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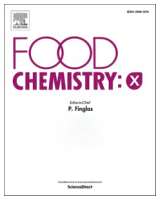

Unveils key proteins in Xinjiang goat muscle linked to post-mortem meat quality: A TMT-based proteomic analysis

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ABSTRACT

An extensive proteomic analysis utilizing the tandem mass tag (TMT) method was conducted to investigate the changes in protein expression in the longissimus dorsi muscle of Xinjiang goats over various post-mortem intervals: immediately after death within 0 h, 12 h, 24 h and 48 h. The investigation carefully identified around 108 proteins that showed significant changes in expression during these intervals. Among these proteins, six were highlighted for their crucial roles in muscle growth and differentiation of muscle fibers post-mortem. These proteins, namely COL12A1, MRPL46, CTNNB1, MYH1, CAPZA1, and MYL9, have a direct effect on the meat's quality attributes, such as tenderness and color. Further discuss observed a progressive increase in the expression of proteins linked with oxidative metabolism (MSRB2, ENOX1, LOC102170282, GSTM1, and AOC3) as the post-mortem aging period extended, particularly between 24 h to 48 h. These proteins are instrumental in defining the color and flavor profiles of goat meat, underscoring the importance of precise processing and storage conditions to preserve meat quality during the critical aging phase. This enhanced understanding of protein expression dynamics offers significant implications for optimizing meat quality and provides a scientific basis for post-mortem handling practices in the goat meat industry.

Abbreviations used

LC	liquid chromatography
MS	mass spectrometry
TMT	Labeled quantitative proteomics techniques
TEAB	tetraethyl ammonium bromide
SDS	sodium dodecyl sulfate
TCEP	Tris (2-carboxyethyl) phosphine
FDR	false discovery rate
GO	Gene ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes.

1. Introduction

Muscle tissue's metabolic activity does not cease immediately after an animal is slaughtered for meat (Koohmaraie & Geesink, 2006). Throughout the post-mortem time, the physicochemical properties of the muscle undergo changes that influence the quality of the meat (Paredi et al., 2012). Huff-Lonergan and Lonergan (2023) highlight that the post-mortem metabolism of muscle goes through significant changes within 24 h of the muscle's transformation into the meat, these changes have far-reaching consequences on the meat's key characteristics, including color, ultimate pH, water-holding capacity, and tenderness (Wang et al., 2018). The participation of proteins in these metabolic processes significantly influences the development of meat quality (Huang et al., 2014).

Meat protein content significantly influences meat's structure, nutritional value, and texture (Bax et al., 2013; Xiong, 2004). Protein modification significantly influences the activity and function of proteins through rapid alterations in enzyme activity, target protein stability, or subcellular localization (Graves & Krebs, 1999). Therefore, recently proteomics-based meat quality analysis has gained popularity in the meat science community in recent years (Huff-Lonergan & Lonergan, 2023), particularly for meat from pigs and bovine (Marino

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et al., 2023; Xu et al., 2023). Proteomic data may be able to clarify the intricate molecular processes involved in meat quality variation (D'Alessandro et al., 2011) and muscle-to-meat conversion (Wu et al., 2014). The isobar-tagged tandem mass spectrometry (TMT) -based proteomics technology can comprehensively and accurately analyze low-abundance proteins in cotton fine root tissue samples (Xiao et al., 2020). This allows for quantities analysis with higher-throughput proteomic analysis and reduces experimental errors (Liu, 2019). Liu et al. (2024) TMT quantitative proteomics analysis reveals molecular mechanism of ferroptosis during beef refrigeration 15 differentially expressed proteins (DEPs) were identified as involved in the ferroptosis pathway. These DEPs were involved in ferric ion transport, Fe^{2+} and Fe^{3+} conversion, lipid oxidation pathway, redox regulation, and iron-containing protein degradation. Hou et al. (2020) used TMT quantitative proteomics to analyze the relationship between porcine muscle a and post-mortem meat quality, About 140 differentially expressed proteins were identified. The Xinjiang goats are raised in the mountains at high altitudes. They are favored by consumers at home and abroad for their juicy meat, high nutritional value, and chewiness (Porter, 2020). Research on Xinjiang goats has predominantly concentrated on breed improvement, with comparatively few studies exploring the mechanisms that affect meat quality changes during post-mortem aging. As a result, there is a lack of comprehensive scientific guidelines for optimizing and maintaining goat meat quality. Additionally, there has been minimal documentation on the molecular and proteomic changes that occur in Xinjiang goats throughout the post-mortem aging process, particularly when analyzed using the TMT (tandem mass tag) approach.

At this work, by combined TMT and LC-MS/MS techniques to investigate alterations in the proteome of Xinjiang goats across different period points (0 h, 12 h, 24 h, and 48 h post-mortem) during post-mortem aging. The study focused on identify potential biochemical markers by examining the changes in protein abundance, thereby illuminating the molecular processes that regulate protein modifications in Xinjiang goats during post-mortem aging. The purpose of this protein correlation analysis is to reveal the biochemical activities tied to the aging phenomenon.

2. Materials and methods

2.1. Animal and sample collection

Nine healthy Xinjiang male goats, approximately 1.5 years old with an average weight of 16.72 ± 1.06 kg, were carefully selected from the same breeding farm to minimize stress reactions during the slaughtering process In accordance with the national standard of the People's Republic of China "GB/T 43562-2023 Code of practice for livestock and poultry slaughtering ooperation-Sheep and goat", slaughtering was carried out in the slaughterhouse. All slaughtering procedures were conducted in strict accordance with the guidelines established by the China Animal Welfare Committee [AEC Approval Number: SYXK (Xin) 2021–0002]. Longissimus dorsi (LTL) meat samples were collected from each goat and placed in bags at 4 °C for storage. The samples were then randomly divided into four groups: 0 h, 12 h, 24 h, and 48 h, with three replicates of samples in each group. These samples were intended for subsequent analysis of pH value, color, tenderness, and proteomics research. Additionally, a small portion of each sample at different time points was rapidly frozen in liquid nitrogen and stored at −80 °C for future proteomic studies.

2.2. Quality determination

Meat quality was assessed by measuring the pH, color, and shear stress of the goat meat. The pH measurement method followed the protocol outlined by Batuer et al. (2012), wherein 4 g of goat meat sample was placed in 40 mL of 0.1 mol/L potassium chloride solution at room temperature for 30 min, and the pH value was determined using a pH meter equipped with an E-201F probe (Shanghai Lei Magnetic Co., Ltd.).

The surface color of the samples was evaluated using an NS800 spectrophotometer (Shenzhen 3nh Technology Co., Ltd.). Tenderness assessment was conducted using the Warner-Bratzler shear force method, as described by D'Alessandro et al. (2011). Each muscle sample was precisely cut into 6 × 6 × 3 cm pieces, and the shear force was measured using a blade set (HDP/BS) at a constant speed of 1.0 mm/s, with the first main peak recorded.

Table 1
The software and database used for bioinformatics analysis.

Analysis	Software/database
Hierarchical clustering analysis	Cluster 3.0 (http://bonsai.hgc.jp/~mdehoon/software/cluster/software.htm) and Java Treeview software (http://jtreeview.sourceforge.net)
Protein sequences	InterProScan database
GO annotation	KEGG pathway enrichment
KEGG pathway enrichment	Kyoto Encyclopedia of Genes and Genomes database (http://geneontology.org/)
Protein-protein interaction analysis	IntAct molecular interaction database (http://www.ebi.ac.uk/intact/)

2.3. TMT marker analysis

2.3.1. Protein extraction and digestion

Protein extraction and sample analysis for each group were conducted using SDT (4 % SDS, 100 mM Tris-HCl, 1 mM DTT, pH 7.6) buffer, as per the guidelines outlined by Huang et al. (2014). The protein concentration was determined using the BCA protein assay kit (Bio-Rad, USA). After desalting the sample with an EmporeTM SPE cartridge C18 (standard density, bed diameter 7 mm, volume 3 mL, Sigma), it was centrifuged under vacuum and reconstituted with 40 μL of 0.1 % (*v*/v) formic acid solution.

2.3.2. TMT labeling and peptide fractionation

The digested peptides were labeled with TMT reagent (Applied Biosystems). Subsequently, the labeled peptides were isolated using a Thermo Scientific high pH reversed-phase peptide isolation kit. Following reconstitution and acidification with a 0.1 % TFA solution, the dried peptide mixture was loaded onto a high pH reversed-phase fractionation spin column and concentrated through centrifugation using an EmporeTM SPE cartridge C18 (standard density).

