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Abstract

This study aimed to identify protein biomarkers of meat quality traits from *Longissimus thoracis* (LT) muscle of male goats (*Capra hircus*). Goats of similar age and weight reared under extensive conditions were used to relate the LT muscle proteome with multiple meat quality traits. The early post-mortem muscle proteome analyzed using label-free proteomics was compared among three texture clusters built using hierarchical clustering analysis. Twenty-five proteins were differentially abundant and their mining using bioinformatics revealed three major biological pathways to be involved: 10 muscle structure proteins (MYL1, MYL4, MYLPF, MYL6B, MYH1, MYH2, ACTA1, ACTBL2, FHL1 and MYOZ1); 6 energy metabolism proteins (ALDOA, PGAM2, ATP5F1A, GAPDH, PGM1 and ATP5IF1), and two heat shock proteins: HSPB1 (small) and HSPA8 (large). Seven other miscellaneous proteins belonging to pathways such as regulation, proteolysis, apoptosis, transport and binding, tRNA processing or calmodulin-binding were further identified to play a role in the variability of goat meat quality. The differentially abundant proteins were correlated with the quality traits in addition to multivariate regression models built to propose the first prediction equations of each trait. This study is the first to highlight in a multi-trait quality comparison the early post-mortem changes in the goat LT muscle proteome. It also evidenced the mechanisms underpinning the development of several quality traits of interest in goat meat production along the major pathways at interplay.

Significance

The discovery of protein biomarkers in the field of meat research is an emerging topic. In the case of goat meat quality, very few studies using proteomics have been conducted with the aim of proposing biomarkers. Therefore, this study is the first to quest for biomarkers of goat meat quality using label-free shotgun proteomics with a focus on multiple traits. We identified the molecular signatures underlying goat meat texture variation, which were found to belong to muscle structure and related proteins, energy metabolism and heat shock proteins along with other proteins involved in regulation, proteolysis, apoptosis, transport and binding, tRNA processing or calmodulin-binding. We further evaluated the potential of the candidate biomarkers to explain meat quality using the differentially abundant proteins by means of correlation and regression analyses. The results allowed the explanation of the variation in multiple traits such as pH, color, water-holding capacity, drip and cook losses traits and texture.

Keywords: Goat meat quality; Label-free proteomics; Protein biomarkers; Biochemical pathways; Muscle system process.

1. Introduction

Regarding the current world and climate changes, there is a call for an increase in the supply of agricultural and farming products and a strengthening of the link between production and consumption sites, particularly in the livestock sector. In fact, increasing the supply of animal proteins requires a balanced development between the different animal species (monogastric *versus* ruminant) which do not use the same resources [1]. Thus, the production of small ruminants can be considered a good alternative towards an economic and agroecological production, in particular because of their generally traditional mode of production [2-4]. Goat would therefore be the animal best adapted to valorize/use fodder and non-competitive resources to humans of low nutritional value and many other resources such as shrubby type rangelands or agricultural co-products [5, 6]. In Algeria, research on goats has been largely neglected, despite the economic importance of this domesticated species for the rural livelihoods of nomads in the south of the country (Sahara) and small farmers in the North and semi-desert regions. Among the native goat breeds that are distributed in the different Algerian areas, *Arbia,* is the most widespread in steppic regions, whereas *Naine de Kabylie* is a local breed of the mountainous Northern regions of Kabylia. Regarding the Sahara, *M'zabite* is mainly found in the northern part and *Mekatia* dominates in the areas between the steppic and Saharian regions [7]. The rearing of these breeds (or populations as they are hugely cross-breed with European breeds such as Saanen and/or Alpine) has many advantages for the local farmers including the ability to tolerate harsh climates, good drought recovery capacity, suitability for poor systems due to small size and grassland capacity.

Among the few research studies conducted in Algeria on goat farming and production, most of them focused on the morpho-biometric characterization and the typology of goat production systems [3, 7]. Thus, the research studies on the production, consumption and distribution of goat meat are very limited [8] and seemed necessary to undertake. Accordingly, we aim by this work to support goat farming and production practices through the implementation of innovative characterization strategies in the frame of high-throughput techniques such as Foodomics, for instance by means of proteomics [9, 10], to evaluate the goat populations used for meat production purposes. The use in the recent decades of massive omics methods in meat science such as proteomics allowed to unravel the huge complexity of the muscle proteomes (or sub-proteomes) and provided significant details about the changes that occur early post-mortem or over aging times or even among muscle types and comparisons in the expression pattern of proteins in different production conditions [11-13].

Proteomics also allowed a better understanding of the mechanisms behind several meat quality traits and their defects, mainly because the proteome is the main constituent of muscle tissue and it has pivotal roles in the muscle to meat biochemical changes occurring during aging [10, 14]. The major structural/metabolic alterations in early post-mortem muscle and during aging were further partly revealed thanks to these sophisticated methods [15-17]. Proteomics was further successfully applied to propose explanatory mechanisms at the origin of the variability of different meat-eating quality traits from many species and also to propose biological markers [13, 18, 19]. However, to the best of our knowledge, very few studies aimed to investigate goat muscle/meat proteome in relation to its meat quality [20-22].

Taken all together, our major aim is to strengthen our knowledge using proteomics and catch up on the scientific delay accumulated over the years in understanding consumers' needs and relaunch research on goat meat, which according to our assumptions will play an important role in the coming years in achieving the sustainable development goals of producing localities. Indeed, the livestock sector is known to play a dynamic and significant role in the economic development of rural and desert areas. Thus, this study has an overall attempt of in-depth characterization of goat meat produced by entire male Saanen x Naine de Kabylie crossbred goats reared and produced in Kabylia Mountains (North of Algeria). We further aim to identify candidate protein biomarkers explaining the variation of multiple meat quality traits using label-free proteomics, chemometrics and bioinformatics approaches.

2. Materials and Methods

2.1. Goats and slaughtering procedure

All the experimental procedures used in this trial were in compliance with the Algerian guidelines for the care and use of animals. Twenty entire male goats of the Saanen x Naine de Kabylie crossbred goats reared under extensive production systems in the Kabylia Mountains of Algeria were used in this study. The animals were of equivalent age at slaughter (240 ± 8 days) and similar weight (25 ± 0.8 kg). The goats were transported one day before to the abattoir and kept overnight without feeds but with free access to water. To avoid season effect, the animals were slaughtered during one month of the same season (Spring). The animals were slaughtered under ethical standards in the same slaughterhouse, in compliance with the Halal slaughter method (based on Islamic legislation). In this type of slaughter, the goats were not stunned. The carcasses were dressed following routine commercial

slaughterhouse procedures. After exsanguination, the carcasses were split into two equal halves along the vertebrate column.

2.2. *Longissimus thoracis* muscle and meat sampling

Longissimus thoracis (LT) muscle samples, known as an oxido-glycolytic muscle, were collected from the 6th to 9th thoracic vertebrae and immediately transported in an insulated box filled with ice. After delivery of the samples, the meat chops were trimmed of external connective tissue (epimysium) and visible fat and then fabricated into required pieces and placed in different labeled plastic bags, vacuum packaged and kept at 4°C for 48 h. The samples of LT tissue were analyzed during the post-mortem period at three different time points depending on the quality trait: 30 min, 6 h and 24 h post-mortem for pH measurements; 6 h, 24 h and 48 h post-mortem for myofibril fragmentation index (MFI), 24 h and 48 h post-mortem for water-holding capacity (WHC), drip loss and cook loss determinations, and 24h for color evaluation. For proteomic analysis, muscle samples (10 g) were removed before the measurement of pH 30 min, chopped into small pieces using sterile scalpels, and stored at −80°C until the extraction of muscle proteins.

