

Estimation of the proteome affecting changes in tenderness of yak meat during storage by label-free mass spectrometry

Shengsheng Li^{1,2}  | Shujie Liu^{3,4}

¹Academy of Animal and Veterinary Science, Qinghai University, Xining, P. R. China

²State Key Laboratory of Plateau Ecology and Agriculture, Qinghai University, Xining, P. R. China

³National R&D Center for Yak Meat Processing Technology, Xining, P. R. China

⁴Key Laboratory of Plateau Grazing Animal Nutrition and Feed Science of Qinghai Province, Xining, P. R. China

Correspondence

Shengsheng Li, Academy of Animal and Veterinary Science, Qinghai University, Xining, Qinghai 810016, P. R. China.
Email: lishsh123@163.com

Funding information

Natural Science Foundation of Qinghai Province, Grant/Award Number: 2020-ZJ-961Q

Abstract

Background: Tenderness is the main quality of meat products. However, the meat tenderness formation is a complex biological process, and pathways and proteins that affect the tenderness of yak meat are unknown.

Methods: Label-free proteomics method was used to explore the effects of differentially expressed proteins on the tenderness of yak skeletal muscle (*tenderloin*) during post-mortem storage (0, 3, and 7 days) at $3 \pm 1^\circ\text{C}$.

Results: The tenderness of yak skeletal muscle improved significantly during storage. A total of 91 differentially expressed proteins of yak skeletal muscle during post-mortem storage were identified by the following comparisons: day 3 versus 0, day 7 versus 0, and day 7 versus 3. NDUFS6, CYCS, COX6A2, LDB3, HSPB7, TPM4, TAGLN, COL1A1, LUM, MYH11, ACTC1, and MYOZ1 proteins showed a significant difference during yak skeletal muscle post-mortem storage. Furthermore, bioinformatics analyses revealed that the identified proteins were related to carbon metabolism, citrate cycle, glycolysis, oxidative phosphorylation, and RNA degradation.

Conclusion: The results of the present study could provide proteomic insights into changes in yak skeletal muscle tenderness during storage and may be a valuable resource for future investigations.

KEYWORDS

bioinformatics, label-free, proteomics, tenderness, yak meat

1 | INTRODUCTION

Yak (*Bos grunniens*) is mainly distributed in the Qinghai-Tibet Plateau (S. S. Li et al., 2019). The annual yak meat yield of these regions is approximately 300,000 tons, and the yak meat output value was estimated to be 27 billion yuan in 2019 (Wang et al., 2019). For a long time, yak meat has been favoured by consumers because it has “high protein and low fat”. However, the yak meat has bad tenderness compared to other cattle (e.g., angus, Hereford, Charolais, Simmental, Wagyu) (Bayraktaroglu & Kahraman, 2011; Koohmaraie, 1996). Therefore, the improvements in the quality of yak meat are restricted due to the prob-

lems of “thick muscle fibre and poor tenderness” (M. N. Li et al., 2017; Yang et al., 2020; Zuo et al., 2016).

Tenderness is the main quality of meat products (Bhat & Pathak, 2012; Kemp & Parr, 2012). At present, the formation mechanism of meat tenderness shows that the texture is mainly composed by background toughness and rigor-induced tough (Chang et al., 2013; Eyre et al., 1984). The background toughness is mainly affected by the connective tissue (Canto et al., 2015; Picard et al., 2014). Rigor-induced toughness is mainly caused by the carcass contraction after slaughter of livestock, which can be improved by endogenous enzymes during aging (Huang et al., 2016; Kemp & Parr, 2012). There is research on

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2022 The Authors. *Veterinary Medicine and Science* published by John Wiley & Sons Ltd.

yak meat tenderness based on the myofibrils degraded by the endogenous enzymes. Wang et al. (2019) reported that caspases increased the yak meat tenderness by apoptosis. However, the meat tenderness formation is a complex biological process, and pathways and proteins that affect the tenderness of yak meat are unknown.

Proteomics is an effective technology for understanding the molecular mechanisms associated with meat quality (Bjarnadottir et al., 2012; Wu et al., 2016; Yu et al., 2017; Zuo et al., 2016). Proteomics has been used to explain the changes in beef tenderness after slaughter, and structural proteins, oxidative-resistance proteins, proteolysis, and heat shock proteins were found to affect beef tenderness (Lametsch et al., 2003; Lomiwes et al., 2014; Picard et al., 2014). However, it is still not known whether those proteins are associated with yak skeletal muscle tenderness.

In this study, a proteome study via label-free mass spectrometry (MS) was used to identify biomarkers in yak skeletal muscle tenderness during refrigerated storage. Bioinformatic analyses were conducted to interpret the underlying mechanisms on the development of yak skeletal tenderness.

2 | MATERIALS AND METHODS

2.1 | Materials

This study was carried out using *tenderloin* from yak skeletal muscle. The yak *tenderloin* was obtained from a commercial abattoir (Qinghai Xia Hua Food Ltd., Haiyan City, Qinghai Province, China). Thirty-two yak bulls (weighing 265.31 ± 19.36 kg) were 45–50 months of age and were fed the same diet from the same batch. The *tenderloin* was immediately removed after slaughtering, trimmed mucosa and fats, vacuum packed, and transported to the laboratory at $3 \pm 1^\circ\text{C}$. The *tenderloin* of each yak was cut into three pieces (160 ± 10 g for each piece). Then, all samples were transferred to a pallet and stored in the refrigerator at $3 \pm 1^\circ\text{C}$ for 7 days (no light during storage) and the samples were taken from the refrigerator at 0, 3, and 7 days for measuring the Warner–Bratzler shear force (WBSF) values. Approximately 5 g sample taken from 160 g sample was frozen immediately in liquid nitrogen until the proteomics analyses.

2.2 | Assessment of meat Warner–Bratzler shear force

Each sample was trimmed after aging (length \times width \times height of 60 mm \times 30 mm \times 30 mm), placed into a plastic bag, and cooked in a water bath at 80°C for 30 min. Subsequently, all samples were cooled in running water until their core temperature was below 30°C . Then, each sample was cut into slices with a length \times width \times height of 50 mm \times 10 mm \times 10 mm (the fibre axis was in the 50 mm direction). Afterwards, each sample was placed in a texture analyzer (CT-3, TexturePro CT; Brookfield Engineering Laboratories, Inc., USA) and tested by using a Warner–Bratzler V-shaped shearing device with a crosshead speed of 1.5 mm/s. The shear force was recorded in newtons (N).