2.3.3. LC-MS/MS analysis

LC-MS/MS analysis was conducted by coupling an Easy nLC system (Proxeon Biosystems, now Thermo Fisher Scientific) to a Q Exactive mass spectrometer (Thermo Scientific) for either 60 or 90 min. The analytical method adhered to the protocol outlined in the literature (Li et al., 2018). A C18 reversed-phase analysis column (Thermo Scientific Easy column, 10 cm long, 75 μm inner diameter, 3 μm resin) was connected to a reversed-phase capture column (Thermo Scientific Acclaim PepMap100, 100 μm*2 cm, nanoViper C18). Peptides were loaded onto the column in buffer A (0.1 % formic acid).

2.3.4. Protein database searching and analysis

Protein database search and analysis were performed using Thermo Electron's Mascot 2.2 and Proteome Discoverer 1.4 software. MS/MS spectral data were analyzed quantitatively and qualitatively. Proteins were identified using the Sequest HT engine against the NCBI Goat_20161109 protein database.

2.3.5. Bioinformatics analysis

Table 1 lists all software and databases used for bioinformatics analysis.

Table 2
Predicted means at each treatment by muscle level for LTL pH, shear force (N), lightness (L*), redness (a*) and yellowness (b*) fresh color parameters within 48 h postmortem aging at 4 °C.

Entry		Post-mortem time period/h			
		0 h	12 h	24 h	48 h
pH value		6.48 ± 0.17^{a}	6.23 ± 0.10^{b}	5.96 ± 0.08^{c}	5.79 ± 0.23^{c}
shear force (kg)		7.95 ± 0.83^{a}	8.23 ± 0.89^{a}	6.96 ± 0.97^{ab}	5.99 ± 0.78^{b}
color	a*	11.61 ± 0.70^{a}	12.66 ± 0.45^{b}	13.92 ± 0.94^{c}	12.74 ± 0.66^{b}
	b*	8.45 ± 0.87^{a}	9.78 ± 0.61^{b}	10.68 ± 0.42^{b}	7.62 ± 0.71^{a}
	L*	32.49 ± 1.09^{a}	37.12 ± 0.77^{b}	41.22 ± 0.89^{c}	43.81 ± 1.12^{d}

2.4. Statistics and analysis

Data processing for pH, color, and shear stress using Microsoft Excel 2021. The significance analysis and Pearson correlation analysis were performed by SPSS 24 software. The resulting data were expressed as mean ± standard deviation. Proteomic and metabolomic data were analyzed using R software. Using the GO database (https://www.geneontology.org/) and KEGG database (https://www.genome.jp/kegg/) to GO and KEGG pathway annotations.3. Results and discussion.

2.5. Quality analysis of goat muscle

During the post-mortem conversion of muscle to meat, the reduction in muscle pH significantly affects meat quality traits, including color, tenderness, and juiciness (Huang et al., 2011). The changes in quality characteristics of goat muscles within the first 48 h post-mortem are presented in Table 2. The ultimate pH values in LTL averaged 5.7 (± 0.78). The pH of the muscle decreased rapidly within the first 12 h and then more gradually from 24 to 48 h post-mortem. The variation trend of pH is consistent with the results obtained by Li et al. (2019). At 0 h after slaughter, the pH value of meat was higher, which may be due to the large amount of glycogen consumption caused by the stress reaction before slaughter, which affected the rate and degree of pH decline, resulting in a higher final pH value. At 24 h to 48 h after slaughter, the pH values of goats tended to be flat or even increased slightly, which may be caused by the gradual removal of muscle rigidity with the extension of maturation time (Herrera-Mendez et al., 2006).

Tenderness is an important index affecting the taste of meat after slaughter, and shear force is an important basis for evaluating the tenderness of meat, which is inversely proportional to the tenderness of meat. The Warner-Bratzler shear force (WBSF) value remained stable up to 12 h post-mortem but consistently decreased after 12 h of aging ($P < 0.05$). The color of the meat is primarily influenced by myoglobin and its associated proteins (Khliji et al., 2010) and is also related to animal varieties and fiber types. The data we found in Table 2 showed that there were significant differences both L^* and a^* values within 48 h post-mortem ($P < 0.05$). There was no significant difference in b^* value between 12 h ~ 24 h postmortem, but it significantly decreased during 24 h ~ 48 h postmortem aging ($P > 0.05$).

Data in the figure are represented as mean (±SD). Differences between means with different superscripts exceed the estimate of least significant difference at 5 % critical value. Comparisons were made at the level of a single rows parameter and do not apply to column.

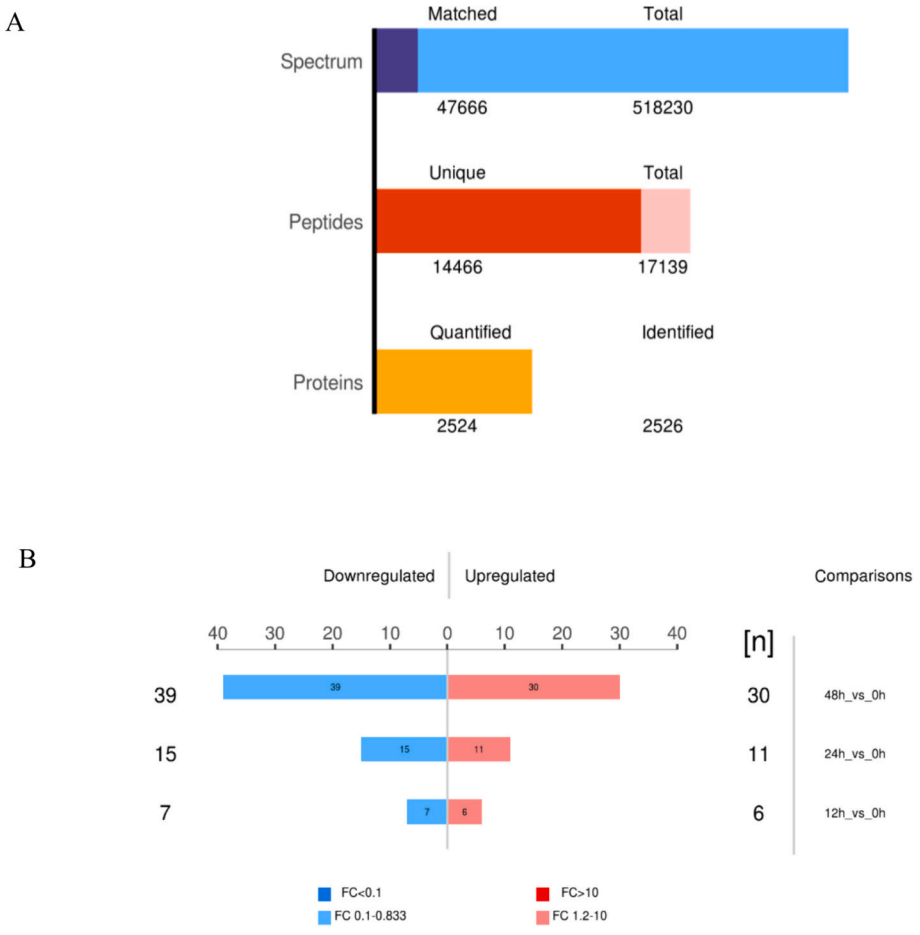

Fig. 1. (A) Proteins identification and quantitative results statistic. (B) Proteins identification and quantitative results statistics.

2.6. Identification of proteins by quantitative proteomics analysis

TMT coupled with LC-MS/MS mass spectrometry was employed to investigate the differentially expressed proteins in longissimus dorsi muscle samples from Xinjiang goats over a 48-h period post-mortem. Mass spectrometry experiments obtained a total of 518,230 secondary mass spectra. A total of 17,139 peptides were identified, with 14,466 of these being unique peptides. Additionally, 2526 proteins were identified, out of which 2524 were quantifiable. Among these, 108 proteins were identified as significantly differentially expressed, as depicted in Fig. 1(B). Yang et al. (2024) used TMT quantitative proteomics to analyze flavor-related proteins in mutton and their related pathways, and identified a total of 2244 proteins for quantitative analysis. This is similar to the number of proteins identified in this study. Fold Change (FC) was used to screen significant DEPs. To differentiate the proteins showing increased expression and decreased expression across different groups, a FC criterion of 1.2 (indicating an increase by more than 1.2-fold or a decrease by less than 0.83-fold) along with a significance level of $P < 0.05$ (determined using a *t*-test or similar statistical test) were employed as the benchmarks. Across the three comparison groups, a total of 13 DEPs were identified in the 0 h/12 h group, with seven proteins up-regulated and six down-regulated, as shown in Fig. 1(B). In the comparison group between 0 h and 24 h, a total of 26 distinct proteins were detected, including 15 that exhibited increased expression and 11 that showed decreased expression. Similarly, in the 0 h and 48 h comparison group, thirty-nine differentially expressed proteins were found, with thirty demonstrating reduced expression and forty-nine showing increased expression.