2.3. Meat quality evaluation

2.3.1. pH

Muscle pH was recorded at 30 min (pH 30 min), 6 h (pH_{6h}) and 24 h (pH_{24h}) post-mortem. The pH 30 min was evaluated directly on the carcass before sampling using a calibrated meat pH meter probe. For pH_{6h} and pH_{24h}, approximately 1 g of tissue muscle from each animal was used in triplicate and mixed with a fresh and cold solution containing 5 mM sodium iodoacetic acid and 150 mM KCl (pH 7.0) at a 1:9 ration (w/v) [23, 24]. Samples were homogenized for two 15 s bursts, with a 5 s interval, using a Polytron homogenizer (model PT2100, Kinematica AG, Littan/Luzern, Switzerland). For pH measurements, an insertion electrode (PHS-3CW microprocessor pH /mV meter, BANTE instrument) was used to record the pH of the tissue homogenates. A two-point calibration was performed with calibration buffers of pH 7.00 and 4.00 at 20 °C.

2.3.2. Color

Color was determined following the AMSA protocol [25]. Briefly, 24 h LT muscle samples were exposed to air for 45 min at 2 – 4 °C to bloom. Then, the instrumental lean color traits were measured using a Minolta colorimeter CR-400 (Konica Minolta, Japan), using illuminant D65, a 10° standard observer and an 8 mm diameter aperture. Lightness (L^*),

redness (*a**), and yellowness (*b**) coordinates values on the bloomed meat surface were determined by averaging values of five readings from five randomly selected areas per steak.

2.3.3. Water holding capacity and drip loss

Water-holding capacity (WHC) was measured at 24 h and 48 h according to the filter paper method of Grau and Hamm [26] with modifications concerning the areas determination using an imaging software. Briefly, a sample of 300 ± 5 mg of meat from the LT muscle was weighed (G) and deposited in the center of a previously desiccated and weighed (P) filter paper Whatman #1 disk (Whatman Laboratory Division, Maidstone, England). Then, the sample on the paper was placed between two plates of Plexiglass and pressed with a constant pressure of fabricated weight of 2.25 kg for 5 min. After that, the areas of meat spot (M) and released juice (T) were drawn on clear plastic and the damp paper filter was weighed (D) after removing the compressed meat sample. WHC was calculated as M × 100 / T of the areas.

The percentage of drip loss (DL) was calculated as (D – P) × 100/G. Traditionally, the stain areas of meat spot (M) and released juice (T) were measured using a Planimeter [26], but the method is not very precise, instrument-dependent and very old. As an alternative, we used a simple previously validated method based on image analysis [27]. Hence, the stain areas drawn in clear plastic were scanned and quantified using the open source ImageJ 1.48 software. The freehand selection option was used for the quantification of the different areas. From each muscle, five samples were analyzed and a mean of these replicates was used.

2.3.4. Cooking loss

The cooking loss was evaluated following the procedure described previously [28]. Briefly, each weighed meat cube (30 ± 2 g) was packed in a polyethylene bag, and then placed in a temperature-controlled water bath set at 80 °C for 45 min. The cooked samples were then cooled at room temperature for 30 min, and then weighed again to calculate the percentage of weight loss, which is the difference in weight between the cooked and raw samples. The analyses were performed in triplicate.

2.3.5. Myofibrillar fragmentation index (MFI)

Myofibril fragmentation index (MFI) was performed in triplicate using the method given by Culler *et al.* [29] and modified by Hopkins *et al.* [30]. Briefly, 1 g of each muscle sample was homogenized using a Polytron homogenizer (model PT2100, Kinematica AG, Littan/Luzern, Switzerland), for 30 s in 10 mL of rigor buffer (pH 7.0) containing 75 mM

KCl, 10 mM KH_2PO_4, 2 mM $MgCl_2$, 2mM EGTA, and 1 mM NaN_3. After centrifugation at 1000 x g for 15 min and at 4°C, the supernatant was discarded. The pellet was re-suspended in 10 mL of the rigor buffer, with stirring, centrifuged again, and the supernatant was discarded. The sediment was re-suspended in 10 mL rigor buffer and filtered through a filter paper to remove connective tissue and fat. An additional 10 mL of the rigor buffer was used to facilitate the passage of myofibrils through the filter paper. The filtrate has been kept in adequate tubes at –20°C. The protein concentration of the protein extract (suspension) was determined using the Bradford protocol [31]. Then, the suspension was diluted with the same buffer to 0.5 ± 0.05 mg/mL protein concentration. Finally, the MFI was determined as the value of the absorbance of the myofibrillar suspension measured at 540 nm using an UV spectrophotometer (UV2550, Shimadzu, Japan) and expressed as the absorbance of a myofibrillar protein solution multiplied by 200.

2.4. Proteomics profiling and characterization of goat *Longissimus thoracis* muscle using shotgun proteomics

2.4.1. Muscle protein extraction and quantification

The goat muscle biopsies taken 30 min post-mortem and stored at -80°C were used to extract the total proteins (i.e., both sarcoplasmic and myofibrillar proteins) following the protocol of Bouley *et al.* [32] with slight modifications [16]. Briefly, 150 mg of the samples from each individual were mixed with 3 mL of fresh buffer containing 2 M thiourea, 1.2% DL-dithiothreitol, 8 M urea, 1% Pharmalyte 3−10 (GE Healthcare, Uppsala, Sweden) and 2% CHAPS. The samples and protein homogenates were handled during the entire extraction process in wet-ice and incubated at 4°C for 15 min before homogenization using a Polytron homogenizer (model PT2100, Kinematica AG, Littan/Luzern, Switzerland) at 20,000 rpm for 45 seconds. Afterwards, the homogenates were kept once again in wet-ice and shacked for 25 min before centrifugation at 10,000 x g at 4°C during 25 min to remove insoluble proteins, connective tissue, fat and other debris. The supernatant was then collected in Eppendorf tubes and stored at -80°C for further analyses in terms of protein quantification, SDS-PAGE analyses and proteome characterization.

The quantification of the total proteins, in triplicate, was performed using the Biorad protein assay kit (Bio-Rad Laboratories, Hercules, CA, USA) based on the Bradford method. Serum albumin from bovine (BSA) was used as a standard at a concentration of 1 mg/mL.

2.4.2. Protein bands preparation for shotgun proteomics using 1D SDS-PAGE electrophoresis

To gather all the proteins in one tiny band before shotgun proteomics [33], the muscle protein extracts were first denatured by mixing them at 1.0:1.0 (v/v) with a standard Laemmli sample buffer 2× concentrate (#S3401, Sigma-Aldrich, Saint Louis, USA) that contains 125 mM Tris (pH 6.8), 20% v/v glycerol, 4% w/v SDS, 10% v/v β-mercaptoethanol and 0.004% bromophenol blue. Then, the samples were vortexed and incubated at room temperature for 5 min before heating at 90 °C for 10 min using a standard block heater (VWR, International). The denatured proteins (40 µg, final volume of 20 µL) were subsequently loaded in standard 12% resolving and 4% stacking gels of sodium dodecyl sulphate polyacrylamide gel electrophoresis (one-dimensional SDS-PAGE) using a Mini-PROTEAN Tetra Cell system (Bio-Rad Laboratories, Hercules, CA, USA) at 4 watts during 15 min to concentrate the proteins in the stacking gel. A TGS running buffer (#T7777, Sigma-Aldrich, Saint Louis, USA), containing 25 mM Tris (pH 8.6), 0.1% SDS and 192 mM glycine was used. Afterwards, the gels were washed several times with Milli-Q water, stained with EZ Blue Gel staining reagent (Sigma-Aldrich, Saint Louis, USA) for around 10 min and subjected to several other washes with Milli-Q water under gentle shaking. The protein bands from each animal were excised from the washed gels using sterile and disposable scalpels, and immediately placed into sterile Eppendorf tubes to be reduced, alkylated, destained and dried, before LC-MS/MS analyses [16].