2.3 | Analysis of proteomics

2.3.1 | Sample preparation

The samples of yak *tenderloin* were collected on days 0, 3, and 7 after slaughter and then subjected to proteomics analyses. All samples were homogenized in lysis buffer (4% SDS, 1 mM DTT, 150 mM Tris-HCl, pH 8.0) and ProteaseArrest (100 \times , G-Biosciences, USA). After 5 min of incubation in boiling water, the homogenate was sonicated (JYQZ-IIN Ultrasonic cell crusher, Ningbo Scientz Biotechnology Co., Ltd. Conditions: ultrasonic time: 2 min, ultrasonic power: 247 kW) on ice. The crude extract was then incubated in boiling water for another 5 min and clarified by centrifugation at 16,000 \times g at 25°C for 10 min. The protein content was determined with the BCA protein assay reagent (Bio-Rad, USA). The supernatants were stored at -80°C until analysis.

2.3.2 | Protein digestion

The protein (200 μg of each sample) was digested according to the filter-aided sample preparation (FASP) procedure described by S. S. Li et al. (2019). The detergent, DTT, and other low-molecular-weight components were removed using 200 μl of UA buffer (8 M urea, 150 mM Tris-HCl, pH 8.0) by repeated ultrafiltration facilitated by centrifugation. Then, 100 μl of 0.05 M iodoacetamide in UA buffer was added to block reduced cysteine residues, and the samples were incubated for 20 min in the darkness. The filter was washed three times with 100 μl of UA buffer and then twice with 100 μl of 25 mM NH_4HCO_3 . Finally, the protein suspension was digested with 3 μg of trypsin (Promega, WI, USA) in 40 μl of 25 mM NH_4HCO_3 overnight at 37°C , and the resulting peptides were collected by filtration. The peptide content was determined by UV spectroscopy at 280 nm using an extinction coefficient of 1.1 in a 0.1% (g/L) solution, and the value was calculated on the basis of the frequency of tryptophan and tyrosine in vertebrate proteins.

2.3.3 | Liquid chromatography–electrospray ionization–tandem MS analysis by Q Exactive

The peptides in each sample were desalted on C18 cartridge (Empore™SPE Cartridges C18 [standard density], bed I.D. 7 mm, volume 3 ml, Sigma, Kawasaki, Japan), concentrated by vacuum centrifugation, and reconstituted in 40 μl of 0.1% (v/v) trifluoroacetic acid. MS experiments were performed on a Q Exactive mass spectrometer that was coupled to an Easy nLC system (Thermo Fisher Scientific, MA, USA). Exactly 2 μg of peptide was loaded onto a C18-reversed-phase column (Thermo Scientific Easy Column, 10 cm long, 75 μm I.D., 3 μm resin) in buffer A (2% acetonitrile and 0.1% formic acid) and separated with a linear gradient of buffer B (80% acetonitrile and 0.1% formic acid) at a flow rate of 300 nl/min. The solvent gradient used was 5%–8% buffer B for 0%–2 min, 8%–23% buffer B for 2–90 min, 23%–40% 100–108 min, and then 100% buffer B for 108–200 min. MS data were acquired using a data-dependent top10 method that dynamically

selected the most abundant precursor ions from the survey scan (300–1800 m/z) for high energy collision dissociation (HCD) fragmentation. The target value was determined based on predictive automatic gain control (pAGC). The dynamic exclusion duration was 25 s. Survey scans were acquired at a resolution of 60,000 at m/z 200, and the resolution for HCD spectra was 15,000 at m/z 200. The normalized collision energy was 30 eV, and the underfill ratio, which specifies the minimum percentage of the target value likely to be reached at the maximum fill time, was defined as 0.1%. The instrument was run with peptide recognition mode enabled. MS experiments were performed in triplicate for each sample.

2.3.4 | Sequence database searching and data analysis

The MS data were analyzed using MaxQuant software version 1.3.0.5 and compared with the UniProt Bos Taurus database (uniprot-Bos Taurus(Bovine)-46716-20200715.fasta). The initial search used a precursor mass window of 6 ppm. The search followed the enzymatic cleavage rule of trypsin/P and allowed a maximum of two missed cleavage sites with a mass tolerance of 20 ppm for fragment ions. Carbamidomethylation of cysteines was defined as a fixed modification, whereas protein N-terminal acetylation and methionine oxidation were defined as variable modifications for database searching. The cut-off value of the global false discovery rate for peptide and protein identification was set to 0.01. Label-free quantification was carried out in MaxQuant as previously described, and the differentially expressed proteins were determined using $p < 0.05$ and a quantitative ratio of >1.5 or <0.667 (Dai et al., 2016; Lubner et al. 2010; Wisniewski et al., 2009; Wong & Cagney, 2010). Protein abundance was calculated on the basis of the normalized spectral protein intensity.

2.4 | Bioinformatics approaches

The gene ontology (GO) program Blast2GO (<https://www.blast2go.com/>) was used to annotate differentially expressed proteins to create histograms of GO annotation. For pathway analysis, the differentially expressed proteins were mapped to the terms in the KEGG database by using the KAAS program (http://www.genome.jp/kaas-bin/kaas_main). Protein–protein interactions (PPI) networks were analyzed using STRING (<http://string-db.org/>).

2.5 | Parallel reaction monitoring

To further check the changes in the levels of protein expression using label-free analysis, additional quantification was conducted using LC-PRMMS analysis (M. N. Li et al., 2017). The label-free protocol was used for the preparation of 23 peptides. Each sample was spiked with the stable isotope-containing AQUA peptide as a standard internal reference. Tryptic peptides were loaded on the stage tips of C18 columns for desalting prior to reversed-phase chromatography on an nLC-1200

Easy system (Thermo Scientific). A gradient of 5%–35% acetonitrile over 45 min was used for liquid chromatography. Q Exactive Plus MS was applied for Parallel reaction monitoring (PRM) analysis. The methods for measuring the energy of the collision, the charge, and the retention time of the most crucial peptides were optimized using experiments involving unique peptides with high intensities. The raw data were analyzed via Skyline in which the intensity of the signal produced by a certain peptide sequence can be quantified according to each sample and referenced to standards via normalization of each protein.

2.6 | Statistical analysis

The yak *tenderloin* WBSF values for the different storage periods (0, 3, and 7 days) were analyzed by one-way analysis of variance (ANOVA) using SPSS 22.0 software (SPSS, Inc., IL, USA). Tukey–Kramer test was carried out to analyze significant differences among mean values at $p < 0.05$. Moreover, hierarchical clustering analysis (HCA) was carried out with the R language version 4.1.1 (Kick Things).

