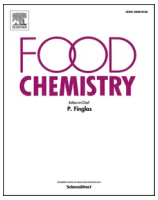
Food Chemistry 427 (2023) 136739

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem



Validation of protein biological markers of lamb meat quality characteristics based on the different muscle types

Caiyan Huang [a,b], Dequan Zhang [a], Zhenyu Wang [a], Yingxin Zhao [c], Christophe Blecker [b], Shaobo Li [a], Xiaochun Zheng [a], Li Chen [a,*]

[a] Institute of Food Science and Technology, Chinese Academy of Agriculture Sciences, Key Laboratory of Agro-Products Quality & Safety Harvest, Storage, Transportation, Management and Control, Ministry of Agriculture and Rural Affairs, Beijing 100193, China
[b] University of Liège, Gembloux Agro-Bio Tech, Unit of Food Science and Formulation, Avenue de la Faculté d'Agronomie 2, Gembloux B-5030, Belgium
[c] Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta T6G 2P5, Canada

ARTICLE INFO

Keywords:
Protein biomarkers
Glycolytic enzymes
Structural proteins
Meat quality

ABSTRACT

This work investigated the ability of 8 potential biomarkers (phosphoglycerate kinase-1 (PGK1), pyruvate kinase-M2 (PKM2), phosphoglucomutase-1 (PGM1), β-enolase (ENO3, myosin-binding protein-C (MYBPC1), myosin regulatory light chain-2 (MYLPF), troponin C-1 (TNNC1) and troponin I-1 (TNNI1)) to characterize meat quality by analyzing their relative abundance and enzymatic activity. Two different meat quality groups (*Quadriceps femoris* (QF) and *Longissimus thoracis* (LT) muscles) were selected at 24 h postmortem from 100 lamb carcasses. The relative abundance of PKM2, PGK1, PGM1, ENO3, MYBPC1, MYLPF, and TNNI1 was significantly different between LT and QF muscle groups ($P < 0.01$). Moreover, PKM, PGK, PGM, and ENO activity in LT muscle group was significantly lower than that in QF muscle ($P < 0.05$). Suggesting that PKM2, PGK1, PGM1, ENO3, MYBPC1, MYLPF, and TNNI1 can be used as robust biomarkers of lamb meat quality, providing the reference for understanding the molecular mechanism of postmortem meat quality formation in future.

1. Introduction

Meat quality characteristics, especially for the tenderness, color, and water-holding capacity (WHC) of fresh meat, directly affect and determine the consumers' overall preference and the economic benefit of the meat industry (Matarneh, Silva, & Gerrard, 2021). In recent years, the economic loss and the resource waste of meat caused by meat quality worsening (such as discoloration, serious juice loss, and texture worsening of postmortem muscle) has become an urgent problem in the meat industry. Statistics from the Food and Agriculture Organization (FAO) revealed that more than 20% of the 263 million tons of meat produced worldwide is lost or wasted annually (http://www.fao.org/3/i4807e/i4807e.pdf). Protein biomarkers or indicators refer to the molecular components of biological processes, showing the differentially expressed proteins related to the phenotype of a specific trait (Huang et al., 2020). Biomarkers provides a new strategy to solve the issue of postmortem meat quality deterioration. Thus, it is essential to certify the biological markers of meat quality in order to characterize and assess it, and elucidate the molecular mechanisms underlying the development of meat quality, which is crucial for reducing the yield of inferior meat in the industry.

A series of complex physiological biochemistry changes and metabolic pathways occur during the process of muscle conversion to meat, including glycolysis, muscle contraction, apoptosis, and post-translational protein modification etc., and thereafter affect meat quality, such as appearance (color), juiciness (water holding capacity) and texture (tenderness) (Matarneh et al., 2021). Glycolysis is an energy metabolic process in postmortem muscle, of which more than ten glycolytic enzymes are involved in enzyme catalysis reactions. Among them, many glycolytic enzymes play a major role in the development of postmortem meat quality. The findings showed that the abundance of PGK1 (phosphoglycerate kinase 1) had a significant difference in meat tenderness, color and WHC (Kim, Jeong, Yang, & Hur, 2019; Silva, Rodrigues, Assis, Benedeti, Duarte, & Chizzotti, 2019). PKM2 (pyruvate kinase M2) is one of the rate-limiting enzymes of glycolysis, and its activity and posttranslational modification directly affect the formation of meat quality after slaughter (Ren, Li, Bai, Schroyen, & Zhang, 2022). Kim et al. (2019) reported that the glycolytic enzymes of PGM1

* Corresponding author at: Institute of Food Science and Technology, Chinese Academy of Agricultural Sciences, No.2 Yuanmingyuan West Road, Haidian District, Beijing 100193, China.
E-mail address: chenliwork@126.com (L. Chen).

https://doi.org/10.1016/j.foodchem.2023.136739
Received 16 February 2023; Received in revised form 20 June 2023; Accepted 25 June 2023
Available online 26 June 2023

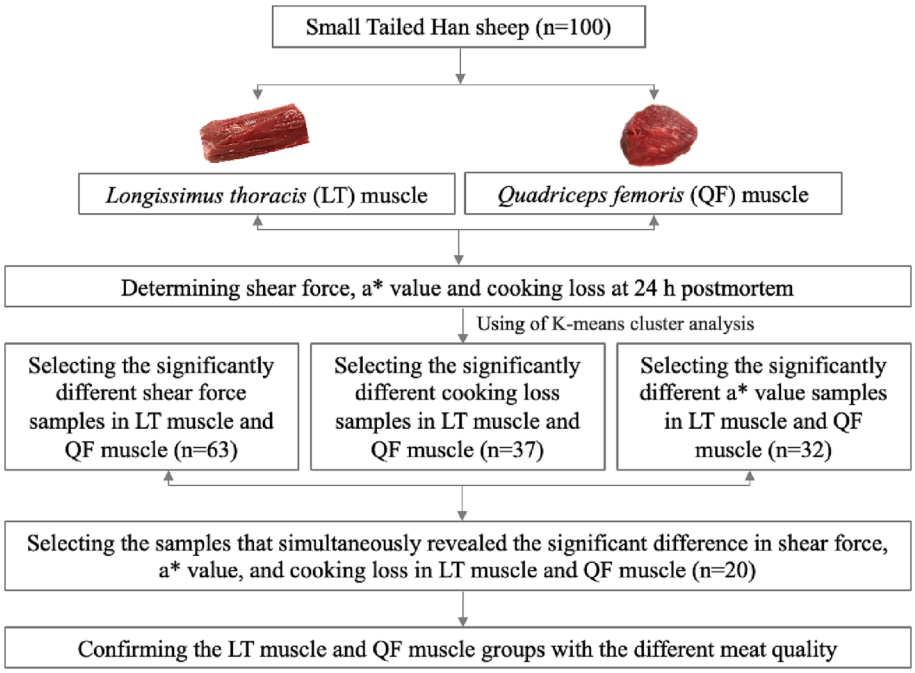


Fig. 1. Experimental design: Selection of two lamb muscle groups from randomly collected 100 carcasses at abattoir according to shear force, a* value, and cooking loss at 24 h postmortem. LT represented *Longissimus thoracis* of lamb muscle, QF represented *Quadriceps femoris* of lamb muscle.