2.4.3. LC-MS/MS analysis, protein identification and preparation of the proteome database

Before the liquid chromatography-tandem mass spectrometry (LC-MS/MS) analyses, the dried protein bands stored at -20 °C were first digested by a sequence grade Trypsin (Promega, USA) following the conditions described in Zhu *et al.* [33]. The samples were analyzed by means of a Dionex UltiMate 3000 system (nanoelectrospray ion source) coupled to a Q Exactive™ HF-X hybrid Quadrupole-Orbitrap mass spectrometer (Thermo Fisher Scientific). A C18 column was used to desalt the peptide fractions before LC-MS/MS analysis according to the manufacturer's instructions. The peptides were loaded with C18 PepMap trap column (Thermo Fisher Scientific) and washed with pure water (98%), acetonitrile (ACN, 2%), and formic acid (0.01%) with a flow rate of 10 µl/min. After 6 min, the trap column underwent reversed-phase high-performance liquid chromatography (RP-HPLC) using an EASY nLC self-filling column (Thermo Fisher Scientific, Germany). Mobile phase A of the reversed-phase HPLC consisted of 0.1% formic acid in pure water, and mobile

phase B consisted of 95% ACN acetonitrile with 0.1% formic acid. The peptides were separated using a 40-min gradient method at a flow rate of 300 nL/min. The peak identification of the peptides and raw files of LC-MS/MS were screened and aligned against *Capra hircus* (goat) database (UP000291000, 32,609 sequences). The digestive enzyme was set to trypsin to search for the mass spectrometry data in the retrieval database. The maximum missing cleavage sites were set to 2, and the tolerances of precursor and fragment ions were 5 ppm and 0.02 Da, respectively. The aminomethylation of cysteine was a fixed modification, and acetylation of the protein N-terminal and lysine and methionine oxidation were variable modifications. The database search results were screened and exported when the false discovery rate (FDR) of the peptide spectrum and protein match level was < 1%.

2.5. Statistical analyses

For the statistical analysis, XLSTAT 2018.1.1 (AddinSoft, Paris, France) software was used. The texture meat quality traits based on the myofibrillar fragmentation index (MFI) were first considered by means of an unsupervised learning method to create from the 20 goats three meat texture clusters of low (Low-Q), medium (Medium-Q) and high-quality (High-Q) using a hierarchical clustering analysis (HCA). The Euclidean distances were used based on standardized data (z-scores). This hierarchical approach is of interest because it allows a naturally cluster formation with valuable use in meat research [34].

Afterwards, a one-way ANOVA was used to compare the evaluated quality traits among the three meat texture clusters. Animals were considered as a random effect. The same model was further used to compare the whole proteome data based on the Log2 relative abundances of the proteins. Differences in protein abundance among the meat texture quality groups were considered significant at $P < 0.05$, and differentially abundant proteins (DAPs) were considered as candidate protein biomarkers and used as a subset for further analyses.

Principal component analysis (PCA), as a common multivariate data analysis technique for dimension reduction, was used to study the distribution of the individuals and the extent of the separation of the MFI meat texture quality clusters using the meat quality traits evaluated in this study. The scree plot of the PCA based on eigenvalues and the squared cosines of the variables were calculated. Further, to examine the suitability of the data for the PCA model, the Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy of each variable and the overall KMO (Bartlett's test of sphericity) were computed. In addition, a linear discriminant

analysis (LDA), a classical feature extraction algorithm, was applied to separate the three texture groups using the relative abundances of the identified DAPs proteins.

Pearson correlations and multivariate regression analyses were further performed using the DAPs proteins. The correlations between the 15 meat quality traits investigated in this study at different post-mortem times (texture parameters evaluated by MFI, pH, color and WHC, drip and cook losses traits) with the DAPs proteins were tested using Pearson correlation coefficients. The correlations were considered significant at $P < 0.05$. On the other hand, step-wise regressions were undertaken to explain/predict each of the 15 quality traits (as dependent variables, y) with the DAPs proteins (as independent variables, x). The regression model allowed the entrance of no more than two proteins as explanatory variables to satisfy the parsimony principle [24]. This approach allowed us identifying the most relevant candidate protein biomarkers for each trait. The proteins with large regression coefficient values (whatever the direction (sign)) were considered as good predictive candidates. Variance inflation factor (VIF), a criteria selection method that maximizes the correlation between independent variables and response variables and minimizes the collinearity between the selected protein biomarkers was set at less than 10 for each model. This allowed us to avoid any multi-collinearity in the validated regression equations.

2.6. Bioinformatics analyses

Bioinformatics analyses were conducted on the DAPs proteins to assess the main molecular functions, the enriched Gene Ontology (GO) terms and to build the protein-protein interactions (PPI) networks. For this purpose, the goat accession numbers of the proteins were first converted into human gene entries by means of the Uniprot Retrieve/ ID mapping tool (https://www.uniprot.org/id-mapping) to take advantage of the most updated information and knowledge concerning the proteins being aware of the weakness of the *Capra hircus* (goat) database. The PPI network between the DAPs proteins was constructed using the open source STRING 11.0 platform (https://string-db.org/). We used the default settings of confidence of 0.4 and 7 criteria for linkage: text mining; co-occurrence, co-expression, experimental evidence, neighborhood, existing databases and gene fusion. Subsequently, the other bioinformatics analyses were performed following well-described procedures [15] using the Metascape® online platform (https://metascape.org/). The process enrichment analysis allowed to create an ontology network based on the most significant and enriched GO terms considering Gene Ontology (GO) Biological Processes, Reactome gene sets, WikiPathways and Kyoto Encyclopedia of Genes and Genomes (KEGG) on the DAPs proteins as one

dataset. Benjamini-Hochberg p-value correction algorithm was used to statistically validate the top significant enriched ontology terms (parameters fixed to *P*-value <0.05, a minimum count number of 3 proteins, and an enrichment factor >1.5 (ratio between the observed counts and the counts expected by chance). In addition, the densely connected regions (MCODE) known also as molecular complexes were detected from the GO network generated by Metascape®.

3. Results and Discussion

The decision of consumers to purchase meat, including goat meat, is guided by myriad interacting factors including freshness and safety, nutritional profile, sustainability pillars (*i.e.*, environmental, economic, and social) along with a variety of organoleptic characteristics such as color, succulence, tenderness, juiciness and flavor [35]. Thus, meat quality is a complex dimension influenced by both intrinsic and extrinsic interacting factors with key influences coming from on-farm management and post mortem processing. For goat meat quality, the aforementioned factors are also key determinants [36], but very few studies are available in the literature for this species with a significant scarcity of those conducted at the molecular level to decipher the biological pathways at interplay. The increase of our knowledge and evaluation of such important factors along the biochemical mechanisms is then of paramount importance in the case of goat, because of the lower tenderness of its meat compared to lamb, pork, and beef [35, 37]. Therefore, this work intends to broaden our knowledge on the underlying mechanisms of meat quality (on multiple traits) of male goats raised in an extensive production system, using shotgun proteomics that has recently gained popularity as the performance of mass spectrometers has significantly improved [9].

3.1. Meat quality and characteristics of the texture quality clusters

The descriptive statistics of the meat quality traits evaluated in this trial are depicted in **Table 1**. In line with the general assumptions on goat meat texture [36, 38], the findings revealed greater and substantial variation in the MFI values (CV ranging from 25 to 31%) compared to the other quality attributes (CV <3% for pH, <6.5% for color parameters, and from 5 to 14% for water-holding capacity, drip and cook losses traits). MFI is one of the most commonly used indicators to evaluate the extent of proteolysis (myofibrillar protein degradation) in post-mortem meat [30]. Based on the higher variation observed for MFI, the values at 6, 24 and 48 h post-mortem were then used together to build texture quality clusters by means of hierarchical clustering analyses (**Figure 1**). Three texture quality clusters, these

being High-Q (n = 6), Medium-Q (n = 7) and Low-Q (n = 7), were created by this unsupervised learning method (**Figure 1A**). The clustering approach allowed to clearly distinguish the goat meat quality groups (**Figure 1B,C**), which were also significantly different in terms of pH whatever the post-mortem time ($P < 0.05$), lightness 24h ($P < 0.001$), WHC ($P < 0.01$) and drip loss ($P < 0.001$) at both 24 and 48h, and cook loss ($P < 0.001$) at 48h only (**Table 2**). Overall, the High-Q cluster characterized by the highest MFI values (producing meat with better tenderness), had lower pH, lighter meat and high water retention (**Figure 2** and **Table 2**). MFI was found to increase significantly ($P < 0.01$) within the high-Q cluster by increasing post-mortem time compared to Low-Q cluster ($P > 0.05$), which has a numerically very slow trend. Further, the principal component analysis (**Figure 2A,B**) using the whole variables evaluated in this trial highlighted a clear separation of the goat meat texture clusters (**Figure 2B**). The first two principal components explained 66% of variation (**Figure 2C**), with a significant amount in the first principal component (51%). The pH variables were all grouped together with redness (a^*) in the bottom left of the PCA. The variables MFI, WHC and lightness (L^*) were projected together on the right side of the first principal component with the High-Q meat samples, while drip and cook losses and yellowness (b^*), were projected on the left side with the Low-Q meat samples. Among these parameters, pH is an important factor that can better reflect the quality of meat and directly affect several traits likely color, water retention/loss and cooking loss of muscle, thus affecting the final texture of meat. In this study, the mean values of ultimate pH and irrespective of the quality cluster were in the acceptable range of 5.5 – 5.8 for goat meat [39-41], but with lower and favorable values of better quality in the High-Q cluster.