3 | RESULTS AND DISCUSSION

3.1 | Changes in WBSF of yak tenderloin during storage

Tenderness is the main characteristic of meat, and changes in the WBSF can indicate changes in meat tenderness. The WBSF values of yak *tenderloin* during storage are presented in Table 1. WBSF values decreased during the storage period from 55.21 ± 2.36 N at day 0 to 29.58 ± 1.26 N at day 3, and the values were 25.11 ± 0.23 N at day 7 ($P < 0.05$), which is a 54.52% decrease. Bjarnadottir et al. (2012) reported that bovine longissimus thoracis muscle tenderness increased during storage similar with these results. This could be associated with the proteolysis of myofibrillar proteins of meat.

3.2 | Changes in proteome profiles during storage

The changes in the proteome of yak skeletal muscle during storage were investigated using label-free MS. A total of 91 differentially expressed proteins were identified on day 3 versus 0, day 7 versus 0, and day 7 versus 3 as determined using $p < 0.05$ and a quantitative ratio

TABLE 1 Changes in the Warner–Bratzler shear force (WBSF) values of yak tenderloin during the storage period (0, 3, and 7 days) at $3 \pm 1^\circ\text{C}$

Index	Storage time/day		
Attribute	0	3	7
WBSF/N	55.21 ± 2.36^c	29.58 ± 1.26^b	25.11 ± 0.23^a

Note: The results are expressed as the mean standard deviation. Means with different letters (a, b, and c) are different ($p < 0.05$).

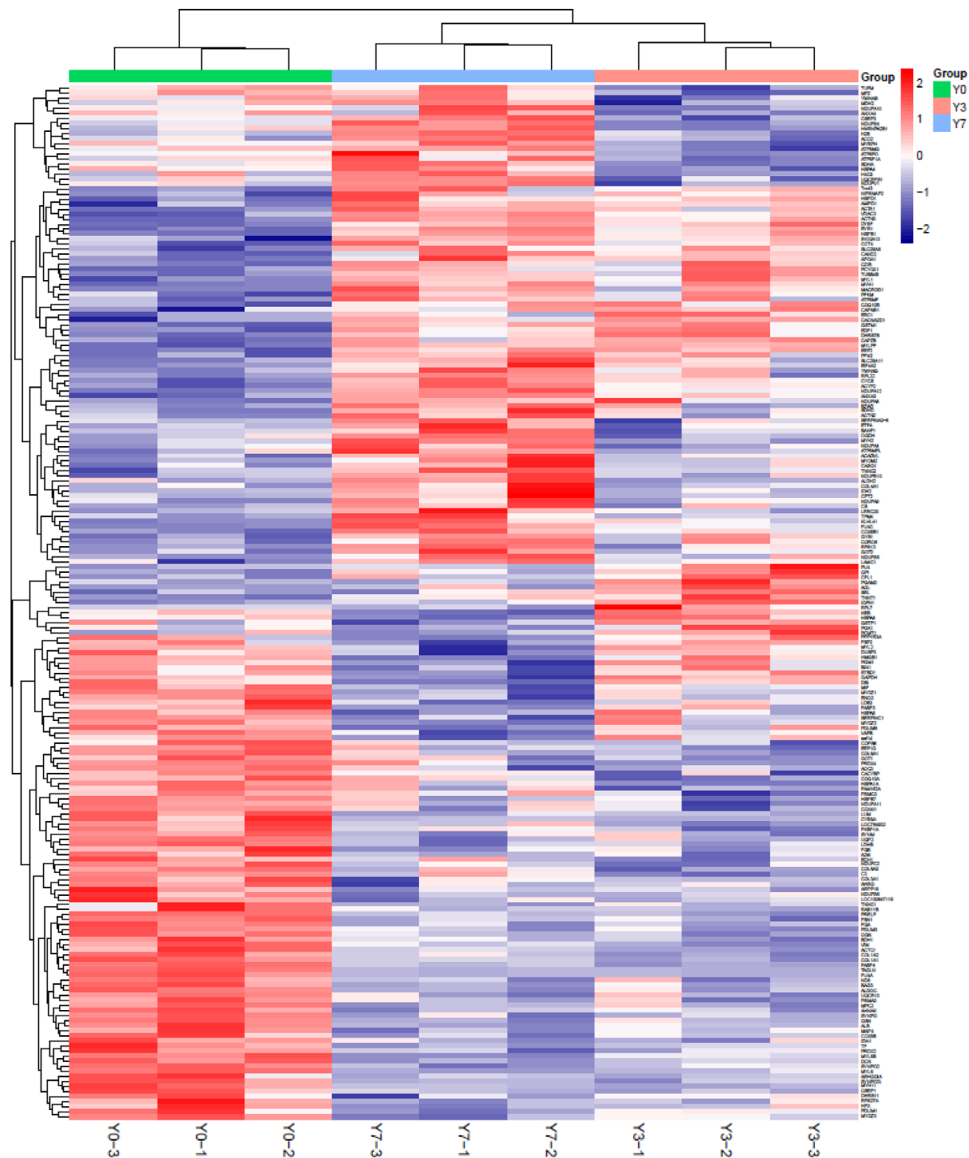


FIGURE 1 Hierarchical clustering analysis (HCA) of differentially abundant proteins, including three biological replicates with yak tenderloin stored at days 0, 3, and 7. The image presents the relative abundance of proteins using different colours, where dark red represents higher intensity and dark purple represents lower intensity

of >1.5 or <0.667 . These proteins are displayed in Table S1. The results showed 50 differentially abundant proteins on day 3 versus 0 (10 up-regulated and 40 down-regulated). Sixty-one differentially abundant proteins were identified on day 7 versus 0 (20 up-regulated and 41 up-regulated), and 16 differentially abundant proteins were detected on day 7 versus 3 (12 up-regulated and four down-regulated).

To compare the dynamic changes of each protein, HCA was used for further analysis (Figure 1). The HCA plot presents the data visually, and each colour in the figure corresponds to the concentration of the protein. Samples stored for different periods (0, 3, and 7 days) are shown by different colours, and the three biological replicates of each sample group displayed similar colours. These results indicated that characteristic changes occurred in the proteomes of the samples stored for different periods. The samples from days 3 and 7 were aggregated

into a cluster and separated from day 0 samples due to their increased Euclidean distance. In summary, the results of HCA indicated that the identified proteins did change in response to the extended storage of yak skeletal muscle.