(phosphoglucomutase 1) abundance were most closely associated with intramuscular changes in meat quality. ENO3 (enolase 3) is one of the key enzymes of the glycolysis pathway and is closely associated with meat tenderness (Malheiros et al., 2019) and color (Yu, Wu, Tian, Hou, Dai, & Li, 2017). Skeletal muscle is the most important component of the body's organs, accounting for approximately 40% of body mass in mammals (Janssen, Heymsfield, Wang, & Ross, 2000). Myofibrillar proteins are the largest protein category in skeletal muscle and are responsible for the contractile characteristics of the muscle, which accounting for approximately 55–60% of the total protein content. After slaughter, the depletion of ATP and the liberation of Ca^{2+} from the sarcoplasmic reticulum lead to the cross connection of sarcomeric thin and thick filaments to continuously contract the muscle (Muroya, Ohnishi-Kameyama, Oe, Nakajima, Shibata, & Chikuni, 2007). Myofibrillar proteins regulating muscle contraction could affect the development of meat quality. A series of studies found that the differential abundance of structural proteins, for example, MYBPC1 (myosin-binding protein C, slow-type) (Tong et al., 2014), MYLPF (myosin regulatory light chain 2, fast skeletal muscle isoform) (Kim et al., 2019), TNNC1 (troponin C, type 1) (Pierzchala et al., 2014) and TNNI1 (troponin I, slow skeletal muscle) (Pierzchala et al., 2014) proteins were closely associated with postmortem meat quality characteristics. In addition, our previous study also found that key glycolytic enzymes (PKM2, PGK1, PGM1, and ENO3) and muscle contractile proteins (MYBPC1, MYLPF, TNNC1 and TNNI1) were intimately associated with the lamb meat quality characteristic (tenderness, color and water-holding capacity) (Huang et al., 2023). Although, these abovementioned proteins (PKM2, PGK1, PGM1, ENO3, MYBPC1, MYLPF, TNNC1 and TNNI1) have been reported to be closely related to the postmortem meat quality, it is currently unclear whether these proteins can be used as the robust indicators to characterize or monitor the formation of lamb meat quality traits.

Skeletal muscle is made up of different types of fibers, which are influenced by multiple factors, especially muscle type. Muscle fibers are the basic unit of muscle, which typically make up 75–90% of muscle volume. The type and characteristics of muscle fibers can directly impact the tenderness, color and WHC of meat (Lee, Joo, & Ryu, 2010). Muscle types of the carcass are different in tissue composition, which leads to differences in meat quality attributes. Therefore, the purpose of the current study was to assess the ability of 8 potential biomarkers (PKM2, PGK1, PGM1, ENO3, MYBPC1, MYLPF, TNNC1 and TNNI1) to characterize lamb meat quality in two different muscle types (*Longissimus thoracis* (LT) and *Quadriceps femoris* (QF) muscles) samples (with different meat quality traits) were selected from 100 lamb carcasses, and confirm the protein biomarkers of postmortem lamb meat quality combined with multivariate statistical analysis. Furthermore, to investigate whether these potential biomarkers of meat quality attributes reported in previous studies are affected by muscle types. The data will provide valuable insights into the development of postmortem meat quality and contribute to the advancement of meat quality detection and identification technologies.

2. Materials and methods

2.1. Animals and samples collection

A total of 100 Small Tailed Han sheep with the same feeding conditions were supplied by Jinhong halal meat Co., Ltd, Hebei, China (6–8 months old, carcass weight of 38.37 ± 2.83 kg). The experimental design was shown in Fig. 1. The 100 *Longissimus thoracis* (LT) and *Quadriceps femoris* (QF) muscles from the left carcass side were selected within 45 min after bleeding and enveloped in an oxygen-permeable polyethylene (PE) film with an oxygen transmission rate of $cm^3/(m^2 \cdot 24\ h \cdot atm)$ and a moisture transmission amount of 68.5 $g/(m^2 \cdot 24\ h)$. Subsequently, the muscle samples were stored in a refrigerated room at a temperature of 2 ± 2 °C for 24 h. And then the pH value, *Warner-Bratzler* shear force, a* value and cooking loss of LT and QF muscles were measured at 24 h postmortem immediately, and 50 g samples were frozen in liquid nitrogen simultaneously. For the sample grouping, firstly, the samples with significant differences in shear force, a* value and cooking loss between LT and QF muscles were selected by K-means clustering analysis (Table S1), respectively. Subsequently, to select the samples that simultaneously showed significant difference in shear force, a* value, and cooking loss in LT and QF muscles as the two different meat quality groups (LT muscle and QF muscle groups), each group with 20 biological replicates. Finally, the relative abundance of 8

potential biomarkers (PKM2, PGK1, PGM1, ENO3, MYBPC1, MYLPF, TNNC1, and TNNI1) and the activity of glycolytic enzymes were measured at 24 h postmortem in two different meat quality groups.

2.2. Meat quality measurement

2.2.1. pH value

The pH value of LT and QF muscle samples was detected by using a portable pH meter (Testo 205, Lenzkirch, Germany). The pH meter probe was calibrated with commercial buffers of pH 4.0, 7.0, and 10.0 before using in the chilling room (2 ± 2℃). And then the probe was inserted into muscle samples about 2 cm deep, with three measurements for each sample.

2.2.2. a* Value

The a* value of LT and QF muscle samples was determined on the fresh muscle samples, at which time the measurement was conducted on fresh-cut surface after blooming 45 min in the chilling room (2 ± 2℃) by using the spectrophotometer (Minolta CM-600D, Konica Minolta Sensing Inc., Osaka, Japan). The calibration of colorimeter was consistent with our previous research (Huang et al., 2023). An aperture size of 8 mm with a D65 illuminant and a 10° standard observer were used in the whole experiment. The average was calculated according to the a* value from four random sites for each muscle cut sample, avoiding the visible fat and connective tissue.

2.2.3. Cooking loss and Warner-Bratzler shear force

The LT and QF muscles samples were weighed (W1, about 60 g) and cooked in the thermostat water bath (HH-4, Shenzheng, China) for 35 min at 71℃ (Huang et al., 2023). Subsequently, the samples were dried by the neutral filter paper and reweighed (W2). The percentage of cooking loss was counted with the following formula:

$$Cooking\ loss\ \% = \frac{W1 - W2}{W1} \times 100$$

The cooked samples were chilled at 4℃ overnight, and then were cut into 1 × 1 × 1.5 cm cube for each to measure the *Warner-Bratzler shear force* by the tenderometer (C-LM4, Harbin, China). Ten technical replicates were performed for each sample and the average was calculated and expressed as N.

2.3. Western blotting analysis

LT and QF muscle samples were chopped into meat paste and added the RIPA lysis buffer (Sigma, USA) with protease inhibitor cocktail (Sigma, USA) to lyse it on ice. The protein concentration of samples was detected using a BCA Protein Assay Kit (Thermo Fisher Scientific, 23225). Equal quantities of protein lysates were loaded onto a SDS-PAGE gel system consisting of a separating gel with a range of 10%-12% and a stacking gel with a concentration of 4%, and then converted to the PVDF membrane (Merck-Millipore, 0.2 μm, Millipore, Germany). After that, the PVDF membrane was soaked into the TBST (Tris Buffered Saline with Tween® 20) solution (137 mM NaCl, 20 mM Tris, 0.1% Tween-20) with 5% defatted milk powder. The PVDF membrane was incubated at 4℃ overnight and the following first antibodies: PKM2 (A16700, 1:1000, Abcam Inc, Cambridge, MA), PGK1 (AV48140, 1:1000, Sigma-Aldrich, St. Louis, MO), ENO3 (ab126259, 1:1000, Abcam Inc, Cambridge, MA), MYLPF (ab79935, 1:1000, Abcam Inc, Cambridge, MA), MYBPC1 (ab124196, 1:1000, Abcam Inc, Cambridge, MA), TNNI1 (A9664, 1:1000, ABclonal, Wuhan, China), TNNC1 (A1927, 1:1000, ABclonal, Wuhan, China), and GAPDH (AC001, 1:1000, ABclonal, Wuhan, China). Subsequently, the PVDF membrane was associated with the HRP Goat Anti-Rabbit IgG (H + L) (AS014, ABclonal, Wuhan, China) at room temperature for 80 min. Finally, each band was detected using the ECL detection kit (Bio-Rad, Hercules, USA) with the ChemiDocTMMP imaging system. Calculating the abundance of bands by using Quantity One software (Version 4.62, Bio-Rad, Hercules, USA). The equal amount of all samples from two muscle groups were mixed as a reference sample. Each gel was loaded with a same reference sample. For the relative abundance of glycolytic enzymes (PKM2, PGK1, PGM1 and ENO3) and structural proteins (MYBPC1, MYLPF, TNNC1 and TNNI1), the targeted bands intensity was quantified by a relative ratio compared with reference protein of GAPDH band intensity firstly, and then compared with the reference sample band intensity again to calculate the relative abundance of PKM2, PGK1, PGM1, ENO3 MYBPC1, MYLPF, TNNC1 and TNNI.