The primary structural proteins in muscle consist of actin, myosin, troponin, tropomyosin, desmin, connectin, among others (Yuan et al., 2010). Degradation of structural proteins is the main cause of the postmortem muscle tenderization (Lana & Zolla, 2016). In this research, 27 DEPs associated with muscle contraction and cell structure were identified. Among these, DEPs such as CAPZA1, CARNS1, ACTR3B, MYH1, DYNC1I2, MYL9, DYNLL1, MYH4, and STRN3, which are linked to muscle fiber composition, were down-regulated in the 0 h/48 h comparison group. Conversely, HIST1H1B and VPREB1 were found to be up-regulated in the 0 h/24 h comparison group. Furthermore, the expression level of COL12A1 increased in the comparison between the 0 h and 12 h groups, as shown in Supplementary Table S2. The expression levels of DEPs related to muscle fiber composition were higher at the early stage of postmortem aging and lower at 48 h after maturity. The expression levels of these DEPs would affect the quality of goat meat, and this is consistent with previous research (de Oliveira et al., 2019).

In addition, this study found that ECHS1, ACTR3B, COL6A1 and STRN3 can simultaneously characterize tenderness and color. Sarcoplasmic proteins are significant indicators for various meat quality issues, including discoloration, variations in color within and between muscles, and the oxidation of meat proteins in pork and beef (Kim et al., 2018).

Skeletal muscle, heart, and smooth muscle are the primary tissues that contain ACTR3B protein. The two main constituents of the myofibrillar protein filament were actin and myosin (myosin 1 and 2). This phenomenon may be attributed to myosin and actin being the key components of crude myofibrils and their microfilaments, suggesting that the strong interaction between these two proteins significantly impacts meat tenderness (Gagaoua, Bonnet, De Koning, & Picard, 2018; Gagaoua, Bonnet, Ellies, et al., 2018; Gagaoua et al., 2017). Meat color may be influenced by the ultrastructural alterations in proteins brought on by hydrolysis and degradation, which alter light scattering (Hughes et al., 2017).

Myosin heavy chain 4 (MYH4 or MYHC IIb) fibers contribute to differential muscle growth, and can be used as an indicator of muscle protein synthesis and muscle growth rate (Morales et al., 2015). Myosin light polypeptide 1 (MYL1) was also supposed to play an important role in determining meat quality (Wang et al., 2011). Moreover, there was a

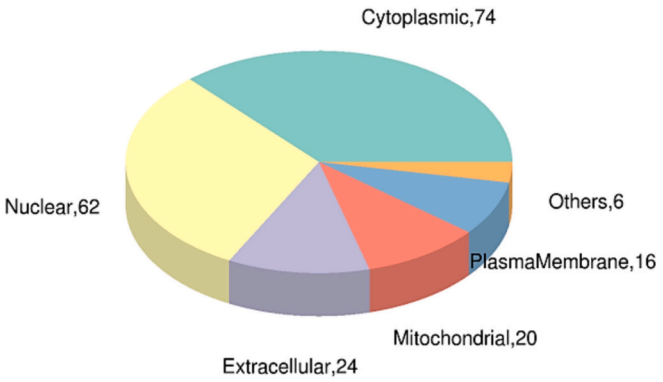

Fig. 2. Pie map of subcellular localization of differentially expressed proteins.

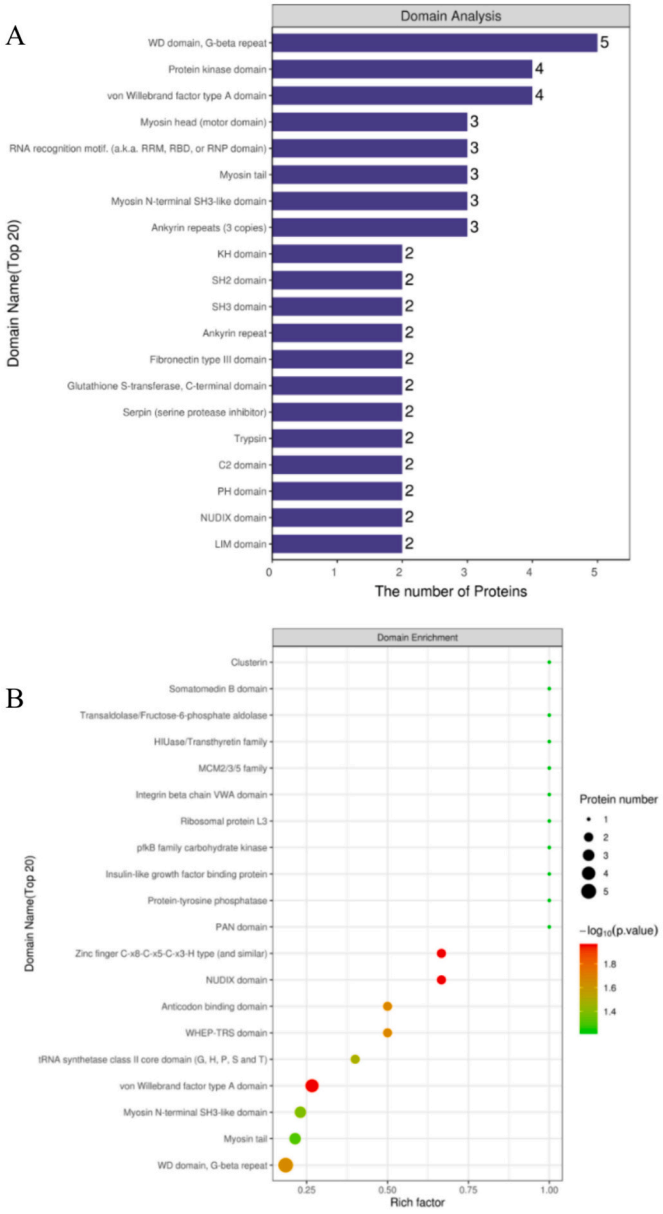

Fig. 3. (A) Domain analysis of differentially expressed proteins. (B) Domain enrichment analysis diagram.