3.3 | Statistic of the differentially abundant proteins of yak tenderloin

All differentially abundant proteins listed in Table S1 were statistically analyzed according to their function. The results are presented in Figure 2. These proteins can be categorized into the four following groups according to their function: metabolic enzymes, structural proteins, metal binding proteins, and other proteins

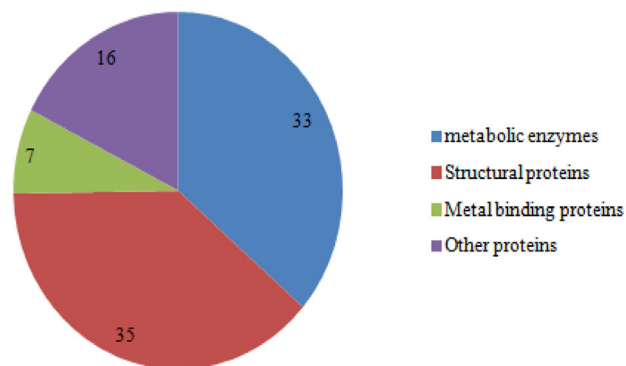


FIGURE 2 Quantitative analysis of proteins identified by proteomic analysis

3.3.1 | Metabolic enzymes

Thirty-three proteins were classified as metabolic enzymes based on their functions. Nine proteins (A7MBI3, B9W0B4, O97725, P07471, P23934, P35816, Q02375, Q0QE4, and Q9BG12) were involved in protein oxidation. It is reported that protein oxidation can affect the muscle tenderness by affecting the activity of endogenous enzymes and hydrolysis structure proteins (Boehm et al., 1998; Huang et al., 2016; Kemp et al., 2010). Five proteins (A0A3Q1NE41, E1BF95, F2Z4I4, P32007, and Q3ZBY4) were related to protein phosphorylation, and phosphorylation was found to be the key process in the Chianina *Bos taurus longissimus dorsi* from muscle to meat (Lana & Zolla, 2016). Three proteins (A0A3Q1LHB1, A0A3Q1MGQ5, and A0A3Q1MR43) were related to cell apoptosis, and apoptosis itself was thought to be closely related to the tenderness of skeletal muscle (Wang et al., 2019).

Therefore, protein modification (e.g., oxidation phosphorylation) and cell apoptosis may play an important role in the development of tenderness in the yak skeletal muscle.

3.3.2 | Structural proteins

In this study, 35 proteins were classified as muscular structural based on their annotation. Those structural proteins can be divided into myofibrils and connective tissue.

At present, the formation mechanism of meat tenderness shows that the texture is mainly composed by background toughness and rigor-induced toughness (Delgado et al., 2001; Kristensen & Purslow, 2001). The background toughness is mainly affected by the connective tissue, and collagen can affect the strength of the connective tissue (Blank et al., 2017; Cross et al., 1973; D'Alessandro et al., 2012). Strange et al. (1977) reported that the degradation of muscle bundle membrane and intramuscular membrane during aging increased the solubility of collagen, and improved the muscle tenderness. Those collagen proteins (such as, A0A3Q1NA44, P02453, P04258) were down-regulated during storage, which indicated that collagen degraded after slaughter. The results showed that connective tissue weakening may

play an important role in the tenderness formation of yak skeletal muscle.

The rigor-induced toughness was mainly affected by myofibrils, and myofibrils can be degraded by calpain, cathepsin, and apoptotic enzymes, and improve the meat tenderness (Muroya et al., 2007; Otsuka & Goll, 1987). Geesink et al. (2006) indicated that endogenous enzymes can degrade muscle fibres during aging, which promoted the improvement of muscle tenderness. These myofibril proteins (such as, A0A3Q1MG42, A0A452DID9, A6QP99, F6QQ60) were down-regulated during storage, which indicated that myofibrils degraded after slaughter. The results showed that degraded myofibrils also play an important role in the tenderness formation of yak skeletal muscle.

3.3.3 | Metal ion-binding proteins

Seven proteins (A0A3Q1M7H9, A0A3Q1MH84, A0A3Q1MIY3, A0A452DIG0, P62894, Q29R16, and Q3TOV7) were classified as metal ion-binding proteins. It is reported that metal ion plays an important role in many metabolic processes. For example, calcium ions participate in muscle contraction and can activate Ca^{2+} -dependent cysteine proteases (Kapprell & Goll, 1989; Lonergan et al., 2010). Most of the metal ion-binding protein of yak skeletal muscle showed up-regulation during storage, which showed that those metal ion participated in the degradation of muscle structure by endogenous enzymes. The results suggest that some metabolic processes that require metal ions contribute to the development of yak skeletal muscle tenderness.

3.3.4 | Other proteins

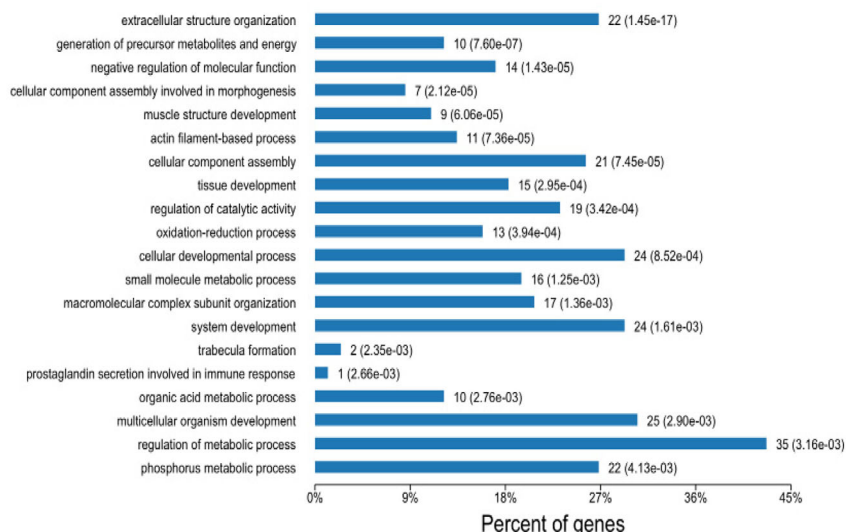
Aside from metabolic enzymes, muscular structural proteins, and metal-ion binding proteins, 16 proteins showed significantly different expressions during yak smooth muscle refrigerated storage.