2.4. Enzyme activity measurement

2.4.1. Pyruvate kinase (PKM)

PKM activity was measured by the pyruvate kinase assay kit (BC0545, Solarbio, Beijing, China) according to manufacturer's instructions. 100 mg muscle samples were added with 1 mL extraction buffer and homogenized on ice using grinding rod (D9030, Solarbio, Beijing, China). Subsequently, the homogenate was centrifuged for 10 min at 4℃ and 8,000 g. The supernatant was collected and its absorbance at 340 nm was measured using a microplate reader (Multimode Microplate Reader, Spark®, Tecan, Switzerland). The activity of PKM (units) was expressed as U/mg protein.

2.4.2. 3-Phosphoglycerate kinase (PGK)

PGK activity was measured by the 3-phosphoglycerate kinase assay kit (BC2255, Solarbio, Beijing, China) according to manufacturer's instructions. 100 mg muscle samples were added with 1 mL extraction buffer and homogenized on ice using grinding rod (D9030, Solarbio, Beijing, China). After that, the homogenate was centrifuged at 4℃ and 10,000 g for 10 min. The supernatant was collected and its absorbance at 340 nm was measured using a microplate reader (Multimode Microplate Reader, Spark®, Tecan, Switzerland). The activity of PGK (units) was expressed as U/mg protein.

2.4.3. Phosphoglucomutase (PGM)

PGM activity was measured by phosphoglucomutase colorimetric assay kit (MAK105, Sigma-Aldrich, St. Louis, MO, United States) according to manufacturer's instructions. 50 mg muscle samples were homogenized in 200 mL of PGM assay buffer on ice and centrifuged the samples at 4℃ and 13,000 g for 5 min. The supernatant was collected and its absorbance at 340 nm was measured using a microplate reader (Multimode Microplate Reader, Spark®, Tecan, Switzerland). The activity of PGM (units) was expressed as milli U/μg protein.

2.4.4. Enolase (ENO)

Enolase activity was determined by the enolase activity assay kit (MAK178, Sigma-Aldrich, St. Louis, MO, United States) according to manufacturer's instructions. 10 mg muscle samples were homogenized in 100 mL of ENO assay buffer on ice and centrifuged the samples at 4℃ and 10,000 g for 5 min. The supernatant was collected and its absorbance at 340 nm was measured using a microplate reader (Multimode Microplate Reader, Spark®, Tecan, Switzerland). The activity of ENO (units) was expressed as milli U/μg protein.

2.5. Statistical analysis

Date analysis was performed using SPSS 22.0 (IBM Corp., New York, USA). Student's T-test ($P < 0.05$) was considered to determine significant differences in the a* value, shear force, and cooking loss, relative abundance of proteins and enzymes activity between the LT muscle and QF muscle groups. The K-means cluster analysis, hierarchical cluster analysis, correlation analysis and principal component analysis were performed by Origin 2022b (Originlab Corp., Northampton, USA). All data were presented as the mean ± SEM (standard error of mean).

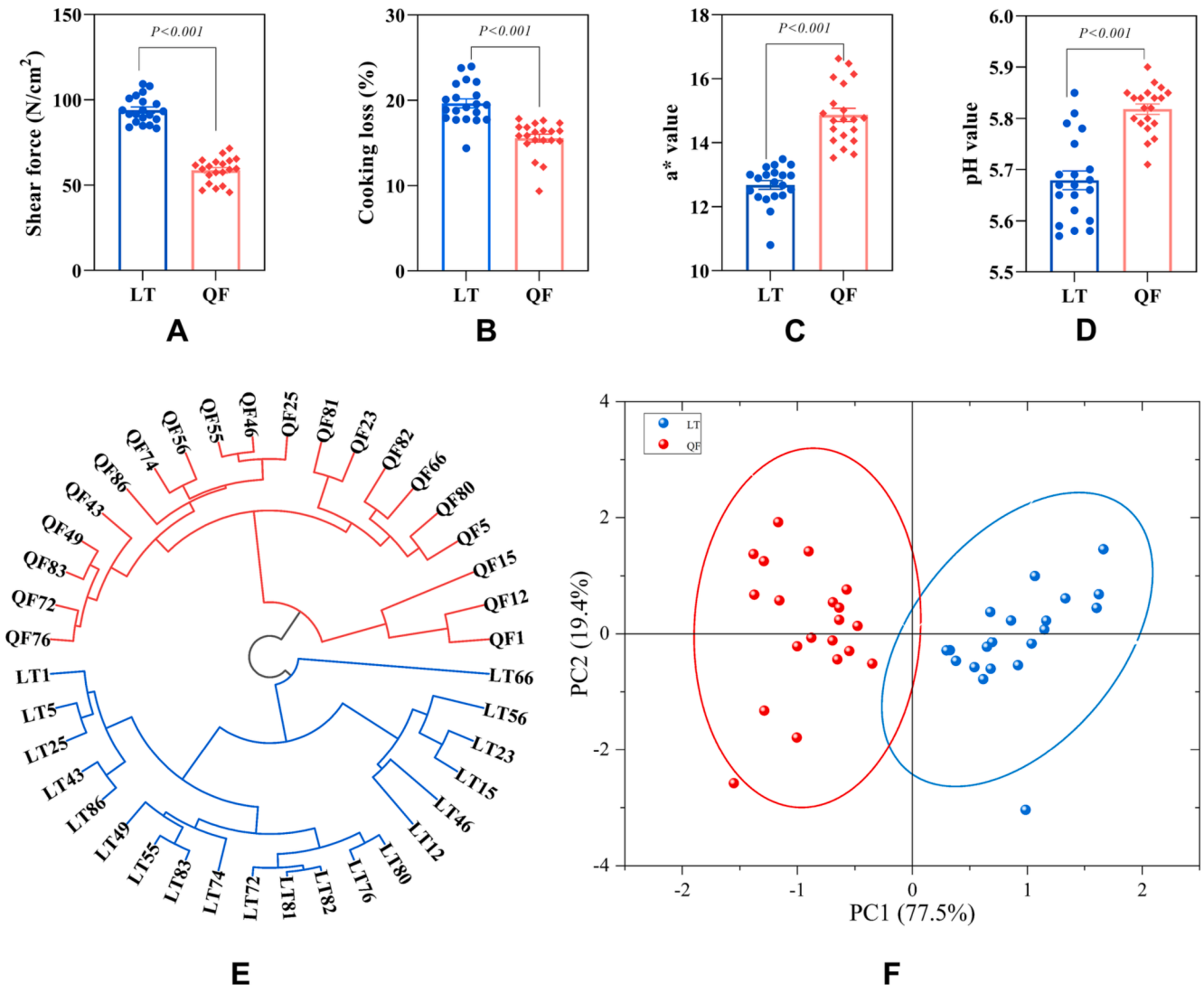


Fig. 2. The difference of meat quality traits in LT and QF muscle groups at 24 h postmortem. A - D: The difference of shear force, a* value, cooking loss, and pH value in LT and QF muscle groups; E and F: Hierarchical cluster analysis and Principal component analysis for LT and QF muscle groups according to shear force, a* value and cooking loss of lamb meat at 24 h postmortem, respectively.

3. Results and discussion

3.1. Samples grouping and meat quality characteristics

In this study, 20 LT and QF muscles were selected respectively from 100 lamb carcasses as two different meat quality traits groups based on the values of lamb meat color, tenderness, and water-holding capacity at 24 h postmortem (Fig. 1). As shown in Fig. 2, the shear force and cooking loss in LT muscle group was significantly higher than QF muscle group at 24 h postmortem ($P < 0.001$; Fig. 2A and B). The a* value presented a remarkably lower level in LT muscle group compared to those of QF muscle group at 24 h postmortem ($P < 0.001$; Fig. 2C). Moreover, the hierarchical cluster analysis (Fig. 2E) and principal component analysis (PCA) analysis (Fig. 2F) presented a clear boundary between LT muscle group and QF muscle group based on shear force, a* value and cooking loss. It was indicated that the sample grouping (LT muscle and QF muscle groups) met the requirements of the differential lamb meat quality attributes.