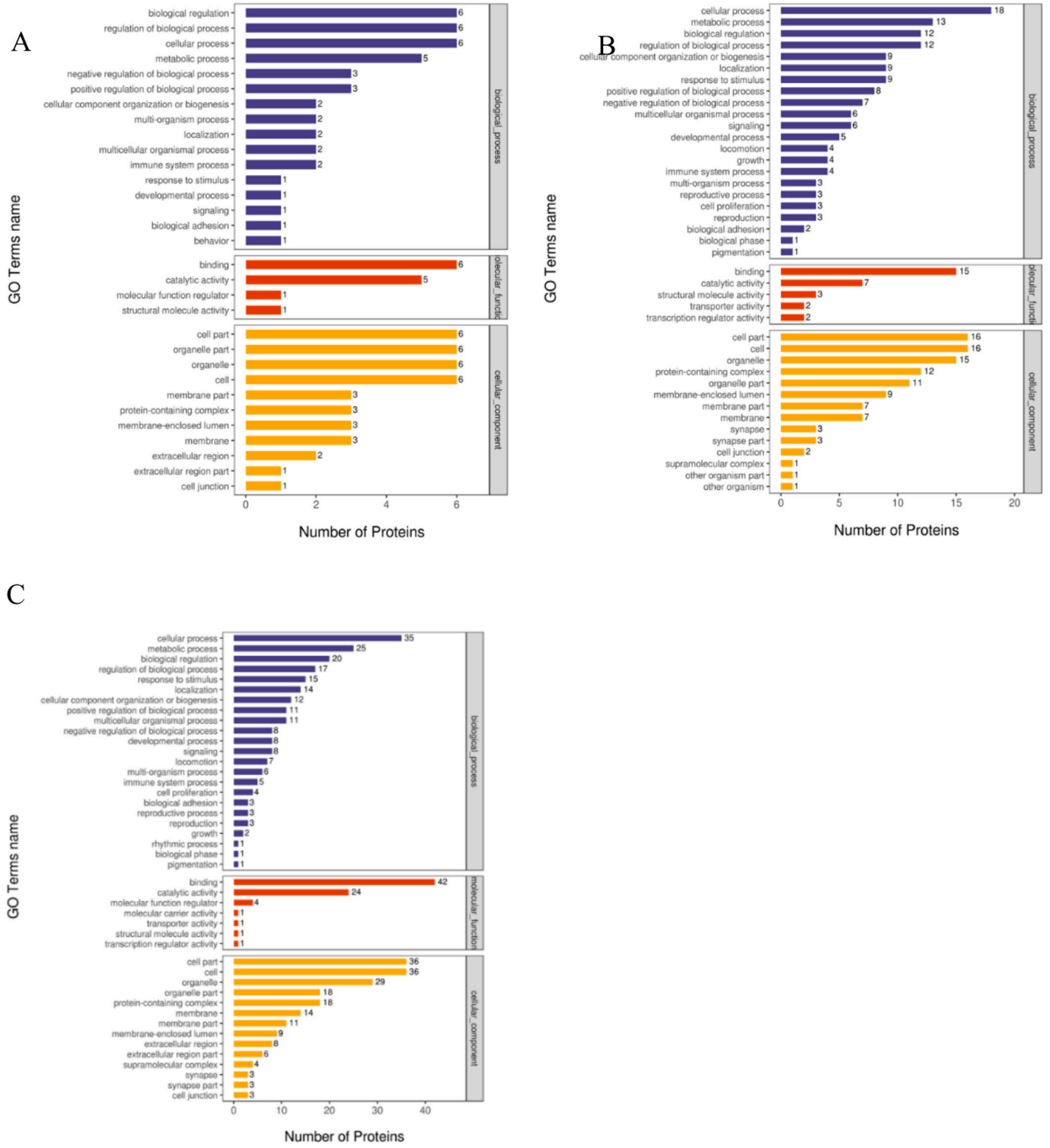

Fig. 4. Results of GO functional annotation of DEPs during postmortem in goats; (A) 12 h postmortem of goat. (B) 24 h postmortem of goat. (C) 48 h postmortem of goat.

notable increase in the expression of ALB In the evaluation of the 0 h and 48 h groups, with the function of ALB in meat browning yet to be explored. ALB was also found in the study on the proteome profile of bovine muscle (Bouley et al., 2004). In this study, cysteine and glycine-rich proteins (CARNS1 and GARS1) were down-regulated in the 0 h/48 h group, which were related to muscle structural proteins and could be used as candidate molecular marker for test the meat quality (Xu et al., 2010). Western blot validation tests could be performed to verify the screened differential proteins later, which was also the limitation of this study.

2.7. *Subcellular localization analysis of DEPs*

An organelle refers to a small structure within the cytoplasm, such as mitochondria or endoplasmic reticulum, each with distinct morphology and function. These organelles serve as vital sites where proteins carry

out various functions within the cell. Each subcellular organelle typically executes specific cellular tasks. Therefore, analysis of protein subcellular localization helps us to explore the function of proteins in cells further. In this work, the subcellular structure prediction software CELLO (http://cello.life.nctu.edu.tw/) was used to analyze the subcellular localization of all differentially expressed proteins, and the results were presented in the form of the pie chart. The number and distribution of proteins in each subcellular organelle were showed in Fig. 2: nucleus (62), cytoplasm (74), plasma membrane (16), mitochondria (20), extracellular (24), and others (6).

2.8. Internal domain analysis of DEPs

Domain prediction is crucial for identifying key functional regions of proteins and understanding their potential biological roles (Wang et al., 2021). A protein domain consists of two or more localized regions on a large protein molecule that are spatially distinct due to the close linkage of adjacent super-secondary structures along the polypeptide chain. Typically comprising tens to hundreds of amino acid residues, each domain possesses a unique three-dimensional structure and performs distinct biological functions (Barik, 2022). Generally, protein interactions with other proteins or small molecules typically occur at the domain level, where alterations or modifications in amino acids within these domains can lead to functional changes in the protein. Consequently, experiments focusing on amino acid mutations can provide valuable insights for understanding these functional changes.

In this study, domain prediction for differentially expressed proteins was conducted, and Fisher's exact test was utilized for enrichment analysis of these predicted differentially expressed proteins. Fig. 3(A) presents the top 20 predicted domains in the form of bar graphs. These include the WD domain, G-beta repeat, protein kinase domain, myosin head (motor domain), RNA recognition motif (RRM) domain, myosin tail, among others.

Fig. 3(B) presents the results obtained from analyzing the enrichment of domains in the differentially expressed proteins. The domains that showed enrichment include the NUDIX domain, Zinc finger C-x8-C-x5-C-x3-H type (and similar), WHEP-TRS domain, Anticodon binding domain, WD domain (G-beta repeat), tRNA synthetase class II core domain (G, H, P, S, and T), and the Myosin N-terminal SH3-like domain.

2.9. Functional analysis of DEPs

A comprehensive analysis of Gene Ontology (GO) annotations was performed on the entire protein composition of goat meat, utilizing protein databases. The purpose of this analysis was to elucidate the functions, locations, and biological pathways related to various proteins found in goat meat. A set of dynamically updated standardized terminology is provided by the GO functional classification system, which is used to characterize the characteristics of genes and gene products in living things. Gene Ontology (GO) The categorizations include Molecular Function (MF), Cellular Localization (CL), and Biological Activity (BA). The DEPs in the 0 h/12 h, 0 h/24 h, and 0 h/48 h comparison groups from Xinjiang goats were analyzed using secondary classification within the GO framework, based on the results from the protein GO annotation. The DEPs exhibited notable variances in biological functions, molecular properties, and cellular structures of goat meat across various time intervals during post-mortem processing. Lamri et al. (2023) performed proteomic analysis of goat meat at 30 min, 6 h and 24 h after slaughter, and the functional properties of DEPs in goat meat were different at different time points.

The results of GO functional annotation of DEPs within 12 h after slaughter and maturation of goat were showed in Fig. 4(A). Based on the classification of biological processes, the proteins identified within the first 12 h of post-mortem aging are categorized into 16 different biological process groups, involving a total of 44 proteins. This includes 6 proteins that are active in biological regulation, regulation of biological

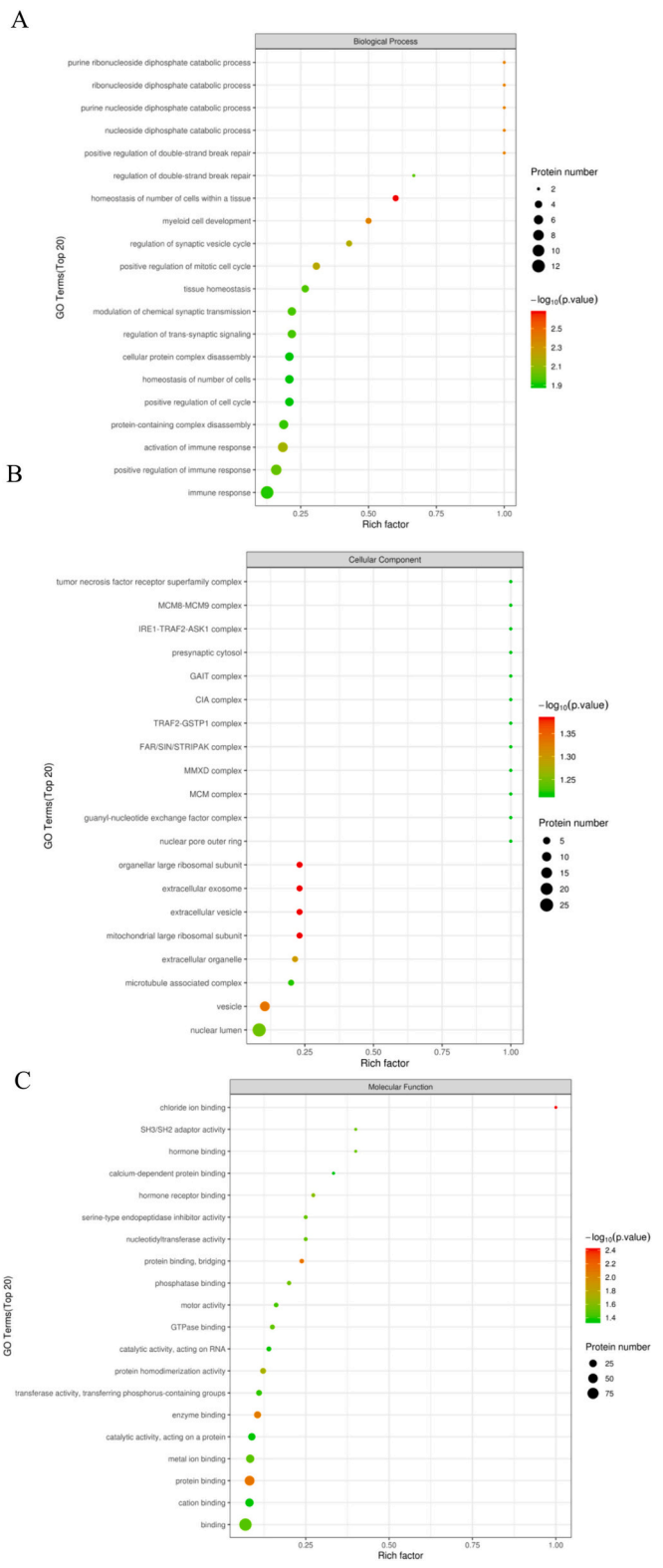

Fig. 5. GO functional enrichment bubble diagram under Biological Process (BP) classification (A), Cellular Component (CC) classification (B), Molecular Function (MF) classification (C).