Three proteins (A0A3Q1MAU9, Q2T9 × 2, and Q5EAC6) related to ATP binding. Energy metabolism significantly affects the tenderness of meat (Polati et al., 2012; Scheffler & Gerrard, 2007). These proteins (A0A3Q1MB96, A0A3Q1NI96, A0A452DI15, E1BPW2, F1MIU2, and P68250) were involved in signal transduction and nucleic acid change, which initiates muscle cell apoptosis, and apoptosis were thought to be closely related to the tenderness of skeletal muscle (Laville et al., 2009). P48035 related to long-chain fatty acid binding, and this protein down-regulated during storage. The results support that the lipid content can affect meat tenderness (Blank et al., 2017; S. S. Li et al., 2019).

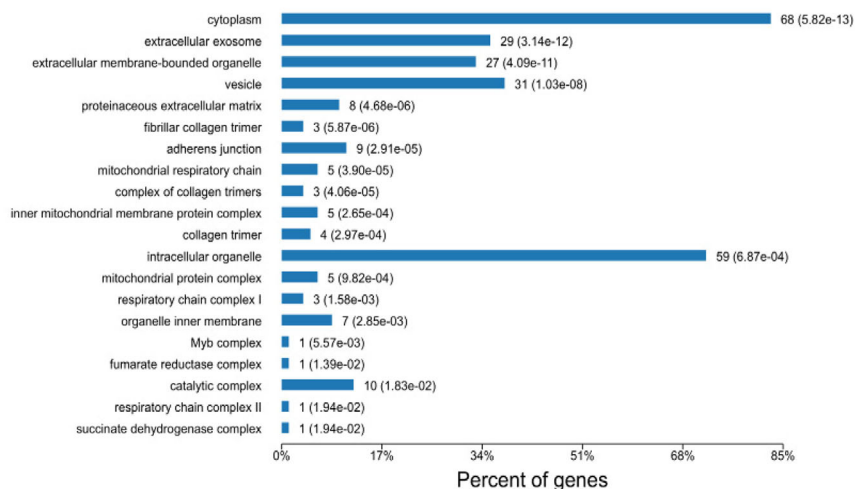
3.4 | Bioinformatics analyses

Bioinformatics analyses were applied to elucidate the properties and functions of the proteins. GO annotation provided biological process, cellular components, and molecular function, which serve as three detailed and structured parameters of the proteins. The results of GO enrichment are shown in Figure 3. With

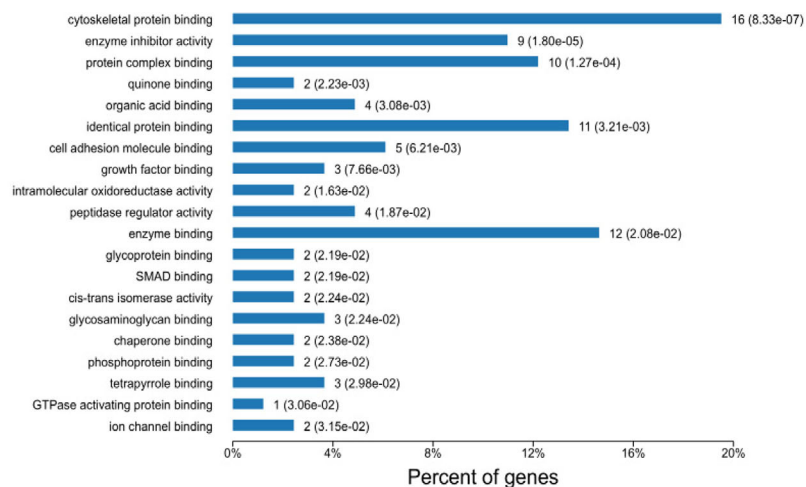
FIGURE 3 Classification of differential expression proteins identified by gene ontology (GO) functional classification



(a) Biological processes



(b) Cellular component



(c) Molecular function

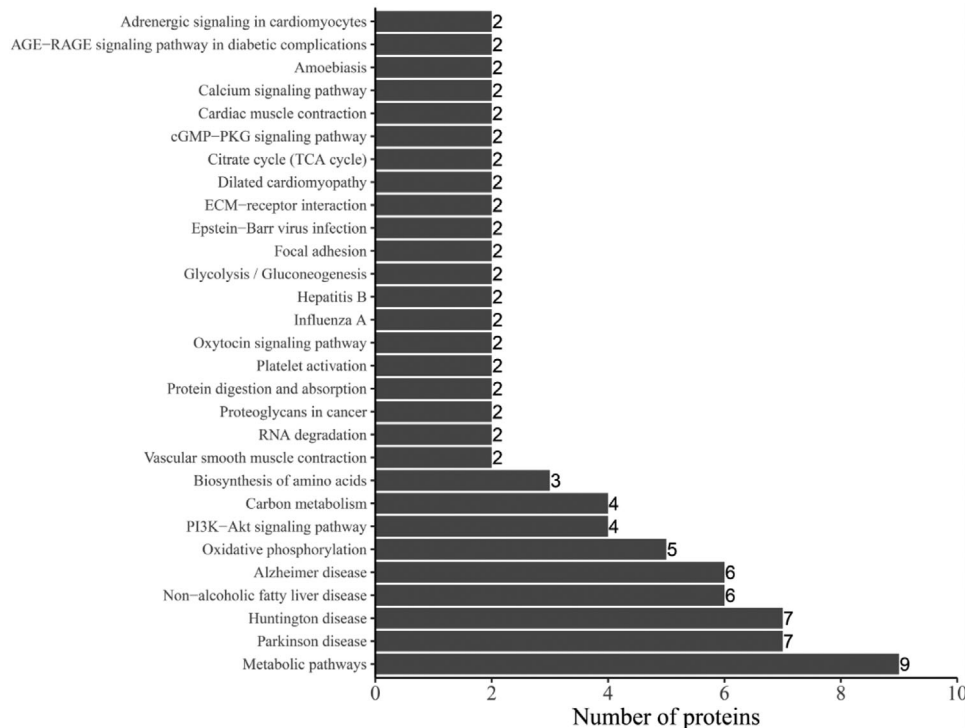


FIGURE 4 Classification of differential expression proteins identified by KEGG pathway enrichment analysis

respect to biological processes, regulation of metabolic process (GO:0019222), multicellular organism development (GO:0007275), system development (GO:0048731), cellular developmental process (GO:0048869), extracellular structure organization (GO:0043062), phosphorus metabolic process (GO:0006793), and cellular component assembly (GO:0010927) were enriched above 20 proteins. For the cellular components, the major classes of these proteins were cytoplasm (GO:0005737), intracellular organelle (GO:0043229), vesicle (GO:0031982), extracellular exosome (GO:0070062), and extracellular membrane-bounded organelle (GO:0065010). For molecular function, most proteins in the categories of cytoskeletal protein binding (GO:0008092), enzyme binding (GO:0019899), identical protein binding (GO:0042802), protein complex binding (GO:0032403), and enzyme inhibitor activity (GO:0004857) were enriched.