Based on the color and pH results, the meat samples of High-Q cluster can be valued as pale red, a desirable quality searched by consumers [8]. The greater L^* values can be ascribed to higher retention of water (high WHC and low drip loss), in agreement to what has been reported in earlier studies [40, 42]. Higher L^* values is known to be caused by scattering of light within the muscle structure as well as the present fluid on the surface of the meat matrix [43]. Consistent to this, it is worthy to note that L^* is in this database correlated irrespective of sampling time positively with WHC and MFI and negatively with drip loss (**Table 3**). From the meat texture point of view, the degradation of proteins is known to be related to the extent and rate of pH decline [16, 44]. Indeed, fast pH decline was thought as a favorable condition for enhanced proteolysis and degradation of myofibrillar muscle proteins [45]. The extent of the structural proteins denaturation and degradation during the post-mortem process

would affect the protein density along the sarcomere, hence influencing myofilament lattice spacing and muscle fiber shrinkage, which impacts also light scattering [43]. Higher pH in the goat carcasses of Low-Q cluster early post-mortem or during aging may reduce the activity of μ-calpain activity, thereby decreasing the rate and extent of the tenderization process [44]. Another contributing factor may include a higher activity of calpastatin inhibitor in goat muscles (especially in the Low-Q cluster) compared, for instance, to lamb [46]. Thus, the significantly higher MFI values at 6, 24 and 48 h post-mortem times ($P < 0.001$) observed in the High-Q cluster could be due to faster pH decline during the post-mortem period. The increased protein degradation at lower pH might further lead to increased light scattering properties of meat [43], consequently increasing L^* values observed for the High-Q texture samples. The equivalent redness/vividness of the goat meat samples among the quality texture clusters could be explained by the type of production system used in this study that is extensive feeding [47]. Nevertheless, further investigations are worthy to conduct for a better understanding of such stability in a^* and b^* values observed in the goat carcasses of this trial.

In the current work, greater MFI values in the High-Q cluster can be further ascribed to changes in the myofilament spacing. In fact, lower pH impacts the physical state of proteins that will be below their isoelectric point, thereby reducing the association with muscle water and consequently impacting positively the tightness (decreased compactness) of the muscle fibers [48]. In agreement with earlier studies [49], the higher WHC in the High-Q cluster was further found to be characterized by lower drip and cooking losses. The increase of drip loss in the Low-Q cluster might be partly related to the reduction of unit cell volume, thereby increasing the elastic pressure within the sarcomeres and the denaturation of proteins induced by pH decline, elevated pre-rigor temperature and cellular stress [50]. Accordingly, we could assume that the goats of the High-Q cluster experienced reduced cellular stress in muscle, thereby keeping more water in their muscle compared to their counterparts in the Low-Q cluster. It is worthy to note that earlier studies suggested that post-mortem proteolytic degradation of myofibrillar proteins is able to improve WHC [51, 52].

The cook loss of meat has been suggested to depend on the ultimate pH of the carcasses [53]. In this study and within the three clusters, cooking loss values were different at 48h post-mortem only and ranged from 23.04% (High-Q) to 26.99% (Low-Q). Overall, these values are in the range of those reported in earlier studies for goat meat [36, 39, 47]. The high ultimate pH in the Low-Q texture cluster (4 animals from 7 had pH ≥ 5.9) might be partly

related to potential animal stress at slaughter, hence explaining the lower WHC values and the high drip and cook losses (**Table 2**). It is known in the literature that goats, especially young males as in this study, are highly prone to stress [36]. Finally, the sudden variation in pH decline was postulated to reflect variation in the post-mortem muscle metabolism among animals [24, 54]. Early post-mortem metabolism seemed to be of a pivotal role in goat meat quality determination. Further details about this aspect and interrelated pathways are comprehensively discussed thanks to the proteomics results presented in the following sections.

3.2. Differentially abundant proteins discriminating goat meat texture clusters and related proteomics signatures

From the goat muscle proteomics database built in this trial (n = 835 quantified proteins), 25 DAPs ($P < 0.05$, FDR 1%, minimum of 2 peptides per protein) were identified to differ among the three goat meat texture clusters (**Table 4**). The proteins were manually annotated and grouped into three main biological pathways plus a group of miscellaneous proteins (**Table 4**). The first pathway "muscle structure and related proteins" contained 10 putative protein biomarkers: Myosin light chain 1/3 (MYL1), Myosin light chain 4 (MYL4), Myosin regulatory light chain 2 (MYLPF), Myosin light chain 6B (MYL6B), Myosin-1 (MYH1), Myosin-2 (MYH2), Actin, alpha skeletal muscle (ACTA1), Beta-actin-like protein 2 (ACTBL2), Four and a half LIM domains protein 1 (FHL1) and Myozenin-1 (MYOZ1). The second "energy metabolism" pathway with both glycolytic and oxidative sub-pathways included 6 putative biomarkers: Fructose-bisphosphate aldolase A (ALDOA), Phosphoglycerate mutase 2 (PGAM2), Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), Phosphoglucomutase-1 (PGM1), ATP synthase subunit alpha, mitochondrial (ATP5F1A) and ATPase inhibitor, mitochondrial (ATP5IF1). The third "response to cellular stress" pathway grouped two heat shock proteins: small Heat shock protein beta-1 (HSPB1) and large Heat shock cognate 71 kDa protein (HSPA8). Finally, 7 other "miscellaneous" proteins with multiple functions belonging to pathways such as regulation, proteolysis, apoptosis, transport and binding, tRNA processing or calmodulin-binding were further identified to play a role in the variability of goat meat quality characteristics. This fourth category grouped Elongation factor 2 (EEF2), Serpin family A member 1 (SERPINA1), Ewing's tumor-associated antigen 1 homolog (ETAA1), Leucine carboxyl methyltransferase 2 (LCMT2), Histone H2A type 1-H (H2AC12), Caveolae-associated protein 4 (CAVIN4) and Tripartite motif containing 72 (TRIM72).

The linear discriminant analysis (LDA), a powerful statistical chemometrics methodology, was further used to confirm the robustness of the 25 DAPs to separate the three texture quality clusters (**Figure 3**). The putative protein biomarkers were used for LDA as the variables and the three goat meat texture clusters (High-Q, Medium-Q and Low-Q) were considered as classes. A high prediction accuracy was achieved within the x-axis (**Figure 3**). In line with the data of **Table 4**, the High-Q cluster had higher abundances of 9 proteins: MYL1, MYL6B, MYH1, FHL1, PGM1, ATP5F1A, HSPB1, EEF2 and SERPINA1. The Low-Q cluster had higher abundances of 10 proteins: MYL4, MYH2, ACTA1, ACTBL2, GAPDH, PGAM2, HSPA8, ETAA1, TRIM72 and LCMT2. Finally, the intermediate Medium-Q cluster had higher abundances of 6 proteins: MYLPF, MYOZ1, ATP5F1, ALDOA, CAVIN4 and H2AC12.