3.2. pH Value

The rate and extent of pH decline significantly affects the overall meat quality at postmortem. After bleeding of animals, the lack of oxygen results in the internal environment of hypoxia, and thereafter the energy supply of animals must rely on anaerobic glycolysis. In the glycolysis pathway, the degradation of glycogen, the production of lactate and the accumulation of hydrogen ion (H^+) lead to the decrease of pH value (Matarneh et al., 2021). As presented in Fig. 2D, our result found that the pH value of LT muscle was significantly lower than that in the QF muscle group at 24 h ($P < 0.001$). Chaosap, Sitthigripong, Sivapirunthep, Pungsuk, Adeyemi, & Sazili (2020) found that LT muscle had a lower ultimate pH (pHu) than other muscles, which is consistent with our result. This may be due to that LT muscle belongs to the glycolytic muscle and has high glycolytic potential, which leads to a faster pH drop than QF muscle group (Shen et al., 2016). The previous study also demonstrated that the rate of glycolysis is influenced by pH, especially at low pH values (Wang, Matarneh, Gerrard, & Tan, 2021). Therefore, it was suggested that the pH value of meat was influenced by muscle types. In addition, the pHu of meat is commonly used as an indicator or reflector of meat quality. Bernad, Casado, Murillo, Picallo, Garriz, and Maceira (2018) found that cooking loss increased in lower pHu value of meat, which was due to the increase of protein denaturation and the decrease of protein water retention under the lower pHu value. The study showed that there was a positive relationship between

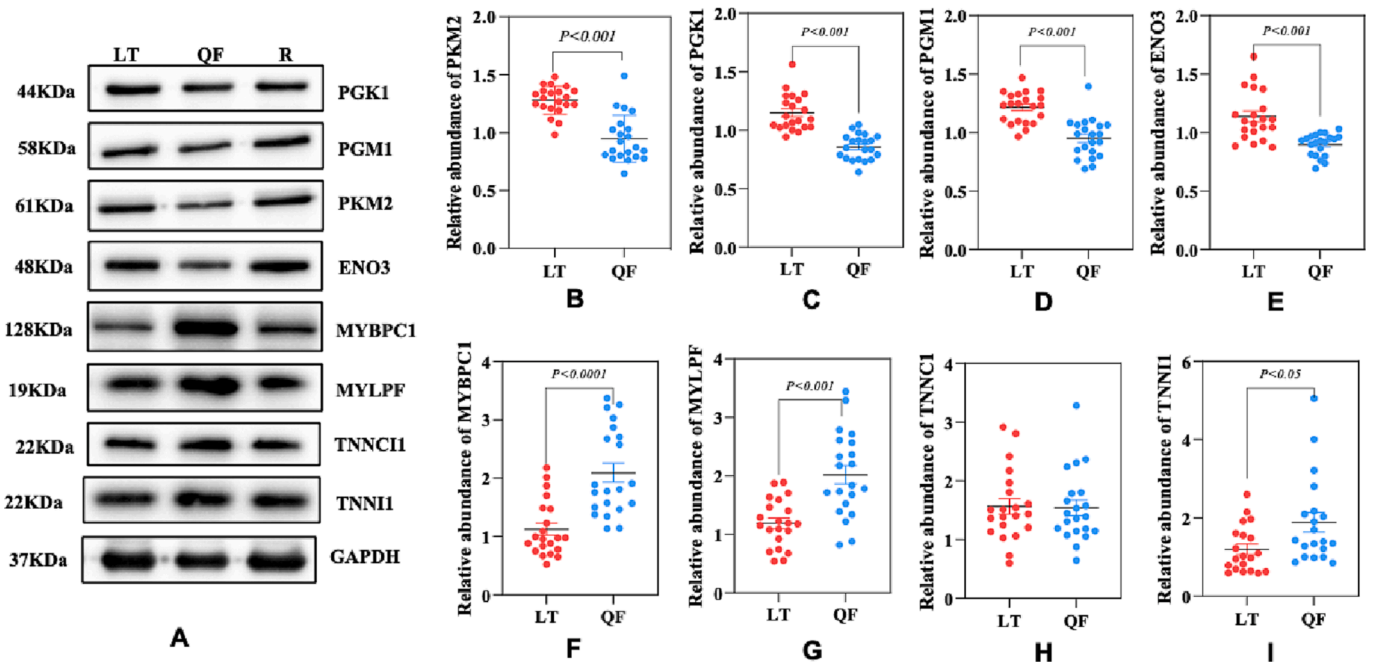


Fig. 3. Relative abundance of PKM2, PGK1, PGM1, ENO3, MYBPC1, MYLPF, TNNC1 and TNNI1 in LT and QF muscle groups. R represented the reference samples. A: Western blotting images of PKM2, PGK1, PGM1, ENO3, MYBPC1, MYLPF, TNNC1 and TNNI1 in LT and QF muscle groups; B-I: The relative level abundance PKM2, PGK1, PGM1, ENO3, MYBPC1, MYLPF, TNNC1 and TNNI1 in LT and QF muscle groups.

pHu and tenderness (Bouton, Harris, & Shorthose, 1971). Yang et al. (2021) studied the physicochemical of beef steak in different pHu values and showed that the lower pHu beef had a lower a* value. These findings agreed with our results. Therefore, we inferred that in the normal ultimate pH value of meat, the higher pHu value may be revealed the better meat tenderness, color and WHC.

3.3. *The relative abundance of 8 potential protein indicators*

Muscle postmortem glycolysis is considered as one of the important metabolic processes during the transformation of muscle - to - meat, which directly affects and determines the development of final meat quality (Honikel, 2014). Moreover, the previous study also reported that structural proteins, including actin, myosin, titin, nebulin, desmin and troponin, were closely related to changes in postmortem meat quality, especially under the effect of protein degradation (Huang et al., 2020). Among them, the key enzymes (PKM2, PGK1, PGM1 and ENO3) during glycolysis and structural proteins (MYBPC1, MYLPF, TNNC1 and TNNI1) have been found, which has the capability of being used as potential protein biomarkers associated with lamb meat quality (Huang et al., 2023). To confirm that these proteins whether can be used as robust biological markers to evaluate and forecast the lamb meat quality characteristics, we selected the two different muscle types samples with different meat quality characteristics for verification. The relative abundance of 8 potential indicators of glycolytic enzymes (PKM2, PGK1, PGM1 and ENO3) and structural proteins (MYBPC1, MYLPF, TNNC1 and TNNI1) was determined by using western blotting analysis and presented in Fig. 3A.

3.3.1. *Glycolytic enzymes*

For glycolytic enzymes, our study found that the relative abundance level of PKM2, PGK1, PGM1 and ENO3 in the LT muscle group was remarkably higher than that in the QF muscle group ($P < 0.001$; Fig. 3B, 3C, 3D and 3E). Glycolysis is one of the significant biological processes affecting the formation of meat quality. The rate of ATP consumption affects the rate of glycolysis in postmortem (Hamm, 1977). ATP content plays a key role in the transformation of muscle - to - meat, causing meat quality changes (Honikel, 2014). PGK1 and PKM catalyze the two glycolytic reactions that produce ATP in the glycolytic pathway (Bernstein & Hol, 1998). PGK1 is the first ATP-generating enzyme in the glycolysis pathway and can catalyze the transformation of 1,3-diphosphoglycerate to 3-phosphoglycerate, leading to the ATP generation. PKM produces pyruvate and ATP by transferring the phosphoenolpyruvate to ADP, which is the second ATP-generating enzyme during the glycolytic process. Shen et al. (2022) revealed that PKM gene expression in pork had a negative correlation with the final pH value, which was in accordance with our results. However, Fuente-García, Sentandreu, Aldai, Oliván, and Sentandreu (2021) studied the preslaughter stress of beef cattle was determined by proteomics and a higher abundance level of PGK1 was found in normal beef (pHu = 5.53 ± 0.14) that in high pHu (6.56 ± 0.25) beef. Generally, the fast and inordinate glycolysis results in PSE (pale, soft and exudative) meat, while inadequate glycolysis leads to DFD (dark, firm, and dry) meat. Wang et al. (2021) also found that pH changes in postmortem muscle were affected by glycolysis. Therefore, the results indicated that PGK1 relative abundance may also be affected by the pHu value of meat. The previous study have proved that postmortem muscle glycolysis transfers the glucose into lactate and H^+, with a drop in pH, which could affect meat quality attributes (Ijaz et al., 2020). PGM1 is one of the key glycolytic enzymes that interconverts glucose-6-phosphate and glucose-1-phosphate, which plays a prominent role in the regulation of glycogen metabolism process (Radenkovic et al., 2019). The observation found that PGM1 can be used as a candidate biomarker to discriminate beef samples according to the incidence of preslaughter stress, and it was negatively correlated with the a* value (Díaz et al., 2020). PGM1 was also reported as a biomarker of beef tenderness downregulated in tender meat compared to tough meat (Picard & Gagaoua, 2020). Moreover, Antonelo et al. (2022) revealed that the abundance of PGK1 and PGM1 in high growth rate of cattle with higher level than low growth rate of cattle group, and concluded that PGM1 and PKM as the candidate biological markers for meat color and tenderness of beef, respectively. This may be due to PKM and PGM1 could regulate the process of glycolysis, and then influence the lateral contraction of muscle fibers and the extension of sarcomeres (Ouali et al., 2013). Enolase (ENO) is a terminal enzyme during glycolysis that