To clarify the metabolic pathways in which these proteins were involved, KEGG enrichment was performed, and the two proteins pathway are shown in Figure 4. The results showed that metabolic pathways (ko01100), oxidative phosphorylation (ko00190), carbon metabolism (ko01200), biosynthesis of amino acids (ko01230), RNA degradation (ko03018), glycolysis (ko00010), citrate cycle (ko00020), and calcium signalling pathway (ko04020) were enriched. These biological processes may influence the tenderness improvement of yak skeletal muscle.

Proteins serve as fundamental parts of protein complexes in the living cell but do not act independently. Some proteins acted as core proteins and are shown by gene name in biological interaction networks (Figure 5). In this network, the p -value of PPI enrichment is 1.0×10^{-16} , and the average local clustering coefficient is 0.442. The proteins that

were implicated, such as NDUFS6, CYCS, COX6A2, LDB3, HSPB7, TPM4, TAGLN, COL1A1, LUM, MYH11, ACTC1, and MYOZ1, play an important role in yak meat tenderness formation. In biological interaction networks, gene names of differentially expressed proteins could be summarized into three crucial clusters. One cluster showed the enzymes related to energy metabolism and oxidative phosphorylation, the other was proteins related to myofiber, and the third was related to connective tissue. The myofiber and connective tissue clusters are the proteins that form a muscle structure, which may be related to the change in tenderness during aging. However, some proteins related to tenderness among the non-interacting proteins that were not classified into the three clusters (Figure 5).

3.5 | Validation of proteins by PRM

Ten proteins (HSPB7, MYH11, COL1A2, MYL6, COL1A1, COL3A1, AHSG, NDUFS6, MYL6B, and ALDOC) were selected by PRM to verify the label-free results (Table 2) based on the differential expression at various storage days. The results showed that HSPB7, MYH11, COL1A2, COL1A1, COL3A1, and MYL6B PRM results were consistent with the results of label-free analysis. The MYL6, AHSG, NDUFS6, and ALDOC were down-regulated according to the label-free analysis, and PRM results showed that those proteins were down-regulated except between days 7 and 3. However, there were only small differences in the results of the label-free and PRM analyses of MYL6, AHSG, NDUFS6, and ALDOC four proteins. In general, the selected proteins detected by PRM were almost the same as those determined

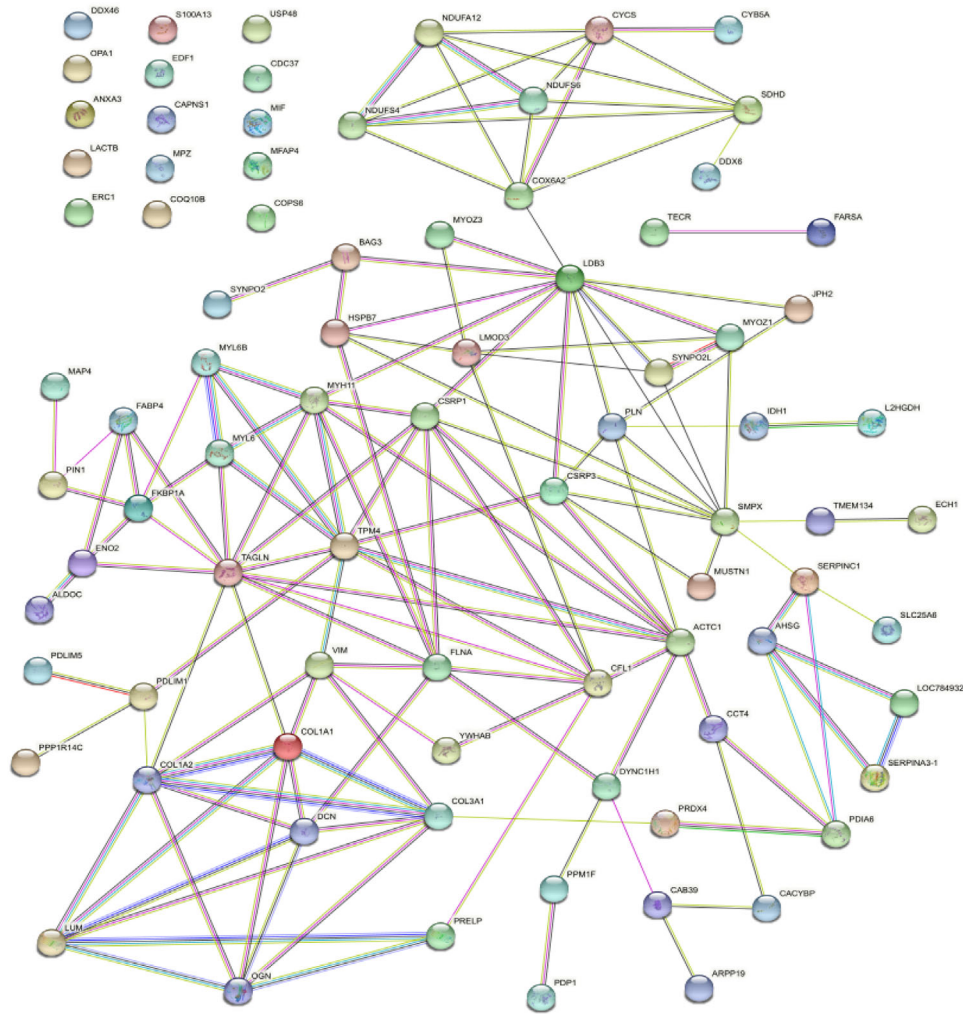


FIGURE 5 Protein-protein interaction networks of the differentially abundance proteins of yak tenderloin during postmortem storage based on the analysis by String10.0 software. The network nodes represent proteins from the *Bos Taurus* database, and the lines are the predicted functional annotations (red line: the presence of fusion evidence; green line: neighbourhood evidence; blue line: cooccurrence evidence; purple line: experimental evidence; yellow line: text mining evidence; light blue line: database evidence; and black line: coexpression evidence)

TABLE 2 Parallel reaction monitoring (PRM) profiles of proteins at days 0, 3, and 7. Three biological replicates were run for each sample