In the protein network (**Figure 4)**, the three main biological pathways were found to be significantly interconnected in one network. The enrichment analysis on the 25 putative protein biomarkers using Metascape® database, allowed the identification of 9 significantly enriched Gene Ontology (GO) terms (**Figure 5A,B**). The top GO term was "GO:0003012: Muscle system process" followed by "GO:0007517: Muscle organ development", "GO:0046034: ATP metabolic process", "R-HSA-397014: Muscle contraction", "GO:0030036: Actin cytoskeleton organization", "GO:0048545: Response to steroid hormone", "WP4754: IL-18 signaling pathway", "GO:0042060: Wound healing" and "GO:1901137: Carbohydrate derivative biosynthetic process" (**Figure 5A**). The GO terms were interconnected in an enriched network (**Figure 5B**) with two conserved and distinct significant molecular complex detection sub-networks (**Figure 5C**) corresponding to "muscle contraction" (MCODE1, n = 8 proteins) and "ATP metabolic process" (MCODE2, n = 5 proteins).

3.2.1. Pivotal role of muscle system process in goat meat texture determination

The identification of a high number of muscle structure and myofibrillar proteins as discriminating biomarkers validates the experimental approach we used, *i.e.,* focusing on texture through an in-depth evaluation of myofibrillar fragmentation index and water retention/loss. Muscle system process was the top most enriched pathway (**Figure 5**). These findings are consistent with established literature on the role that muscle structure and related pathways play in the determination of texture [55], further revealed by several proteomics studies [15]. MFI as a texture indicator used to evaluate goat meat texture was significantly correlated with several cytoskeleton proteins (**Figure 6**), from which several are known as

primary features changing during post-mortem aging [56]. Indeed, the breakdown of structural proteins by endogenous proteolytic systems is a key inducing factor [57] and it has a major role in determining meat tenderness [58, 59]. Apoptosis onset and proteolysis processes occurring early post-mortem with continuous effect during ageing lead to a loss in the integrity of several parts of the sarcomere and consequently to the breakdown of the myofibrils [13-15], thereby explaining the identification of six protein members as related to the contractile proteins of heavy and light myosin chains. Myosin is one of the major structural proteins in muscle, and its content, composition, and rate of degradation are the major factors affecting final meat tenderness [56, 60]. In more detail, the post-mortem structural changes and their extent have been attributed to multi-enzymatic processes, including calpains, caspases, and cathepsins, which all play in a sophisticated manner significant roles in the development of meat texture [16, 58]. The known evidence of the relationship between proteolysis and meat tenderization [59], was further confirmed in this trial by the significant enrichment of 4 from 9 GO terms (**Table 5** and **Figure 5**) exclusively related to "GO:0003012: Muscle system process", "GO:0007517: Muscle organ development", "R-HSA-397014: Muscle contraction" and "GO:0030036: Actin cytoskeleton organization".

During the post-mortem storage, the myofibrils of goat meat seemed to be weakened into shorter segments and certain proteins of interest identified in this trial support the increase during aging of MFI values through their positive associations (**Figure 6**), especially for the High-Q cluster. Z-disk degradation was suggested since the earlier studies as one of the major factors contributing to meat tenderization [61]. This has been evidenced in this study by changes in the abundances of ACTA1, ACTBL2, FHL1 and MYOZ1, all located in the Z-disk [15, 62]. However, the proteolytic breakdown in link with meat tenderization can be also manifested with breaks at the junction of the I-band and the Z-disk adjacent to the Z-disk at N2 lines [63], in support of the identification of other myofibrillar proteins outside the Z-disk degradation from the thick filament (**Table 4** and **Figure 4**).

3.2.2. Significant role of energy metabolism in the interconnected pathways driving the conversion of goat muscle into meat

Studies investigating modifications in post-mortem muscle, namely within the 48 h, have described changes in energy metabolism compared to that in the live animal [14, 64]. The advances of meat research proteomics in the last decade strengthened the evidence and importance of post-mortem metabolism in meat quality determination, mainly through better

analyses of the dynamic changes occurring during the rigor and tenderization phases [15, 65]. In line with this body of knowledge, our study revealed 6 proteins from energy metabolism and ATP metabolic process (**Table 4** and **Figure 4**). This is the second most important pathway impacting goat meat texture development in this study (**Figure 5**). Four proteins are glycolytic enzymes (PGM1, ALDOA, GAPDH and PGAM2), and two with ATP synthase function (ATP5F1A and ATP5IF1) are from mitochondria involved in the oxidative phosphorylation pathway [15, 66]. The identification of more proteins involved in glycolysis rather than those involved in the TCA cycle and oxidative phosphorylation pathways may be explained by decreased aerobic and increased anaerobic metabolic capacity in the muscle [67]. Nevertheless, proteome changes during the rigor development with a shift in post-mortem muscle energy metabolism towards the glycolytic pathway and to some extent to aerobic energy metabolism have been previously reported [68]. The changing enzymes in this trial, especially those from the energy-yielding phase of glycolysis, might be explained by the aim of replenishing the ATP levels in the muscle in an attempt of maintaining muscle in a relaxed state [69]. In fact, ATP production is needed to keep cellular ATP constant, hence allowing a continuous (short) contraction of muscle immediately after animal bleeding. Furthermore, the production of ATP in post-mortem muscle has been described to have a key role in the apoptosis onset among other cell-death processes, known as energy-dependent mechanisms [14]. The identification of PGM1, PGAM2, GAPDH and ATP5F1A among others as changing proteins with impact on goat meat quality is in agreement with previous studies from goat [20, 22] and other species such lamb [70], beef [71, 72], horse [73] and donkey [17]. Consistent with our findings, several studies reported relationships between the above enzymes and the glycolytic rate with impact on post-mortem tenderization [12, 14, 74, 75], hence confirming that energy metabolism and ATP metabolic process are key in understanding the differences in goat meat quality.

3.2.3. Response to cellular stress and protein folding

Extremely conserved within species, chaperone proteins known also as heat shock proteins (HSPs) play a pivotal role in protein folding, protein transport, assembly of protein complexes, cell cycle control and signaling, but also in the protection of cells against cellular stress and/or apoptosis [12]. In addition, the involvement in this study of two HSPs (HSPB1 and HSPA8) in the development of goat meat quality and texture is very relevant. This is in agreement with most meat research proteomics studies that often confirmed differential abundances across treatments of HSPs [12, 15].

Changes in the abundances of HSP members can be partly related to the cellular stress induced by animal death, hence they are a way to respond to the lack of blood supply post-mortem. The small chaperone HSPB1 would play a protective role in muscle protein degradation and/or adaptation to the glycolysing conditions of the post-mortem muscle [76]. Even related to meat quality in different directions, *i.e.*, negative and/or positive, depending on studies/factors; HSPB1 is one of the most frequently identified proteins in the literature of meat research proteomics [9, 15]. It has been reported to associate and/or protect myofibrillar proteins such as desmin, actin and troponins from μ-calpain action as well as in inhibiting caspase-3 activation [77].

HSPA8, a large 70 kDa inducible protein, was in this study more abundant in the Low-Q cluster. In fact, HSPA8 could have accumulated in the cytoskeletal and myofibrillar proteins intending to improve muscle structure stability. This can occur through two main mechanisms, firstly due its role in maintaining muscle cell integrity and repair of denatured proteins [78]. Second, to the response to stress load before or during slaughter or other pre-, peri- and post-slaughter stress factors including transport and mixing with unfamiliar animals [13, 79], thereby showing higher abundances in Low-Q samples (**Figure 3**). In fact, HSPA8 accumulates in the cytoskeletal and myofibrillar proteins with the aim of improving muscle structure stability [11, 80], and by doing so, mainly in the Low-Q texture cluster, it could lead to reduced fragmentation of the myofibrils. Interestingly, both HSPB1 and HSPA8 were in this study connected to each other and with proteins from the energy metabolism and muscle structure pathways (**Figure 4**). Taken all together, further research is necessary to better understand the specific role of HSPs in goat meat quality determination.