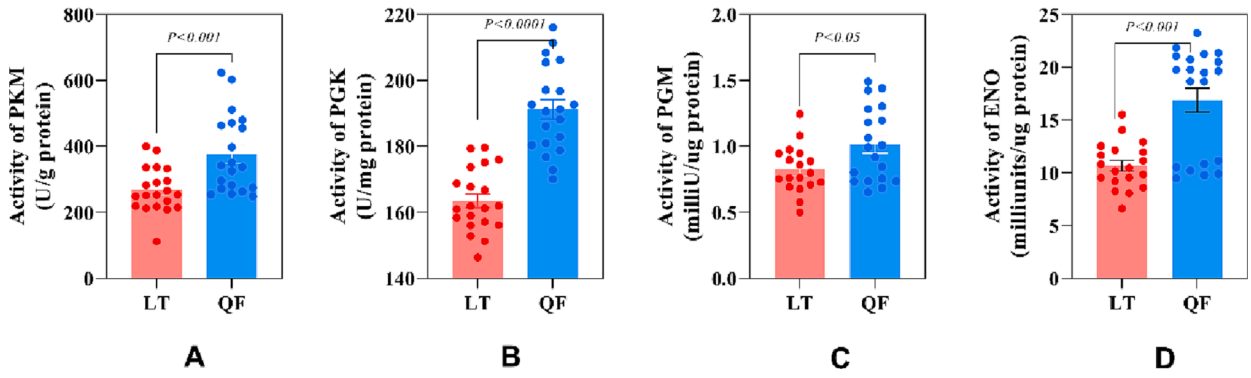


Fig. 4. The change of PKM, PGK, PGM and ENO activity in LT and QF muscle groups. A: PKM; B: PGK; C: PGM; D: ENO.

produces the phosphoenolpyruvate from 2-phosphoglycerate, which consists of three subunits (α, β, γ). Among them, the β subunit is encoded by the ENO3 gene and is the main subunit of muscle. The study found that ENO3 had a different abundance in tender and tough beef and could explain up to 50% of the shear force variability (Bonnet et al., 2020). Malheiros et al. (2019) observed that ENO3 caused a decrease in oxidative damage in the tender beef. Moreover, the study also showed that ENO3 was a pHu related protein (Huang et al., 2020), which could result in a change in the postmortem muscle glycolysis rate and then impact the variation in meat quality. Shen et al. (2022) reported that the PKM2, PGM1 and ENO3 proteins had a higher expression level in the *longissimus thoracis* muscle but revealed a lower a* value and faster pH decrease when compared to the *psoas major* muscle. In addition, our previous study also found that the relative abundance of PKM2, PGK1, PGM1, and ENO3 was higher level in the low lamb meat quality group than in the middle and high lamb meat quality groups using of the proteomics combined with western blotting analysis (Huang et al., 2023). Therefore, it was indicated that PKM2, PGK1, PGM1 and ENO3 proteins can be used as the robust biomarkers of lamb meat quality by affecting the process of glycolysis and then deciding the development of meat quality, and their abundance was not influenced by muscle types.

3.3.2. *Structural proteins*

For structural proteins, it was shown that the relative abundance level of MYBPC1, MYLPF and TNNI1 in the LT muscle group was remarkably lower than that in the QF muscle group ($P < 0.001$; Fig. 3F, G and I). However, the relative abundance of TNNC1 was not significantly different between the in LT and QF muscle groups ($P > 0.05$; Fig. 3H). MYBPC1 is the slow skeletal muscle isoform of the major myosin-binding proteins in vertebrate striated muscles and plays a key role in energy metabolism and muscle contraction. Tong et al. (2014) observed that MYBPC1, as a potential marker, was positively associated with beef marbling of the intramuscular fat. Li et al. (2020) found that MYBPC1 was upregulated with the increase in intramuscular fat at the developmental stage in chicken, and improved meat quality by regulating muscle growth. MYLPF plays a prominent role in the formation of fast and slow skeletal muscle fibers and is significantly expressed in fast muscle fibers. The study found that MYLPF was closely related to the pork pHu and drip loss of Large White pig (Ryan et al., 2016). Antonelo et al. (2022) investigated the changes in meat color and tenderness of beef with the different growth rates and feeding regimes by proteomics, and showed that MYLPF had a negative correlation with shear force and can be used as a candidate marker for the beef tenderness. Moreover, previous study found that MYLPF abundance was positively associated with the a* value and was regarded it as a good marker for meat color (Canto et al., 2015). These results agree with our present study. TNNI1 is the inhibitory subunit of the troponin complex, which exists in the sarcomere thin filaments of striated muscle and regulates muscle contraction and under the influence of calcium concentration. The previous research found that the abundance level of TNNI1 was higher level in PM (*psoas major*) muscle than that in LL (*longissimus lumborum*) muscle of beef (Yu, Tian, Shao, Xu, Dai, & Li, 2018), which was due to the larger proportion of the type I (slow-twitch oxidative) fibesr in PM muscle. Moreover, Hwang, Kim, Jeong, Hur, and Joo (2010) revealed that the proportion of type I fiber was positively associated with fat content and L* value, and negatively associated with shear force, which due to the type I fiber contains a high mitochondrial concentration, myoglobin and intramuscular fat content, which was can improve meat tenderness, color and WHC. These observations were consistent with our results. This result suggested that the MYBPC1, MYLPF and TNNI1 proteins may be regarded as the robust biomarkers to characterize the postmortem lamb meat quality (Huang et al., 2023). The molecular mechanism may be due to that the structural proteins regulating muscle contraction could affect the development of meat quality. ATP is the dominant energy source in animal muscle contraction. After bleeding, ATP can only be produced through glycolysis due to the interruption of oxygen supply, which is not very effective (Matarneh et al., 2021). In addition, skeletal muscle can be divided into oxidative muscle (type I fiber) and glycolytic muscle (type II fiber) on the basis of glycolytic potential. Generally, glycolytic skeletal muscle has thicker fibers, greater glycolytic capability, lower redness, and rapider pH decline, while oxidative skeletal muscle has the opposite features (Shen et al., 2016). Therefore, it was indicated that postmortem muscle contraction could be affected by the glycolytic pathway and then determine the change of meat quality.