Accession	Gene names	label-free			PRM		
		Ratio 3/0	Ratio 7/0	Ratio 7/3	Ratio 3/0	Ratio 7/0	Ratio 7/3
A0A3Q1LP42	HSPB7	0.55	0.66	1.18	0.79	0.89	1.13
A0A3Q1MG42	MYH11	0.16	0.24	1.48	0.21	0.22	1.04
A0A3Q1NA44	COL1A2	0.37	0.56	1.52	0.35	0.59	1.67
A0A452DID9	MYL6	0.54	0.45	0.83	0.49	0.57	1.16
P02453	COL1A1	0.39	0.53	1.37	0.37	0.53	1.43
P04258	COL3A1	0.39	0.61	1.59	0.24	0.61	2.57
P12763	AHSG	0.72	0.62	0.87	0.63	0.66	1.06
P23934	NDUFS6	0.67	0.56	0.83	0.42	0.51	1.25
Q148H2	MYL6B	0.57	0.33	0.58	0.44	0.32	0.74
Q3ZBY4	ALDOC	0.63	0.54	0.85	0.71	0.76	

by the proteomic analysis. The PRM assay illustrated that the label-free results were reliable for further analyses.

4 | CONCLUSION

Ninety-one proteins of yak skeletal muscle were identified during post-mortem storage by label-free MS. NDUFS6, CYCS, COX6A2, LDB3, HSPB7, TPM4, TAGLN, COL1A1, LUM, MYH11, ACTC1, and MYOZ1 may be candidates biomarkers for yak skeletal muscle tenderness. This study provides proteomic insights into yak skeletal muscle tenderness and is useful for future studies focusing on improving the tenderness of yak meat.

ACKNOWLEDGEMENTS

This work was supported by the program of the Natural Science Foundation of Qinghai Province (2020-ZJ-961Q), Qinghai Province "Kunlun Talents Innovation and Entrepreneurial Talents". We thank the editor and referees for their valuable comments and suggestions on an earlier draft of this manuscript. We also gratefully thank the Shanghai Bio-profile Biotechnology Co., Ltd. (Shanghai, China) for providing technical assistance in LC-MS/MS and LC-PRM/MS.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Validation, Writing original draft, Writing review & editing: Shengsheng Li. Conceptualization, Investigation, Resources, Validation, Writing review & editing: Shujie Liu.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author, [Shengsheng Li], upon reasonable request.

ORCID

Shengsheng Li  <https://orcid.org/0000-0002-1957-8609>

REFERENCES

- Bayraktaroglu, A. G., & Kahraman, T. (2011). Effect of muscle stretching on meat quality of biceps femoris from beef. *Meat Science*, *88*, 580–583.
- Bhat, Z. F., & Pathak, V. (2012). Quality evaluation of mutton HARRISA during one week refrigerated storage. *Journal of Food Science and Technology-Mysore*, *49*, 620–625.
- Bjarnadottir, S. G., Hollung, K., Hoy, 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*, 2035–2043.
- Blank, C. P., Russell, J., Lonergan, S. M., & Hansen, S. L. (2017). Influence of feed efficiency classification and growing and finishing diet type on meat tenderness attributes of beef steers. *Journal of Animal Science*, *95*, 2986–2992.
- Boehm, M. L., Kendall, T. L., Thompson, V. E., & Goll, D. E. (1998). Changes in the calpains and calpastatin during postmortem storage of bovine muscle. *Journal of Animal Science*, *76*, 2415–2434.
- Canto, A. C. V. C. S., Suman, S. P., Nair, M. N., Li, S., Rentfrow, G., Beach, C. M., Silva, T. J. P., Wheeler, T. L., Shackelford, S. D., Grayson, A., McKeith, R. O., & King, D. A. (2015). Differential abundance of sarcoplasmic proteome explains animal effect on beef longissimus lumborum color stability. *Meat Science*, *102*, 90–98.
- Chang, Y., Stromer, M. H., & Chou, R. R. (2013). μ -Calpain is involved in the postmortem proteolysis of gizzard smooth muscle. *Food Chemistry*, *139*, 384–388.
- Cross, H. R., Carpenter, Z. L., & Smith, G. C. (1973). Effects of intramuscular collagen and elastin on bovine muscle tenderness. *Journal of Food Science*, *38*, 998–1003.
- Dai, P., Wang, Q., Wang, W., Jing, R., Wang, W., Wang, F., Azadzo, K. M., Yang, J. H., & Yan, Z. (2016). Unraveling molecular differences of gastric cancer by label-free quantitative proteomics analysis. *International Journal of Molecular Sciences*, *17*, 69.
- D'Alessandro, A., Marrocco, C., Rinalducci, S., Mirasole, C., Failla, S., & Zolla, L. (2012). Chianina beef tenderness investigated through integrated omics. *Journal of Proteomics*, *75*, 4381–4398.
- Delgado, E. F., Geesink, G. H., Marchello, J. A., & Koohmaraie, M. (2001). The calpain system in three muscles of normal and callipyge sheep. *Journal of Animal Science*, *79*, 398–412.
- Geesink, G. H., Kuchay, S., Chishti, A. H., & Koohmaraie, M. (2006). μ -Calpain is essential for postmortem proteolysis of muscle proteins. *Journal of Animal Science*, *84*, 2834–2840.
- Eyre, D. R., Koob, T. J., & Van, K. P. (1984). Quantization of hydroxypyridinium crosslinks in collagen by high-performance liquid chromatography. *Analytical Biochemistry*, *137*, 380–388.
- Huang, F., Huang, M., Zhang, H., Zhang, C. J., Zhang, D. Q., & Zhou, G. H. (2016). Changes in apoptotic factors and caspase activation pathways during the postmortem aging of beef muscle. *Food Chemistry*, *190*, 110–114.
- Huang, F., Huang, M., Zhou, G. H., Xu, X. L., & Xue, M. (2011). In vitro proteolysis of myofibrillar proteins from beef skeletal muscle by caspase-3 and caspase-6. *Journal of Agricultural and Food Chemistry*, *59*, 9658–9663.
- Kapprell, H. P., & Goll, D. E. (1989). Effect of Ca²⁺ on binding of the calpains to calpastatin. *The Journal of Biological Chemistry*, *264*, 17888–17896.
- Kemp, C. M., & Parr, T. (2012). Advances in apoptotic mediated proteolysis in meat tenderization. *Meat Science*, *92*, 252–259.
- Kemp, C. M., Sensky, P. L., Bardsley, R. G., Buttery, P. J., & Parr, T. (2010). Tenderness an enzymatic view. *Meat Science*, *84*, 248–256.
- Koohmaraie, M. (1996). Biochemical factors regulating the toughening and tenderization processes of meat. *Meat Science*, *43*, 193–201.
- Kristensen, L., & Purslow, P. P. (2001). The effect of ageing on the water-holding capacity of pork: Role of cytoskeletal proteins. *Meat Science*, *58*, 17–23.
- Lametsch, R., Karlsson, A., Rosenvold, K., Andersen, H. J., Roepstorff, P., & Bendixen, E. (2003). Postmortem proteome changes of porcine muscle related to tenderness. *Journal of Agricultural and Food Chemistry*, *51*, 6992–6997.
- Lana, A., & Zolla, L. (2016). Proteolysis in meat tenderization from the point of view of each single protein: A proteomic perspective. *Journal of Proteomics*, *147*, 85–97.
- Laville, E., Sayd, T., Morzel, M., Blinet, S., Chambon, C., Lepetit, J., Renand, G., & Hocquette, J. F. (2009). Proteome changes during meat aging in tough and tender beef suggest the importance of apoptosis and protein solubility for beef aging and tenderization. *Journal of Agricultural and Food Chemistry*, *57*, 10755–10764.