3.3. Putative protein biomarkers correlated with the goat meat quality traits

For a better management of meat quality traits, proteomics has been further used beyond the objective of unveiling the underlying mechanisms of muscle-to-meat conversion as discussed above, but also with the aim of identifying potential candidate biomarkers to explain/predict the variability in meat eating quality [9]. Referring to this lofty goal, the 25 differentially abundant proteins (DAPs) were correlated with the multiple goat meat quality traits (q = 15) evaluated in this study (**Figure 6**). The total number of correlations for each protein irrespective of the quality trait and *vice versa* were also depicted. Furthermore, best regression equations were built for each quality trait when possible following a parsimony rule of a maximum of two candidate protein biomarkers (**Table 6**). In the following sections, the correlated and retained proteins in each category of quality traits are discussed.

3.3.1. Myofibrillar fragmentation index

As expected, the highest number of correlated proteins were for MFI quality traits with 10, 14 and 23 correlations for 6h, 24h and 48h post-mortem times, respectively (**Figure 6**). Eight proteins from the four interconnected biological pathways (**Figure 4**) were common to the three post-mortem times and in the same direction, these being MYL1, MYL6B, PGM1 and EEF2 (all positive) and MYL4, GAPDH, HSPA8, and H2AC12 (all negative). The regression models irrespective of the post-mortem time explained from 57% to 62% of MFI variation and retained among the common proteins MYL1, PGM1 and HSPA8 (**Table 6**). Further, MYOZ1 was common to MFI 6h and 24h models, while SERPINA1 was retained with PGMI in the best MFI 48h model explaining 62% of the variability (**Table 6**).

Several of these proteins were previously reported as biomarkers of high and low goat meat quality using proteome and/or phosphoproteome studies [20-22]. Among them, myosin light chains were proposed as biomarkers of meat eating quality traits within different species [11, 17, 73]. MYL1 encoding for a myosin alkali light chain and expressed in fast-twitch muscle fibres was in this study the top protein correlated 9 times with several quality traits (**Table 6**). MYL1 was previously shortlisted by an integromics study as a top biomarker of beef tenderness [15]. The positive correlation of MYL1 with MFI is in the same trend observed for MYH1 (fast myosin IIX fibres), which can be related to increased abundance of MYL1 in muscles of leaner animals [11] and consistent with the characteristics of goat meat known to have less fat [81]. PGM1, a key enzyme in glycolysis and glycogenesis, was also shortlisted by the integromics study of Gagaoua *et al.* [15] as a candidate biomarker of meat texture. Anderson *et al.* [82] reported in agreement with our findings greater abundances of PGM1 in tender meat samples. A similar trend has been recently confirmed in Chinese indigenous cashmere goat for the two isoforms of PGM [20]. The negative correlation of the 70 kDa chaperone (HSPA8) is in agreement with the body of knowledge on the anti-apoptotic and negative roles of heat shock proteins in meat tenderization [12, 76, 78, 83, 84], thereby explaining the low MFI values for Low-Q texture cluster. In support of this, H2AC12, a histone h2a family member from the core component of nucleosome and involved in cellular response to DNA damage, was negatively correlated to MFI whatever the post-mortem time. The involvement of histones agrees with earlier proteomics studies that identified members as biomarkers of beef tenderness [83]. During cellular stress, members of histone H2A family might recruit metabolic enzymes to trigger energy production and increase protein synthesis,

and by doing so, they involve proteins of response to stress such as HSPA8 to protect from degradation muscle cells proteins [85].

Identification of EEF2 as a meat quality biomarker in small ruminants, is to the best of our knowledge never reported. Elongation factor 2 (EEF2) is thought to play a role in the ribosome to translate mRNA into protein, mainly by the catalysis of the GTP-dependent ribosomal translocation step [86]. The several correlations of EEF2 beyond the positive relation with MFI irrespective of post-mortem time (**Figure 6**), allow us to propose this protein as a potential biomarker of goat meat texture. The other role EEF2 might play in phosphorylation activity as a response to cellular stress asks for more studies to decipher the mechanisms.

3.3.2. Water-holding capacity, drip and cook losses

A significant number of proteins from the four biological pathways were correlated with drip loss: 17 and 19 correlations for 24h and 48h post mortem times, respectively (**Figure 6**). For cook loss, 16 correlations were found for 48h and one positive correlation (CAVIN4) only was observed for 24h. CAVIN4 known also as MURC (muscle-restricted coiled-coil protein) is the only protein related to goat meat cook loss at 24h and 48h. CAVIN4 localized to the sarcolemmal membrane, is a caveolin protein involved in the caveola biogenesis and function with key protecting role of the cell surface of the skeletal muscle against mechanical stress damage [87]. For drip and cook losses, an overlap of 15 proteins was observed (**Figure 6**), and they were always in the same direction, suggesting interrelated biochemical mechanisms and pathways. For WHC, very few correlations have been identified with only 3 positive associations for WHC 24h (MYL1, HSPB1 and SERPINA1) and 7 correlations for WHC 48h (**Figure 6**).

The involvement of several proteins in the variability of drip loss is consistent with the body of knowledge and earlier proteomics studies that focused on this key parameter [10, 21, 88]. In agreement to our findings the current knowledge gained by proteomics evidenced myofibrillar and associated proteins, energy metabolism, apoptosis and response to stress proteins as the major pathways contributing to the determination of WHC and drip loss. Degradation of goat meat muscle proteins during the post-mortem period as discussed above can be related to the development of, and the improvement in, texture quality traits including water retention/loss [57]. Multiple post-mortem events such as pH decline, apoptosis, proteolysis, and protein oxidation are major muscle factors impacting water retention/loss [51,

57]. This is further evidenced by the strong and high correlation coefficients observed whatever the post-mortem time between WHC and drip loss values with MFI (**Table 3**).

Overall, poor values of water retention in meat may cause reduced water content, thereby affecting the sensory (juiciness and tenderness), technological (yield and processing) and nutritional (loss of valuable nutrients) qualities [9, 51]. Several of the proteins correlated with WHC, drip and cook losses in this study are known biomarkers of meat quality and goat drip loss, for instance referring to those reported in an earlier proteomics study that investigated three goat breeds (Huang-Huai, Boer, and Boer x Laoshan white) using divergent *Longissimus thoracis* samples of high and low drip loss [21]. Among the 10 DAPs structural proteins, 8 were correlated with drip loss (**Figure 6**), from which MYL1, MYL6B, MYH1, MYH2, ACTA1 and ACTBL2 have been reported by Wang *et al.* [21]. In agreement to our findings, MYH1 (fast myosin IIX fibres) was the only biomarker whatever the breed that was in the same direction (negative) with drip loss. Consistent to this, Bernevic et al. [89] reported in their proteomics study the importance of protein denaturation in pork WHC and revealed correlations with carbonylated proteins mainly with myofibrillar proteins such as actin (ACTA1) and isoforms of myosin. Actin is used as a hallmark of programmed cell death because of its early post-mortem degradation by caspases during the apoptosis onset [14]. However, compared to Wang *et al.* [21], actin was in this trial in the same direction (positive) for Boer goat only, but not for Boer x Laoshan white goat (negative). The issue of the inversion of the associations between protein biomarkers and meat quality traits, for instance within breeds, has been previously pointed out [90, 91].

Among the energy metabolism proteins, 3 candidate biomarkers (PGAM2, ATP5F1A and GAPDH) were identified (**Figure 6**) from which ATP5F1A was also identified by Wang *et al.* [21], but with inverted direction to our findings. Changes in the metabolic enzymes in relation to WHC and drip loss has been intensively documented [88], and thought to be mostly related to the impact on the denaturation of proteins due to the acidic conditions in the post-mortem muscle. For example, a higher abundance of ATP synthase subunit was found in pork muscle classified as high drip loss [92]. Overall, enzymes and proteins implicated in glycolysis, lactic acid production and oxidative phosphorylation pathways are key molecular signatures of water retention/loss in meat [9].