3.4. *Glycolytic enzyme activity*

Glycolysis is the essential and necessary metabolic pathway in the process of converting muscle into meat, which includes a succession of enzymatic reactions that consecutively transfer the glucose into pyruvate (Matarneh et al., 2021). In our study, we found that the relative abundance of glycolytic enzymes (PKM2, PGK1, PGM1 and ENO3) was significantly different between the LT muscle and QF muscle groups. Therefore, we inferred that the abundance of glycolytic enzymes (PKM2, PGK1, PGM1 and ENO3) might be influenced by the glycolysis rate and then influence the meat quality in postmortem. The activity of PKM, PGK, PGM and ENO in LT and QF muscles was determined and presented in Fig. 4. In the LT muscle group, the result of PKM, PGK, PGM and ENO activity showed a lower level compared to QF muscle group ($P < 0.05$; Fig. 4A, B, C and D). Our previous research found that hexokinase (HK), phosphofructokinase (PFK) and pyruvate kinase (PKM) activity was lower in the slow glycolysis rate group (muscle samples with a lower a* value and, higher shear force and cooking loss) (Ren et al., 2022), which was compatible with the present results. Moreover, Wang et al. (2020) reported that PES pork (pale, soft and exudative) had the higher the activity of PKM compared to RFN (red, firm and non-exudative). Schwägele, Haschke, Honikel, and Krauss (1996) also showed that the

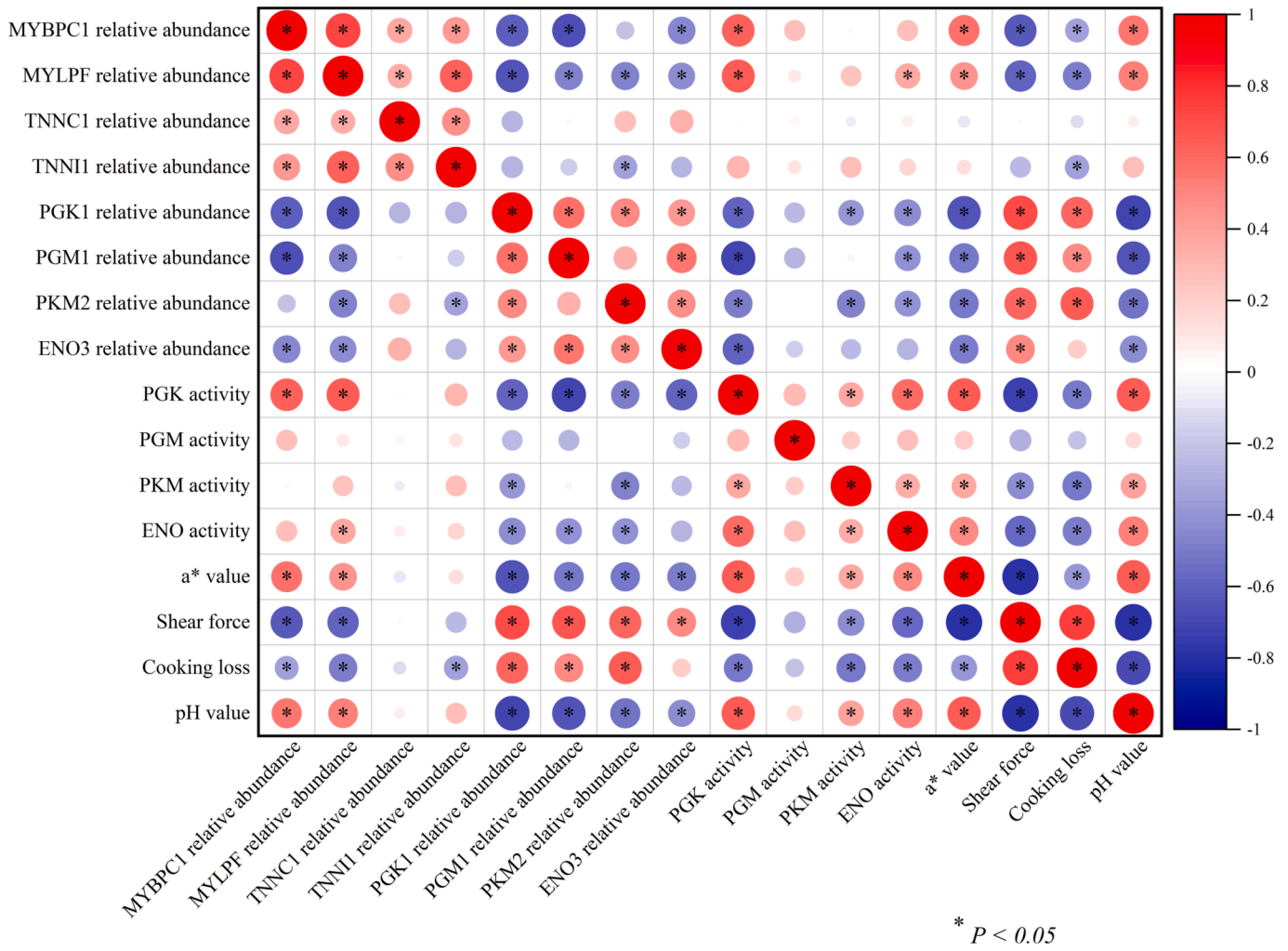


Fig. 5. Relationship between potential biological markers relative abundance, enzymes activity and lamb meat quality traits (shear force, a* value, cooking loss and pH value) by correlation coefficient analysis. * Represented $P < 0.05$.

PKM activity in PES pork had higher level than normal muscle. The activity of PGM1 may be affected by phosphorylation, which can promote a decline in pH value (Gururaj, Barnes, Vadlamudi, & Kumar, 2004). Bai et al. (2020) reported that the fast glycolytic rate group of lamb meat had a lower glycolytic potential and a higher glycogen phosphorylase activity compared to the slow and intermediate glycolytic rate groups. Glycolysis is a succession of enzymatic steps that are decided by the activity of glycolytic enzymes Thus, it is suggested that in the normal pHu rages, the high meat quality (QF muscle group) had a faster glycolytic rate than low meat quality (LT muscle group).

3.5. *Relationships of meat quality traits, relative abundance and enzyme activity of potential protein indicators*

To gain insights into the relationship of lamb meat quality attributes, relative abundance of potential protein indicators and glycolytic enzyme activity, Pearson correlation analysis was used and presented in Fig. 5. The results revealed that the relative abundance level of structural proteins (MYBPC1, MYLPF, TNNC1 and TNNI1) was negatively correlated with shear force and cooking loss, but positively correlated with the a* value. But the relative abundance of PKM2, PGK1, PGM1 and ENO3 had negatively correlated with the a* value, while positively correlated with shear force and cooking loss. Our previous research found that in the *Longissimus thoracis* muscle of Tan sheep with different meat quality, the relationship between these potential indicators and meat quality also had the same results (Huang et al., 2023). These results suggested that the postmortem muscle glycolytic rate may regulate and influence the abundance level of glycolytic enzymes (PKM, PGK1, PGM1 and ENO3) and structural proteins (MYBPC1, MYLPF, TNNC1 and TNNI1) to decide the development of meat quality. Meanwhile, the candidate biological markers of lamb meat quality were not influenced by muscle types. In addition, the activity of glycolytic enzymes (PKM, PGK, PGM and ENO) had a significant positive correlation with the relative abundance of structural proteins but a negative correlation with the relative abundance of glycolytic enzymes. Glycolysis is one of the important biochemical reactions in the process of muscle conversion - to - meat, which includes a succession of enzymatic reactions that consecutively transfer the glucose into pyruvate. Glycolysis is also an important contributor to ATP production in postmortem (Hamm, 1977; Honikel, 2014; Matarneh et al., 2021; Wang et al., 2021). Generally, ATP is the energy currency in cells to maintain cell energy homeostasis in postmortem muscle. However, it was indicated that the key glycolytic enzymes activity can affect and decide the postmortem muscle glycolysis rate, especially for the rate-limiting enzymes of PKM (Ren et al., 2022). Meanwhile, the depletion of ATP in postmortem muscle will lead to the cross connection of sarcomeric thin and thick filaments, which will make the muscle continue to contract (Muroya et al., 2007). Therefore, we inferred that the glycolytic enzyme activity may affect the glycolytic rate of the postmortem muscle, and then influence the production of ATP, which will ultimately affect the relative abundance of structural proteins