processes, and cellular processes. There were 5 proteins involved in the metabolic process, 3 proteins involved in the negative regulation of the biological process 3 proteins involved in the positive regulation of the biological process, and few proteins involved in cell function and composition. The identified proteins were categorized into four

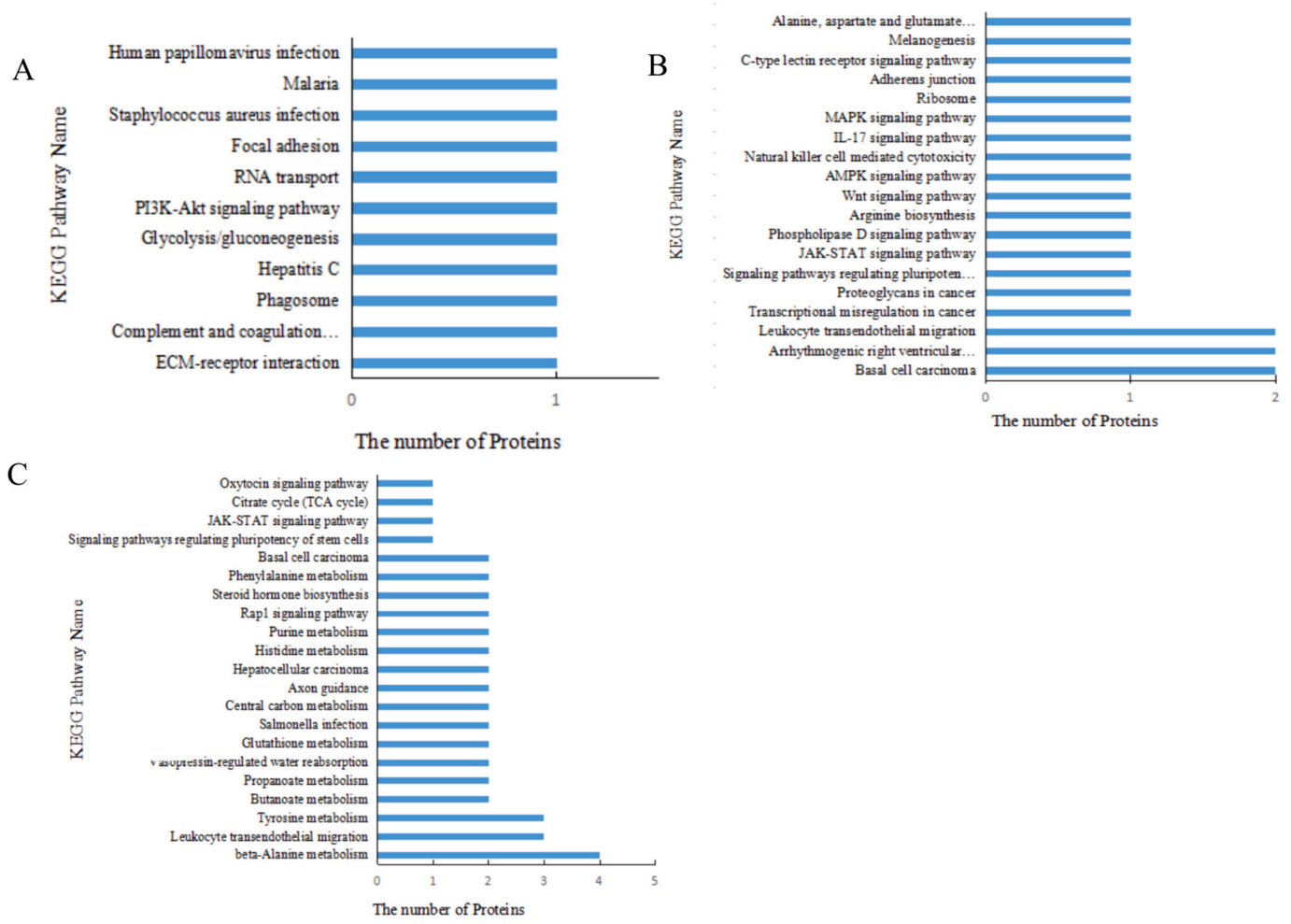

Fig. 6. DEPs are mainly involved in the KEGG metabolic pathway. (A) 0 h/12 h; (B) 0 h/24 h; (C) 0 h/48 h.

functional groups based on their molecular roles. These encompassed six proteins associated with binding function, five proteins involved in catalytic activity, one protein acting as a molecular function regulator, and one protein contributing to structural molecular activity. In the study, 40 proteins were identified, among these 6 proteins associated with cells, cell fractions, organelles, and organelle fractions, alongside 3 proteins related to membranes, membrane fractions, membrane-enclosed cavities, and protein-containing complexes.

The results of the GO functional annotation of DEPs within 24 h during post-mortem aging of goat were showed in Fig. 4(B). Classified according to biological processes, it is found that the identified proteins within 24 h after slaughter and maturation are involved in 22 biological process categories, a total of 142 proteins, including 18 proteins involved in cellular processes and 13 proteins involved in metabolic processes. There are 12 proteins involved in both biological regulation and regulation of biological processes. According to the molecular function, the identified proteins were classified into 5 functional categories, a total of 29 proteins, including 15 proteins involved in binding function, 7 proteins involved in catalytic activity, and 3 proteins involved in structural molecular activity. In total, 104 proteins were identified, among which 16 proteins were associated with cells and cell parts, 15 proteins were linked to organelles, and 12 proteins were part of protein complexes.

The results of GO functional annotation of DEPs in the maturation process of goat within 48 h after slaughter are shown in Fig. 4(C). According to the classification of biological processes, the identified proteins within 48 h after slaughter involve 23 process categories, a total of 223 proteins, of which 35 proteins are involved in cellular processes and 25 proteins are involved in metabolic processes. There were 20 proteins involved in biological regulation, 17 proteins involved in biological process regulation, and few proteins involved in other processes. Classified by molecular function, the identified proteins were involved in 7 functional categories, a total of 74 proteins, of which 42 proteins were involved in binding function, 24 proteins were involved in catalytic activity function, and 4 proteins were involved in molecular function regulator. A 198 proteins were identified, comprising 36 proteins related to cells and cell parts, 29 proteins associated with organelles, and 18 proteins connected to organelle parts and protein-containing complexes.

At the same time, Fisher's Exact Test was used to perform GO functional enrichment analysis of the differentially expressed proteins. All differentially expressed proteins were compared with the annotation results of GO function of all the identified proteins. Fisher's Exact Test was used to obtain the significance of the difference between the two DEPs so as to find out the functional categories of all differentially expressed proteins ($P < 0.05$). Bubble plots were used to show the enrichment of 20 items under three GO categories in the maturation process of goats within 48 h after slaughter. In BP, it is involved in the homeostasis of number of cells within a tissue, ribonucleoside diphosphate catabolic process, the positive regulation of double-strand break repair and other important biological processes, as shown in Fig. 5(A); On CC, it is mainly involved in chloride ion binding, protein binding, bridging, enzyme binding and other molecular functions, as shown in Fig. 5(B). In MF, it is mainly involved in extracellular vesicles, mitochondrial large ribosomal subunit, extracellular exosomes and other