In response to cellular stress, it is very interesting to observe both HSPB1 (negative) and HSPA8 (positive) correlated with water retention/loss parameters, more importantly with drip loss (**Figure 6**). These results are in agreement with proteomics data on goat [20-22] and

other species, especially pork, with the main objective of understanding drip loss [9, 10, 92]. HSPB1 was in this study increasing by decreasing ultimate pH (pH 24h), consequently causing myofibrillar structural damage (positive correlation with MFI and *L**), thereby leading to an increase in drip loss (or a decrease in WHC). The implication of HSPB1 and HSPA8 in drip loss and WHC determination of goat meat could be partly ascribed to their chaperoning function, in essence, to the protection of structural functions such as actin against denaturation and function loss [12]. In this study, both HSPB1 and HSPA8 were interacting in the protein network with ACTA1 (**Figure 4)**, suggesting a pivotal role in controlling actin filament dynamics and degradation. Yu and co-workers investigated in a targeted manner several HSPs, including HSPB1 and HSPA8, to understand the phenomenon of pork drip loss [93]. Consistent to our findings, the authors reported a close association between the abundance of these chaperones and variability of water loss in post-mortem muscle. These findings were further confirmed by another study using two-dimensional difference gel electrophoresis [94]. Thanks to their multifunction roles, HSPs can also contribute to the cellular defence mechanisms against oxidative stress, hence delaying the impact of reactive oxygen species on muscle structure. Despite these interesting findings, more research is required to clarify the role of HSPs in goat meat water retention/loss.

From the miscellaneous proteins, tripartite motif-containing protein 72 (TRIM72) previously reported as an interesting biomarker of meat quality, mainly on beef [18, 95], was positively correlated here with drip loss (24h and 48h) and cook loss 48h (**Figure 6**). In contrast to our study, TRIM72 was under-expressed in high drip loss samples of Boer × Laoshan white goat [21]. Differences in the contractile and metabolic properties in the muscles of the breeds along of the slaughter conditions could be the reason of the disparities between studies [18], especially for TRIM72 known to be involved in oxidative stress and clearance of apoptotic agents [96, 97]. It is worth noting that TRIM72 was in this study a marker of drip and cook losses only, thus further investigation into TRIM72 and its relation to water retention/loss determination in goat meat and other species is necessary. Finally, EEF2 already discussed above, was found to be strongly correlated (negatively) with drip loss 24h and 48h and retained together with Ewing tumor-associated antigen 1 (ETAA1) in their regression equations as the first explanatory proteins (**Table 5**). Both EEF2 and ETAA1 allowed to achieve highest prediction powers of 66% and 70% to explain drip loss 24h and 48h, respectively. Very little is known about ETAA1 in meat research. It was reported by

Wang *et al*. [98] to be associated with fat deposition in sheep. In this specific case, further research is needed to clarify the role of these proteins in goat meat water retention/loss.

3.3.3. Color traits and pH

Certain of the DAPs proteins were also found to be correlated with goat meat color traits, but most importantly with *L** values (**Figure 6**), by the observation of 11 correlations (MYL1, MYL4, MYL6B, GAPDH, PGM1, ATP5IF1, HSPB1, HSPA8, EEF2, H2AC12 and CAVIN4). None of the DAPs was correlated with *b** values and only MYLPF (positive) and PGM1 (negative) were correlated with *a** values. The application of proteomics to investigate meat color has been widely performed and recently reviewed (for review refer to [9, 10, 71]). The comparison of our results to the integromics study by Gagaoua and co-workers on beef color biomarkers [71] and horse color proteomics [99], revealed significant overlap with the proteins retained in this study. The correlations between the proteins with *L** values in this study were in the same direction as those of MFI, which means that the underlying mechanisms are quite similar. Targeted studies on goat meat color and proteomics in the context of stability over aging times are needed in the near future, especially in the context of extensive production systems.

For pH, only 5 proteins were correlated with pH traits (**Figure 6**). MYL6B was negative and common to pH 30min and 6h, while EEF2 was negative with pH 30min and 24h. MYOZ1 (positive), HSPB1 and SERPINA1 (both negative) were specific to pH 30min and pH 24h, respectively. The identification of few related proteins with pH may be due to the experimental design that focused on texture cluster to identify DAPs. Moreover, the very narrow range with respect to ultimate pH of the goat carcasses can be the other reason, as previously reported [34]. It is worthy to note that none of the energy metabolism enzymes was correlated with pH of goat meat in this study. The negative correlation between HSPB1 and SERPINA1 with pH24 is of interest as both of them are related to response to stress and anti-inflammatory properties, respectively. SERPINA1, a member of the serpin superfamily with an evidenced role in muscle to meat conversion [100], was recently identified to be related to lamb [70] and beef quality [33].

4. Conclusion

This study is the first to apply shotgun proteomics with the aim of investigating the proteome basis of goat meat quality and the discovery of protein candidate biomarkers of multiple eating qualities. Molecular signatures related to muscle structure, energy metabolism

with a dominance of glycolysis and response to cellular stress along pathways such as regulation, proteolysis, apoptosis, transport and binding and tRNA processing were at interplay in the development of goat meat quality. This study allowed shortlisting protein candidate biomarkers such as myosin light chains (MYL1, MYL4 and MYL6B), glycolytic enzymes (PGM1 and GAPDH), heat shock proteins (HSPB1 and HSPA8), and other proteins involved in signaling, inflammation, proteolysis regulation, DNA damage repair and tRNA processing/calmodulin-binding (EEF2, SERPINA1 and H2AC12) as putative biomarkers of several goat meat quality traits. These proteins would feed the pipeline of biomarkers discovery for validation using appropriate methods, for instance using targeted proteomics such as parallel, multiple or selected reaction monitoring methods (PRM, MRM and SRM) and SWATH-MS (Sequential Window Acquisition of All Theoretical Mass Spectra). Several other factors of variation such as age at slaughter, ageing time, production system/rearing practices and muscle types are important to consider in the future goat meat research proteomics studies.

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CRediT author statement

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Declaration of Competing Interest

Authors declare no conflict of interests.

Figure captions

Figure 1. Hierarchical clustering analyses based on the texture traits evaluated by myofibrillar fragmentation index (MFI) at 6, 24 and 48 h *post-mortem* (**Table 1**). **A)** Dendrogram allowing the separation of three clusters (optimal classification) of goat meat texture categorizing the 20 male goats into three quality categories: High quality (High-Q), Medium quality (Medium-Q) and Low quality (Low-Q). **B)** Results by cluster detailing the main parameters of the clustering analysis. **C)** Comparison of the MFI values at different post-mortem times (6, 24 and 48 h *post-mortem*) among the three texture quality categories.

Figure 2. Principal component analysis (PCA) of the meat quality traits and texture clusters characterization. **A)** First two principal components highlighting the loadings of the 15 quality traits evaluated in this study on the 20 entire male goat. **B)** Bi-plot of the PCA allowing seeing the separation among the three quality traits (Low-Q, Medium-Q and High-Q). **C)** Scree plot of the PCA showing that three principal components have eigenvalues of > 1.0. **D)** Squared cosines of the variables highlighting the input of each of them in the three principal components. **E)** Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy of each variable in the PCA and the overall KMO of the PCA (Bartlett's test of sphericity was significant, $P < 0.001$). Only the variable b* (yellowness) evaluated at 24 h *post-mortem* is not eligible (KMO < 0.5) to consider in the analysis.

Figure 3. Linear discriminant analysis (LDA) for best classification of the three texture quality clusters identified based on myofibrillar fragmentation index using the 25 differentially abundant proteins (DAPs). The x-axis allows alone to separate the groups and proteins with high prediction accuracy. An insert, bi-plot, at the bottom right of the LDA is showing the level of the separation of the three clusters and degree of clones of the individuals within each group. The proteins abundant in each quality cluster are highlighted with the corresponding colors: green (High-Q), orange (Medium-Q) and red (Low-Q).