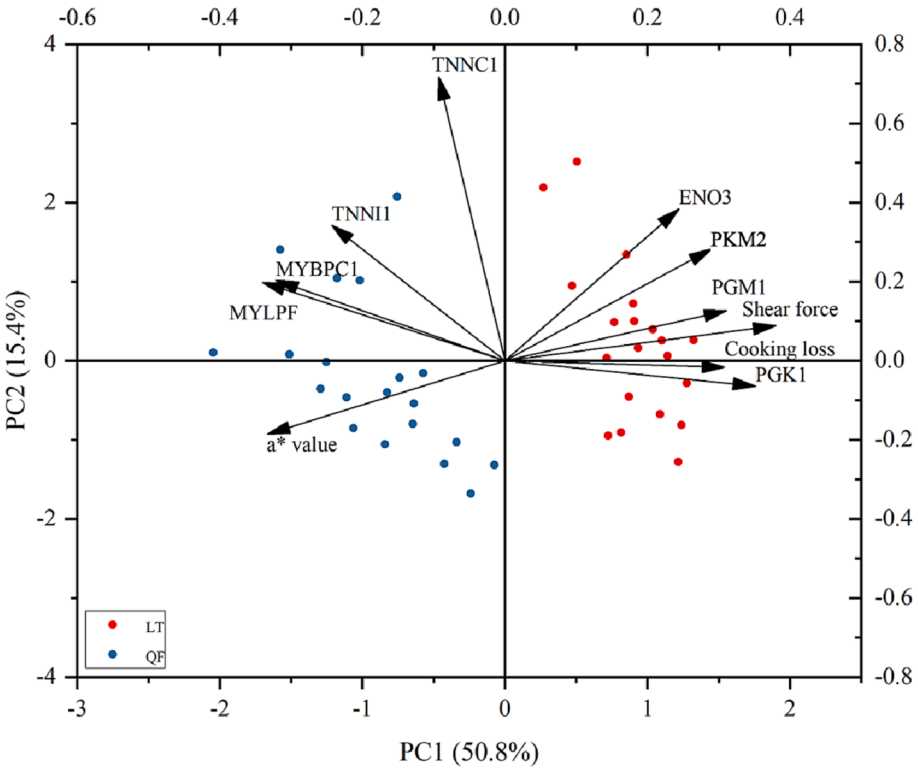


Fig. 6. Principal component analysis summarizing the relationships between potential biological markers and lamb meat quality.

and glycolytic enzyme changes.

3.6. Principal component analysis (PCA)

The PCA was used to evaluate the relationship between 8 potential indicators (PKM2, PGK1, PGM1, ENO3, MYBPC1, MYLPF, TNNC1 and TNNI1) and meat quality characteristics (shear force, a* and cooking loss). PC1 and PC2 explained 50.8% and 15.4% of the total variance, respectively (Fig. 6). LT and QF muscles were clearly distinguished by the positive and negative quadrants of PC1. Therefore, PCA can identify these two muscle groups to a certain extent. In addition, the distances of proteins (PKM2, PGK1, PGM1, ENO3, MYBPC1, MYLPF and TNNI1) and meat quality characteristics (shear force, a* value and cooking loss) was near in the score chart, suggesting significant correlations. Thus, the results showed that PKM2, PGK1, PGM1, ENO3, MYBPC1, MYLPF and TNNI1 proteins can be considered as the robust indicators to characterize and assess lamb meat quality characteristics in the postmortem.

4. Conclusions

The current study verified the ability of 8 potential indicators (PKM2, PGK1, PGM1, ENO3, MYBPC1, MYLPF, TNNC1 and TNNI1) of lamb meat quality characteristics (tenderness, color and water-holding capacity) based on the different muscle types (LT and QF muscles). It is concluded that PKM2, PGK1, PGM1, ENO3, MYBPC1, MYLPF, and TNNI1 proteins can be used as robust biomarkers to characterize and forecast the postmortem lamb meat quality characteristics, which may be influenced by the postmortem glycolytic rate and then decide the final postmortem meat quality. In addition, the glycolytic enzyme biomarkers negatively regulated the lamb meat quality characteristics, while the structural proteins biomarkers positively regulated the lamb meat quality characteristics, and these biomarkers were not influenced by muscle types. However, the molecular mechanism of these protein biomarkers used to characterize lamb meat quality needs to be investigated in the future, and the online detection technology in the lamb meat industry should be developed for them.

CRediT authorship contribution statement

Caiyan Huang: Methodology, Investigation, Data curation, Software, Resources, Writing – original draft. **Dequan Zhang:** Conceptualization, Funding acquisition, Supervision. **Zhenyu Wang:** Investigation, Validation. **Christophe Blecker:** Supervision. **Shaobo Li:** Resources. **Xiaochun Zheng:** Resources, Software. **Li Chen:** Conceptualization, Supervision, Visualization, Validation.

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

No data was used for the research described in the article.

Acknowledgements

This study was financially supported by the Key Program of the National Natural Science Foundation of China (32102018), and the Agricultural Science and Technology Innovation Program, Institute of Food Science and Technology, Chinese Academy of Agricultural Sciences (CAAS-ASTIP-2023-IFST).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2023.136739.

References

Antonelo, D. S., Gómez, J. F. M., Silva, S. L., Beline, M., Zhang, X., Wang, Y., … Balieiro, J. C. C. (2022). Proteome basis for the biological variations in color and tenderness of longissimus thoracis muscle from beef cattle differing in growth rate and feeding regime, 110947 *Food Research International, 153*. https://doi.org/10.1016/j.foodres.2022.110947.

Bai, Y., Li, X., Zhang, D., Chen, L., Hou, C., Zheng, X., … Ijaz, M. (2020). Role of phosphorylation on characteristics of glycogen phosphorylase in lamb with different glycolytic rates post-mortem, 108096 *Meat Science, 164*. https://doi.org/10.1016/j.meatsci.2020.108096.

Bernad, L., Casado, P. D., Murillo, N. L., Picallo, A. B., Garriz, C. A., & Maceira, N. O. (2018). Meat quality traits in the Greater rhea (Rhea americana) as influenced by muscle, sex and age. *Poultry Science, 97*(5), 1579–1587. https://doi.org/10.3382/ps/pey005

Bernstein, B. E., & Hol, W. G. (1998). Crystal structures of substrates and products bound to the phosphoglycerate kinase active site reveal the catalytic mechanism. *Biochemistry, 37*(13), 4429–4436. https://pubmed.ncbi.nlm.nih.gov/9521762.

Bonnet, M., Soulat, J., Bons, J., Léger, S., De Koning, L., Carapito, C., & Picard, B. (2020). Quantification of biomarkers for beef meat qualities using a combination of Parallel Reaction Monitoring- and antibody-based proteomics, 126376 *Food Chemistry, 317*. https://doi.org/10.1016/j.foodchem.2020.126376.

Bouton, P. E., Harris, P. V., & Shorthose, W. R. (1971). Effect of Ultimate pH upon the water-holding capacity and tenderness of mutton. *Journal of Food Science, 36*(3), 435–439. https://doi.org/10.1111/j.1365-2621.1971.tb06382.x

Canto, A. C. V. C. S., 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

Chaosap, C., Sitthigripong, R., Sivapirunthep, P., Pungsuk, A., Adeyemi, K. D., & Sazili, A. Q. (2020). Myosin heavy chain isoforms expression, calpain system and quality characteristics of different muscles in goats, 126677 *Food Chemistry, 321*. https://doi.org/10.1016/j.foodchem.2020.126677.

Díaz, F., Díaz-Luis, A., Sierra, V., Diñeiro, Y., González, P., García-Torres, S., … Oliván, M. (2020). What functional proteomic and biochemical analysis tell us about animal stress in beef?, 103722 *Journal of Proteomics, 218*. https://doi.org/10.1016/j.jprot.2020.103722.

Fuente-García, C., Sentandreu, M. A., Aldai, N., Oliván, M., & Sentandreu, E. (2021). Proteomic pipeline for biomarker hunting of defective bovine meat assisted by liquid chromatography-mass spectrometry analysis and chemometrics, 104153 *Journal of Proteomics, 238*. https://doi.org/10.1016/j.jprot.2021.104153.

Gururaj, A., Barnes, C. J., Vadlamudi, R. K., & Kumar, R. (2004). Regulation of phosphoglucomutase 1 phosphorylation and activity by a signaling kinase. *Oncogene, 23*(49), 8118–8127. https://doi.org/10.1038/sj.onc.1207969

Hamm, R. (1977). Postmortem breakdown of ATP and glycogen in ground muscle: A review. *Meat Science, 1*(1), 15–39. https://doi.org/10.1016/0309-1740(77)90029-8

Honikel, K. O. (2014). Conversion of muscle to meat | Glycolysis. In M. Dikeman, & C. Devine (Eds.), *Encyclopedia of Meat Sciences* (Second ed., pp. 353–357). Oxford: Academic Press.