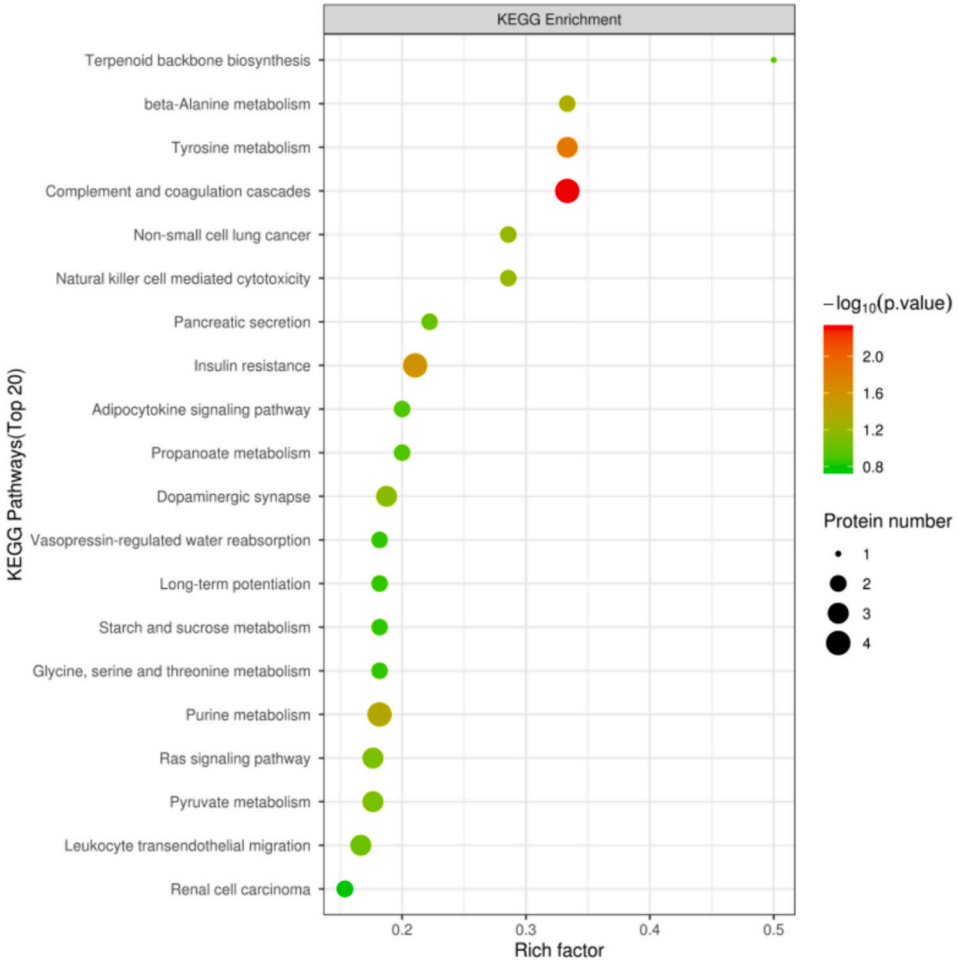

Fig. 7. KEGG pathway enrichment bubble diagram within 48 h postmortem.

functions. As shown in Fig. 5(C).

2.10. Kyoto encyclopedia of genes and genomes pathway analysis of DEPs

To analyze the biological processes involved in proteins more systematically and comprehensively, the changes in metabolic pathways were explored. Then, the rules of meat quality changes were expounded from the perspective of a series of protein coordination. Hence, in our research, the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database was employed to examine the biological pathways associated with the identified DEPs. The results showed that 108 differentially expressed proteins were involved in 142 metabolic pathways within 48 h after goat slaughtering at 4 °C. The results of the 0 h/12 h comparison group showed that 13 DEPs were involved in 10 metabolic pathways. The results of the 0 h/24 h comparison group showed a total of 26 differentially expressed proteins involving 56 metabolic pathways. The results of the 0 h/48 h comparison group showed a total of 69 differentially expressed proteins, involving 91 metabolic pathways.

As shown in Fig. 6(A), for the 0 h/12 h comparison group during goat slaughter maturation, among the annotated metabolic pathways, the identified DEPs were mainly involved in ECM-receptor interaction. Complement and coagulation cascades, Glycolysis/gluconeogenesis, PI3K - Akt signaling pathway, and other important pathways changed dramatically. The DEPs identified in the 0 h/24 h comparison group were mainly involved in Basal cell carcinoma, Arrhythmogenic right ventricular cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy, and arrhythmogenic right ventricular cardiomyopathy. Leukocyte trans endothelial migration, WNT signaling pathway, MAPK signaling pathway and other important pathways (Fig. 6(B)). The DEPs identified in the 0 h/48 h comparison group were mainly involved in Beta-alanine metabolism, Leukocyte trans endothelial migration, Tyrosine metabolism, Propanoate metabolism, Vasopressin-regulated water reabsorption, Glutathione metabolism, etc. (Fig. 6(C)).

Bubble Fig. 7 shows KEGG pathway enrichment analysis of differentially expressed proteins in the process of slaughter maturation within 48 h. The enriched metabolic pathways are Complement and coagulation cascades, Tyrosine metabolism, Insulin resistance, Purine metabolism, beta-alanine metabolism, Pyruvate metabolism, Propanoate metabolism, and Terpenoid backbone biosynthesis.

Bioinformatics methods were used to conduct GO and KEGG analysis on TMT data. Proteins related to the tricarboxylic acid cycle (TCA cycle), oxidative phosphorylation, glycolysis, and amino acid metabolism were found. The process of glycolysis converts glucose to pyruvate, which produces minute amounts of ATP (energy) and NADH (reducing power). The process of gluconeogenesis synthesizes glucose from non-carbohydrate precursors, suggesting a connection between energy metabolism and the ability of muscles to expand (Jiang & Ge, 2014).

Besides, adenosine kinase is associated with energy metabolism and plays an important role in maintaining energy homeostasis in muscles (Li et al., 2016). In the present study, a total of 23 differentially expressed proteins involved in energy metabolism were identified. Among them, part of the DEPs involved in oxidative phosphate and glycolytic pathway (MRPL41, NQO2, MRPS15, TRPT1, MRPL22, IDH2, SIPA1L1, RBP4, LDHB, ART1) higher expression in 0 h/48 h and 0 h/24

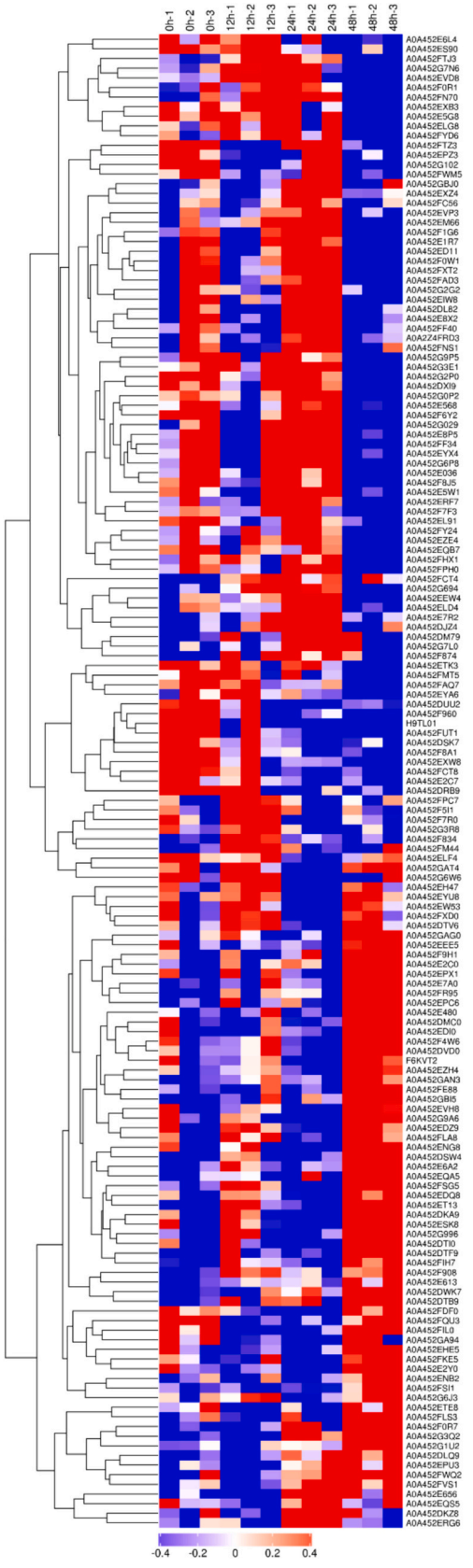

Fig. 8. Clustering analysis of three groups of differentially expressed proteins.

h groups, while a small amount of DEPs related to the TCA cycle (GARS1, PC, PGM2, PKIA) were down-regulated only in the 0 h/48 h comparison group. An increase in these DEPs suggested that an enhanced aerobic energy metabolism occurs in the muscles 12 h after slaughter, which was consistent with some studies (Jia et al., 2006). The decrease of these DEPs involved in the TCA cycle and oxidative phosphorylation pathways and increase of DEPs involved in the glycolysis process may be associated with decreased aerobic metabolic capacity and increased anaerobic metabolic capacity in muscles (Kim et al., 2019).