- Li, M. N., Zhang, K., Long, R. C., Sun, Y., Kang, J. M., Zhang, T. J., & Gao, S. H. (2017). iTRAQ-based comparative proteomic analysis reveals tissue-specific and novel early-stage molecular mechanisms of salt stress response in *carex rigescens*. *Environmental and Experimental Botany*, *143*, 99–114.
- Li, S. S., Yu, Q. L., Han, L., Zhang, Y. M., Tian, X. J., & Zhao, S. N. (2019). Effect of proteome changes on the tenderness of yak rumen smooth muscle during postmortem storage based on the label-free mass spectrometry. *Food Research International*, *116*, 1336–1343.
- Lomiwes, D., Hurst, S. M., Dobbie, P., Frost, D. A., Hurst, R. D., Young, O. A., & Farouk, M. M. (2014). The protection of bovine skeletal myofibrils from proteolytic damage post mortem by small heat shock proteins. *Meat Science*, *97*, 548–557.
- Lonergan, E. H., Zhang, W. G., & Lonergan, S. M. (2010). Biochemistry of post-mortem muscle—Lessons on mechanisms of meat tenderization. *Meat Science*, *86*, 184–195.
- Luber, C. A., Cox, J., Lauterbach, H., & Mann, M. (2010). Quantitative proteomics reveals subset-specific viral recognition in dendritic cells. *Immunity*, *32*, 279–289.
- Muroya, S., Ohnishi-Kameyama, M., Oe, M., Nakajima, I., Shibata, M., & Chikuni, K. (2007). Double phosphorylation of the myosin regulatory light chain during rigor mortis of bovine longissimus muscle. *Journal of Agricultural and Food Chemistry*, *55*, 3998–4004.
- Otsuka, Y., & Goll, D. E. (1987). Purification of the Ca^{2+} -dependent proteinase inhibitor from bovine cardiac muscle and its interaction with the millimolar Ca^{2+} -dependent proteinase. *The Journal of Biological Chemistry*, *262*, 5839–5851.
- Picard, B., Gagaoua, M., Micol, D., Cassar-Malek, I., Hocquette, J. F., & Terlouw, C. E. (2014). Inverse relationships between biomarkers and beef tenderness according to contractile and metabolic properties of the muscle. *Journal of Agricultural and Food Chemistry*, *62*, 9808–9818.
- Polati, R., Menini, M., Robotti, E., Millions, R., Marengo, E., Novelli, E., & Cecconi, D. (2012). Proteomic changes involved in tenderization of bovine longissimus dorsi muscle during prolonged ageing. *Food Chemistry*, *135*, 2052–2069.
- Scheffler, T. L., & Gerrard, D. E. (2007). Mechanisms controlling pork quality development: The biochemistry controlling postmortem energy metabolism. *Meat Science*, *77*, 7–16.
- Strange, E. D., Benedict, R. C., Smith, J. L., & Swift, C. E. (1977). Evaluation of rapid tests for monitoring alterations in meat quality during storage. *Journal of Food Protection*, *10*, 843–847.
- Wang, L. L., Ma, G. Y., Zhang, Y. B., Shi, X. X., Han, L., Yu, Q. L., Zhao, Q., & Ma, J. Y. (2019). Effect of mitochondrial cytochrome c release and its redox state on the mitochondrial-dependent apoptotic cascade reaction and tenderization of yak meat during postmortem aging. *Food Research International*, *116*, 1336–1343.
- Wisniewski, J. R., Zougman, A., & Nagaraj, N. (2009). Universal sample preparation method for proteome analysis. *Nature Methods*, *6*, 359–362.
- Wong, J. W. H., & Cagney, G. (2010). An overview of label-free quantitation methods in proteomics by mass spectrometry. *Methods in Molecular Biology*, *604*, 273–283.
- Wu, W., Yu, Q. Q., Fu, Y., Tian, X. J., Jia, F., Li, X. M., & Dai, R. T. (2016). Towards muscle specific meat color stability of Chinese Luxi yellow cattle: A proteomic insight into postmortem storage. *Journal of Proteomics*, *147*, 108–118.
- Yang, Y. Y., Han, L., Yu, Q. L., Gao, Y. F., Song, R. D., & Zhao, S. N. (2020). Phosphoproteomic analysis of longissimus lumborum of different altitude yaks. *Meat Science*, *162*, 1–8.
- Yu, Q. Q., Wu, W., Tian, X. J., Hou, M., Dai, R. T., & Li, X. M. (2017). Unraveling proteome changes of Holstein beef M. semitendinosus and its relationship to meat discoloration during post-mortem storage analyzed by lab-free mass spectrometry. *Journal of Proteomics*, *154*, 85–93.
- Zuo, H. X., Han, L., Yu, Q. L., Niu, K. L., Zhao, S. N., & Shi, H. M. (2016). Proteome changes on water-holding capacity of yak longissimus lumborum during postmortem aging. *Meat Science*, *121*, 409–419.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Li, S., & Liu, S. (2022). Estimation of the proteome affecting changes in tenderness of yak meat during storage by label-free mass spectrometry. *Veterinary Medicine and Science*, *8*, 1640–1649. <https://doi.org/10.1002/vms3.801>