Huang, C., Blecker, C., Chen, L., Xiang, C., Zheng, X., Wang, Z., & Zhang, D. (2023). Integrating identification and targeted proteomics to discover the potential indicators of postmortem lamb meat quality, 109126 *Meat Science, 199*. https://doi.org/10.1016/j.meatsci.2023.109126.

Huang, C., Hou, C., Ijaz, M., Yan, T., Li, X., Li, Y., & Zhang, D. (2020). Proteomics discovery of protein biomarkers linked to meat quality traits in post-mortem muscles: Current trends and future prospects: A review. *Trends in Food Science & Technology, 105*, 416–432. https://doi.org/10.1016/j.tifs.2020.09.030

Hwang, Y.-H., Kim, G.-D., Jeong, J.-Y., Hur, S.-J., & Joo, S.-T. (2010). The relationship between muscle fiber characteristics and meat quality traits of highly marbled Hanwoo (Korean native cattle) steers. *Meat Science, 86*(2), 456–461. https://doi.org/10.1016/j.meatsci.2010.05.034

Ijaz, M., Li, X., Zhang, D., Hussain, Z., Ren, C., Bai, Y., & Zheng, X. (2020). Association between meat color of DFD beef and other quality attributes, 107954 *Meat Science, 161*. https://doi.org/10.1016/j.meatsci.2019.107954.

Janssen, I., Heymsfield, S. B., Wang, Z. M., & Ross, R. (2000). Skeletal muscle mass and distribution in 468 men and women aged 18–88 yr. *Journal of Applied Physiology (Bethesda Md. : 1985), 89*(1), 81–88. https://pubmed.ncbi.nlm.nih.gov/10904038.

Kim, G.-D., Jeong, J.-Y., Yang, H.-S., & Hur, S. J. (2019). Differential abundance of proteome associated with intramuscular variation of meat quality in porcine longissimus thoracis et lumborum muscle. *Meat Science, 149*, 85–95. https://doi.org/10.1016/j.meatsci.2018.11.012

Lee, S. H., Joo, S. T., & Ryu, Y. C. (2010). Skeletal muscle fiber type and myofibrillar proteins in relation to meat quality. *Meat Science, 86*(1), 166–170. https://doi.org/10.1016/j.meatsci.2010.04.040

Li, D., Pan, Z., Zhang, K., Yu, M., Yu, D., Lu, Y., … Du, W. (2020). Identification of the differentially expressed genes of muscle growth and intramuscular fat metabolism in the development stage of yellow broilers. *Genes, 11*(3). https://doi.org/10.3390/genes11030244

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

Matarneh, S. K., Silva, S. L., & Gerrard, D. E. (2021). New insights in muscle biology that alter meat quality. *Annual Review of Animal Biosciences, 9*, 355–377. https://doi.org/10.1146/annurev-animal-021419-083902

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*(10), 3998–4004. https://pubmed.ncbi.nlm.nih.gov/17429980.

Ouali, A., Gagaoua, M., Boudida, Y., Becila, S., Boudjellal, A., Herrera-Mendez, C. H., & Sentandreu, M. A. (2013). Biomarkers of meat tenderness: Present knowledge and perspectives in regards to our current understanding of the mechanisms involved. *Meat Science, 95*(4), 854–870. https://doi.org/10.1016/j.meatsci.2013.05.010

Picard, B., & Gagaoua, M. (2020). Meta-proteomics for the discovery of protein biomarkers of beef tenderness: An overview of integrated studies, 108739 *Food Research International (Ottawa, Ont.), 127*. https://doi.org/10.1016/j.foodres.2019.108739.

Pierzchala, M., Hoekman, A. J. W., Urbanski, P., Kruijt, L., Kristensen, L., Young, J. F., … te Pas, M. F. W. (2014). Validation of biomarkers for loin meat quality (M. longissimus) of pigs. *Journal of Animal Breeding and Genetics = Zeitschrift Fur Tierzuchtung Und Zuchtungsbiologie, 131*(4), 258–270. https://doi.org/10.1111/jbg.12081

Radenkovic, S., Bird, M. J., Emmerzaal, T. L., Wong, S. Y., Felgueira, C., Stiers, K. M., … Ghesquière, B. (2019). The metabolic map into the pathomechanism and treatment of PGM1-CDG. *American Journal of Human Genetics, 104*(5), 835–846. https://doi.org/10.1016/j.ajhg.2019.03.003

Ren, C., Li, X., Bai, Y., Schroyen, M., & Zhang, D. (2022). Phosphorylation and acetylation of glycolytic enzymes cooperatively regulate their activity and lamb meat quality, 133739 *Food Chemistry, 397*. https://doi.org/10.1016/j.foodchem.2022.133739.

Ryan, M. T., O'Halloran, A. M., Hamill, R. M., Davey, G. C., Gil, M., Southwood, O. I., & Sweeney, T. (2016). Polymorphisms in the regulatory region of the porcine MYLPF gene are related to meat quality traits in the Large White breed. *Meat Science, 113*, 104–106. https://doi.org/10.1016/j.meatsci.2015.11.016

Schwägele, F., Haschke, C., Honikel, K. O., & Krauss, G. (1996). Enzymological investigations on the causes for the PSE-syndrome, I. Comparative studies on pyruvate kinase from PSE- and normal pig muscles. *Meat Science, 44*(1), 27–40. https://doi.org/10.1016/S0309-1740(96)00046-0

Shen, L., Du, J., Xia, Y., Tan, Z., Fu, Y., Yang, Q., … Zhu, L. (2016). Genome-wide landscape of DNA methylomes and their relationship with mRNA and miRNA transcriptomes in oxidative and glycolytic skeletal muscles. *Scientific Reports, 6*, 32186. https://doi.org/10.1038/srep32186

Shen, L., Gan, M., Chen, L., Zhao, Y., Niu, L., Tang, G., … Zhu, L. (2022). miR-152 targets pyruvate kinase to regulate the glycolytic activity of pig skeletal muscles and affects pork quality, 108707 *Meat Science, 185*. https://doi.org/10.1016/j.meatsci.2021.108707.

Silva, L. H. P., Rodrigues, R. T. S., Assis, D. E. F., Benedeti, P. D. B., Duarte, M. S., & Chizzotti, M. L. (2019). Explaining meat quality of bulls and steers by differential proteome and phosphoproteome analysis of skeletal muscle. *Journal of Proteomics, 199*, 51–66. https://doi.org/10.1016/j.jprot.2019.03.004

Tong, B., Sasaki, S., Muramatsu, Y., Ohta, T., Kose, H., Yamashiro, H., … Yamada, T. (2014). Association of a single-nucleotide polymorphism in myosin-binding protein C, slow-type (MYBPC1) gene with marbling in Japanese Black beef cattle. *Animal Genetics, 45*(4), 611–612. https://doi.org/10.1111/age.12172

Wang, C., Matarneh, S. K., Gerrard, D., & Tan, J. (2021). Modelling of energy metabolism and analysis of pH variations in postmortem muscle, 108634 *Meat Science, 182*. https://doi.org/10.1016/j.meatsci.2021.108634.

Wang, Y., Liu, R., Hou, Q., Tian, X., Fan, X., Zhang, W., & Zhou, G. (2020). Comparison of activity, expression and S-nitrosylation of glycolytic enzymes between pale, soft and exudative and red, firm and non-exudative pork during post-mortem aging, 126203 *Food Chemistry, 314*. https://doi.org/10.1016/j.foodchem.2020.126203.

Yang, X., Wang, J., Holman, B. W. B., Liang, R., Chen, X., Luo, X., … Zhang, Y. (2021). Investigation of the physicochemical, bacteriological, and sensory quality of beef steaks held under modified atmosphere packaging and representative of different ultimate pH values, 108416 *Meat Science, 174*. https://doi.org/10.1016/j.meatsci.2020.108416.

Yu, Q., Tian, X., Shao, L., Xu, L., Dai, R., & Li, X. (2018). Label-free proteomic strategy to compare the proteome differences between longissimus lumborum and psoas major muscles during early postmortem periods. *Food Chemistry, 269*, 427–435. https://doi.org/10.1016/j.foodchem.2018.07.040

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