zhai, C., Djimsa, B. A., Brown, K., Prenni, J. E., Woerner, D. R., Belk, K. E., & Nair, M. N. (2020). Tandem mass tagged dataset used to characterize muscle-specific proteome changes in beef during early postmortem period. *Data in Brief, 32*, Article 106064. https://doi.org/10.1016/j.dib.2020.106064

Zhang, B., Hao, J., Yin, H., Duan, C., Wang, B., & Li, W. (2021). Effects of dietary nicotinic acid supplementation on meat quality, carcass characteristics, lipid metabolism, and tibia parameters of wulong geese. *Poultry Science, 100*(11), Article 101430. https://doi.org/10.1016/j.psj.2021.101430

Zhang, M., Wang, D., Geng, Z., Bian, H., Liu, F., Zhu, Y., & Xu, W. (2014). The level of heat shock protein 90 in pig longissimus dorsi muscle and its relationship with meat

pH and quality. *Food Chemistry, 165*, 337–341. https://doi.org/10.1016/j.foodchem.2014.05.111

Zhang, M., Wang, D., Xu, X., & Xu, W. (2019). Comparative proteomic analysis of proteins associated with water holding capacity in goose muscles. *Food Research International, 116*, 354–361. https://doi.org/10.1016/j.foodres.2018.08.048

Zheng, K., Yin, Y., Cao, Y., Guo, L., Li, P., Jiang, J., Huang, X., Jiang, Y., & Wu, J. (2022). Proteomic and parallel reaction monitoring approaches to evaluate biomarkers of mutton tenderness. *Food Chemistry, 397*, Article 133746. https://doi.org/10.1016/j.foodchem.2022.133746