This study found that PTPN11, IDH2, SMDT1, LDHB, PPA2, PMPCB and GARS1 can simultaneously characterize tenderness and color. This was consistent with the results of some related studies (Malheiros et al., 2019; Picard et al., 2019). Transformation of pyruvic acid to lactic acid is catalyzed by LDH. Studies have shown that LDH activity in muscle 0 h after death cannot accurately estimate the difference in meat quality (Chulayo & Muchenje, 2016). Still, the covalent binding of 4-hydroxy-2-nonylaldehyde (HNE) and LDH affects the formation of NADH and the reduction of MetMb (Ramanathan et al., 2014), which can promote meat color stability (Kim et al., 2009). Interestingly, the significantly up-regulated expression of LDH could explain the role of LDH in the stabilization of normal meat color.

2.11. Quantification and screening of differentially expressed proteins

Tree heat-map for cluster analysis of differentially quantified proteins. The significantly different proteins (log2 Expression) were shown in different colors in the Heat-map. Clearly up-regulated protein (red), significantly down-regulated protein (blue), and no protein quantitative information (gray).

To analyze the expression patterns of samples within and between groups, test the project's grouping rationality, and clarify whether changes in the expression of DEPs can represent the significant effect of biological treatment on samples, the Hierarchical Cluster algorithm was used to group and classified the differentially expressed proteins in the comparison group. A heatmap was employed to show the outcomes. The findings of the analysis were showed in Fig. 8. Gray indicates no protein information, blue indicates down-regulation, and red indicates up-regulation.

A protein is represented by each row (ordinate is significantly differentially expressed protein), and a group of samples is represented by each column (abscissa is sample information). The three proteins' up- and down-regulation at 24 h following slaughter (24 h-1, 24 h-2, and 24 h-3) were constant. At 48 h during post-mortem (48 h-1, 48 h-2 and 48 h-3), the up-regulation and down-regulation of protein expression of the three proteins were more consistent, and formed clusters that were significantly different from those of (24 h-1, 24 h-2 and 24 h-3).

2.12. Correlation between DEPs during aging and physical and chemical parameters of post-mortem goats

Table 3 showed Pearson's significant correlation analysis between DEPs and meat quality characteristics in the post-slaughter maturation of goats. DEPs are classified into four groups based on specific functions: structural proteins, hydrolytic proteins, metabolic enzymes, and oxidative proteins. * indicates significant correlation between protein and quality traits ($P < 0.05$), ** indicates highly significant association ($P < 0.01$). Most of the DEPs were significantly correlated with multiple quality traits, and these proteins can be used as potential biomarkers for comprehensive evaluation of goat meat quality. Notably, most of the biomarkers were involved in stress tolerance of meat tenderization, which may play an important role in combating the decline in protein quality and serve as a biomarker combination for future studies on meat quality during meat maturation in goat meat.

A large number of studies have agreed that the post-mortem tender process of meat was related to the proteolysis of myofibrillar and related

Table 3
Pearson's correlation between diffferentially expressed proteins (DEPs) and quality characteristics of goat muscle muscles at different maturation times within 48 h.

Accession	Description	Gene Name	pH	shear force	a*	b*	L*
A0A452F3R4	Coiled-coil domain containing 105	CCDC105	0.434	0.463	−0.471	−0.417	−0.688*
A0A2Z4FR65	Beta-catenin	CTNNB1	0.386	0.478	−0.695*	−0.044	−0.705*
A0A452FCT8	Ubiquitin carboxyl-terminal hydrolase 13	USP13	0.585*	0.659*	−0.504	−0.327	0.863**
A0A452FUT1	Von Hippel-Lindau disease tumor suppressor	VHL	0.585*	0.639*	−0.580*	−0.112	−0.844**
H9TL01	Growth factor receptor-bound protein 2	GRB2	0.514	0.591*	−0.573	−0.237	−0.805**
A0A452F960	Tyrosine-protein phosphatase non-receptor type 11	PTPN11	0.515	0.671*	−0.472	−0.063	−0.852**
A0A452DKA9	Four and a half LIM domains protein 1	FHL1	−0.291	−0.289	0.164	−0.597*	0.401
A0A452DTB9	MARVEL domain-containing protein	SYPL1	−0.435	−0.569	0.449	−0.065	0.788**
B3VHM9	Serum albumin	ALB	−0.286	−0.394	0.202	−0.367	0.528
A0A452EDQ8	Myotilin	MYOT	−0.093	−0.331	0.105	−0.556	0.433
A0A452DLQ9	Uncharacterized protein	ECHS1	−0.340	−0.633*	0.433	0.094	0.689*
A0A452FWD7	Carnosine synthase 1	CARNS1	0.192	0.508	−0.049	0.238	−0.594*
A0A452G9L1	Actin-related protein 3B	ACTR3B	0.436	0.680*	−0.253	−0.009	−0.728**
A0A452G9P5	Myosin-1	MYH1	0.280	0.523	0.205	0.585*	−0.377
A0A452G2P0	Cytoplasmic dynein 1 intermediate chain 2	DYNC1I2	0.395	0.508	−0.160	0.565	−0.646*
A0A452E036	Sarcolemmal membrane-associated protein	SLMAP	0.093	0.335	−0.083	0.601*	−0.356
A0A452F8J5	Dynein light chain-1	DYNLL1	0.208	0.377	−0.104	0.619*	−0.431
A0A452DRB9	Collagen alpha-1(VI) chain	COL6A1	0.240	0.641*	−0.441	−0.065	−0.772**
A0A452F8A1	WD_REPEATS_REGION domain-containing protein	STRN3	0.519	0.715**	−0.496	−0.013	−0.831**
A0A452G1Q7	Coiled-coil-helix-coiled-coil-helix domain-containing protein 2	CHCHD2	−0.086	−0.137	0.200	0.733**	0.189
A0A452DUU2	Ankyrin-1	ANK1	0.432	0.561	−0.608*	−0.401	−0.757**
A0A452FLS3	Ecto-NOX disulfide-thiol exchanger 1	ENOX1	−0.163	−0.545	0.161	−0.683*	0.564
A0A452DSW4	Amine oxidase	LOC102168295	−0.499	−0.573	0.096	−0.560	0.653*
A0A452FE88	Aldo_ket_red domain-containing protein	LOC106503221	−0.521	−0.697*	−0.228	−0.577*	0.576
A0A452F9H1	Plasma protease C1 inhibitor	SERPING1	−0.338	−0.516	0.105	−0.550	0.503
A0A452EDN8	Uncharacterized protein	LOC102185621	0.318	0.460	−0.300	−0.215	−0.661*
A0A452FAQ7	Uncharacterized protein	LOC108634933	0.603*	0.765**	−0.130	0.359	−0.763**
A0A452F6T5	Probable ATP-dependent RNA helicase DDX23	DDX23	0.246	0.288	−0.107	0.636*	−0.355
A0A452EYX4	LIM and senescent cell antigen-like-containing domain protein	LIMS2	0.032	0.139	−0.025	0.617*	−0.199
A0A452DRD5	Eukaryotic translation initiation factor 3 subunit E	EIF3E	0.044	0.059	−0.610*	−0.460	−0.452
A0A452E0V0	eRF1_1 domain-containing protein	ETF1	0.180	0.363	−0.462	0.178	−0.579*
A0A452G3E1	Nudix hydrolase domain-containing protein	NUDT18	0.271	0.529	0.088	0.716**	−0.474
A0A452EQL8	C-reactive protein	CRP	0.320	0.578*	−0.376	−0.058	−0.683*
A0A452FMT5	Histone-binding protein RBBP7	RBBP7	0.570	0.690*	0.034	0.263	−0.628*
A0A452G6P8	Nudix hydrolase domain-containing protein	NUDT16	0.125	0.165	0.130	0.698*	−0.190
A0A452E5W1	Y-box-binding protein 3	YBX3	0.000	0.165	−0.192	0.647*	−0.298
A0A452FCY3	Uncharacterized protein	SIPA1L1	−0.414	−0.746**	−0.274	−0.292	0.534
A0A452FU43	Retinol-binding protein 4	RBP4	−0.371	−0.442	0.070	−0.085	0.539
A0A452DQC8	Isocitrate dehydrogenase [NADP]	IDH2	−0.398	−0.794**	−0.168	−0.286	0.619*
A0A452FFA1	Essential MCU regulator	SMDT1	−0.450	−0.627*	−0.066	−0.348	0.614*
A0A452E480	Lactate dehydrogenase B	LDHB	−0.295	−0.699*	−0.140	−0.445	0.598*
A0A452DWK7	Inorganic pyrophosphatase 2,	PPA2	−0.559	−0.814**	0.265	−0.217	0.883**
A0A452F341	Mitochondrial-processing peptidase subunit beta	PMPCB	−0.577*	−0.894**	−0.048	−0.222	0.755**
A0A452EXW8	Glycine–tRNA ligase	GARS1	0.557	0.633*	−0.384	−0.027	−0.866**
A0A452ERF7	Phosphoglucomutase-2	PGM2	0.019	0.204	0.213	0.738**	−0.159
A0A452EXZ4	Mitochondrial ribosomal protein S15	MRPS15	−0.270	−0.359	0.511	0.604*	0.455
A0A452FEN6	Mitochondrial ribosomal protein L22	MRPL22	−0.289	−0.426	0.192	0.613*	0.443
A0A452EM66	Ribosyldihydronicotinamide dehydrogenase [quinone]	NQO2	−0.062	0.246	0.254	0.867**	−0.119
A0A452E7R2	39S ribosomal protein L41, mitochondrial	MRPL41	−0.119	−0.269	0.642*	0.776**	0.470
A0A452GAD8	Alanine aminotransferase 2	GPT2	−0.230	−0.167	0.090	0.634*	0.145
A0A452E5G8	Cell death regulator Aven	AVEN	0.383	0.642*	−0.052	0.549	−0.598*
A0A452ELG8	MCM domain-containing protein	MCM8	0.308	0.445	0.226	0.711**	−0.287
A0A452F4N6	Protein transport protein Sec24B	SEC24B	0.321	0.584*	−0.322	0.059	−0.760**
A0A452FFZ5	Nucleolysin TIAR	TIAL1	−0.433	−0.451	0.419	0.178	0.589*
A0A452FB45	Guanine nucleotide-binding protein-like 1	GNL1	−0.444	−0.590*	−0.154	−0.082	0.569
A0A452DTI0	Catechol *O*-methyltransferase	COMT	−0.062	−0.218	0.116	−0.706*	0.278
A0A452FF34	UDENN domain-containing protein	DENND10	0.049	0.106	−0.035	0.649*	−0.180
A0A452DM79	H15 domain-containing protein	H1–5	−0.078	−0.027	0.756**	0.696*	0.292
A0A452ELF4	Methionine-R-sulfoxide reductase B2	MSRB2	0.421	0.401	−0.656*	−0.592*	−0.525
A0A452F7E0	Myeloid leukemia factor 1	MLF1	−0.154	−0.064	−0.337	−0.660*	−0.141
A0A452EKQ9	ELAV-like protein	ELAVL1	0.495	0.423	−0.233	−0.182	−0.587*
A0A452DSK7	TNF receptor-associated factor 2	TRAF2	0.359	0.596*	−0.698*	−0.342	−0.793**
A0A452FQ83	Uncharacterized protein	ARMC6	0.409	0.592*	−0.574	−0.209	−0.799**

* Signifficant correlation ($p < 0.05$)
** Highly signifficant correlation ($P < 0.01$).

structural proteins. One of the primary metabolic processes that occurs in meat following livestock animal slaughter is proteolysis.

It has been shown that the representative systems in charge of post-mortem proteolysis in meat are cathepsins, caspases, captains, and proteasomes (Huff-Lonergan et al., 2010). In this research, 18 DEPs associated with proteolysis were identified. Particularly, DEPs such as VPS36, PNPT1, RBBP9, and NUDT experienced a significant decrease in expression in the 0 h/12 h group. These DEPs play a crucial role in goat meat quality by engaging in the hydrolysis of phosphoric acid and influencing the conversion of glycogen. In addition, the expressions of NUDT18, NUDT16, USP13, DDX23, LIMS2, CRP, CLIC4 and CACNB3 were significantly down-regulated in 0 h/24 h group and 0 h/48 h, respectively. The results indicated that the proteolytic capacity decreased with the prolongation of postmortem aging time. This

indicated that these DEPs were involved in the degradation of proteins during postmortem aging and were one of the reasons for the difference in postmortem meat quality.In this study, the expression of CLIC4 and CACNB3 were low at the early stage of maturation, which inhibited the hydrolysis of protein to some extent. Laville et al. (2009) showed that voltage-dependent ion channel proteins 1 and 2 were highly abundant in the tender muscle group. NUDT18, NUDT16, and DDX23 were components of the 26S proteasome, which were involved in the ATP-dependent degradation of ubiquitinated proteins and play a key role in maintaining protein homeostasis (www.uniprot.org). Previous reports, the relationship between the degradation of meat proteins or proteolytic peptides and the sensory properties, such as taste, aroma, and bitterness (Zhou et al., 2019). CTNNB1, USP13 and VHL were typical hydrolytic enzymes that improve meat quality through protein hydrolysis. The down-regulated expression of these proteins at the later stage of goat meat maturation, which indicated that the degradation ability of proteins was decreased and the meat quality had gradually completed the tender process.

Proteins known as calcium release channel proteins impact meat quality. Calcium/calmodulin-dependent protein kinase type II (CaMKII) regulates $Ca2^{+}$ release via a calcium release channel, whereas troponin C and parvalbumin collaborate to control muscle contraction and relaxation (Haiech et al., 2019). In this study, DEPs related to the regulation of Ca^{2+} were detected. Significant down-regulation of CACNB3 differential expression was detected in the 0 h/24 h group. KEGG and functional annotation results showed that (CACNB3) was involved in muscle contraction and MAPK signaling pathway.

Apart from the proteins that were previously described, there are certain biochemical pathways that are associated with meat quality, including signaling and cell death. The protein known as four-and-a-half LIM domains 1 (FHL1) is associated with the cytoskeleton and is a member of the FHL protein family within the LIM super protein family (Chu et al., 2000). Numerous distinct functional categories, such as structural proteins, signal transducers, transcription regulators, receptors, and channels, can be associated with these FHL1 relationships (Lee et al., 2015). Because of this, it has been shown that FHL1 and meat softness are positively connected (Gagaoua, Bonnet, De Koning, & Picard, 2018; Gagaoua, Bonnet, Ellies, et al., 2018). This relationship may be explained by FHL1's function in controlling gene transcription, cell proliferation, metabolism, and apoptosis (Shathasivam et al., 2010). It was noteworthy that this study discovered a negative correlation between goat color and FHL1. This may be because of the way that FHL1 interacts with metabolic enzymes to respond to oxidative stress and hypoxia, which can have an impact on glycolysis (Gagaoua, Bonnet, De Koning, & Picard, 2018; Gagaoua, Bonnet, Ellies, et al., 2018).

3. Conclusion

Differential proteomic analysis of the LTL muscle obtained from Xinjiang goats 48 h after slaughter was performed using the TMT technique. A total of 108 DEPs were identified. Six DEPs, namely COL12A1, MRPL46, CTNNB1, MYH1, CAPZA1 and MYL9, have a direct effect on the quality attributes of meat, such as tenderness and color. Further discussion found that the expression of proteins associated with oxidative metabolism (MSRB2, ENOX1, LOC102170282, GSTM1 and AOC3) gradually increased with the extension of postmortem aging time, especially between 24 and 48 h. The early energy metabolism of goats after slaughter is rapid,resulting in muscle glycogen consumption, ATP supplementation, lactic acid is accumulated, and pH value is rapidly decreased. 24 h after slaughter, the muscle contraction response was intensified and the tenderizing degree was accelerated. With the extension of postmortem ripening time, the tenderizing stage of meat began to slow down, and adverse factors hindered the optimization of meat quality appeared at 48 h. This study provides a proteomic perspective for the study of post-slaughter maturation of goat meat, although further work is needed for more comprehensive protein identification.

CRediT authorship contribution statement

Duoduo Zhang: Writing – original draft, Resources, Methodology, Data curation. **Hong Yu:** Writing – review & editing, Software, Data curation. **Minghui Gu:** Writing – review & editing, Methodology, Investigation. **Shiquan Zhang:** Methodology. **Xiaolin Ma:** Visualization, Validation, Software. **Yanlei Zhu:** Methodology. **Majida Al-Wraikat:** Supervision. **Mohamed Aamer Abubaker:** Writing – review & editing, Supervision. **Rui Zhang:** Funding acquisition. **Yongfeng Liu:** 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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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