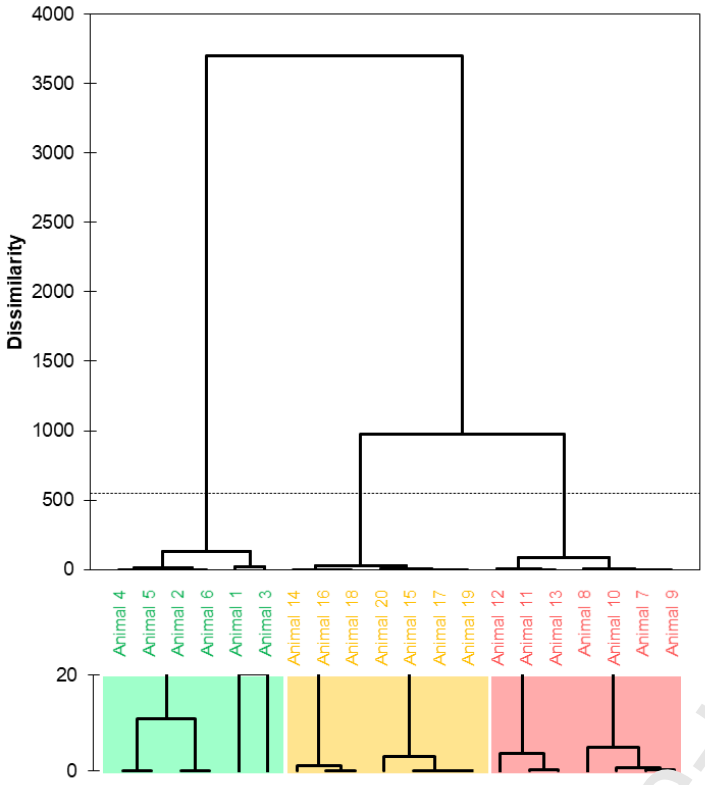
Figure 4. Protein-Protein interaction network built using the web-based search STRING database (https://string-db.org/) based on the 25 differentially abundant proteins (DAPs) (**Table 4**) among the three texture quality traits (**Figure 1**).

Figure 5. Bioinformatics analyses performed by Metascape® (https://metascape.org/) using the 25 differentially abundant proteins (DAPs) (**Table 4**) among the three texture quality traits of the 20 male goats. **A)** Significant enriched TOP 9 Gene Ontology (GO) terms. The bar

graphs are colored according to P-values: terms with a P-value <0.01, a minimum count of 3, and an enrichment factor > 1.5. **B)** Enriched ontology network where each GO cluster term is presented with the corresponding color. The sizes of the nodes reflect the enrichment significance of the terms. **C)** Molecular complex detection (MCODE) enrichment analysis highlighting two key significant modular MCODEs. Each node represents a protein, and the edge between nodes represents the interaction between two connected proteins. The description of the two significant modules from the network is given in the bottom of each MCODE.

Figure 6. Pearson correlation analyses between the quality traits evaluated at different post-mortem times and the 25 differentially abundant proteins (DAPs) (**Table 4**) among the three texture quality traits (**Figure 1**) organized by biological family. The negative and positive significant coefficient of correlation are highlighted with red and green color, respectively. The total number of correlations found for each protein are given on the right and highlighted with a gradual color to indicate the proteins mostly identified. The total number of correlations found for each quality trait are given in the bottom and highlighted with a gradual color.

A)



B)

Cluster	1 High-Q	2 Medium-Q	3 Low-Q
Objects	6	7	7
Sum of weights	6	7	7
Within-class variance	31.65	15.97	5.91
Minimum distance to centroid	1.57	1.11	0.29
Average distance to centroid	4.34	3.47	2.09
Maximum distance to centroid	9.70	5.64	3.00

C)

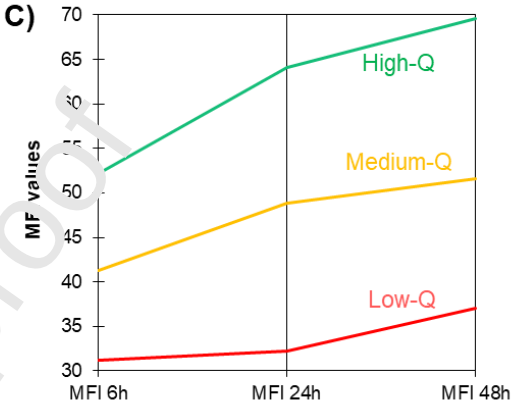
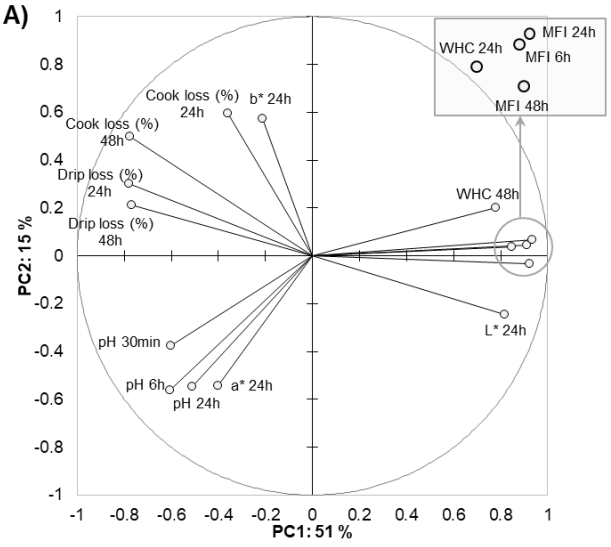


Figure 1.

A)



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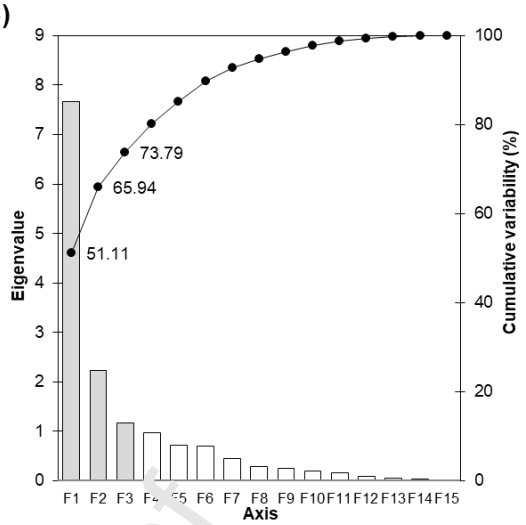
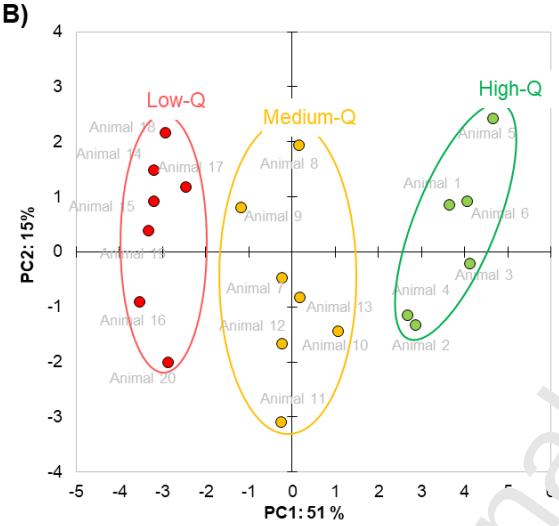


B)



D)

Squared cosines of the variables

Variables	PC1	PC2	PC3
pH 30min	**0.[illegible]**	0.14	0.01
pH 6h	**0.37**	0.31	0.00
pH 24h	0.26	**0.30**	0.03
L* 24h	**0.66**	0.06	0.00
a* 24h	0.16	**0.29**	0.20
b* 24h	0.05	0.33	**0.42**
WHC 24h	**0.72**	0.00	0.02
WHC 48h	**0.61**	0.04	0.06
Drip loss (%) 24h	**0.61**	0.09	0.25
Drip loss (%) 48h	**0.59**	0.04	0.12
Cook loss (%) 24h	0.13	**0.36**	0.05
Cook loss (%) 48h	**0.60**	0.25	0.00
MFI 6h	**0.83**	0.00	0.01
MFI 24h	**0.87**	0.00	0.00
MFI 48h	**0.85**	0.00	0.00

Values in bold correspond for each variable to the factor for which the squared cosine is the largest

E)

Variables	KMO
pH 30min	0.61
pH 6h	0.80
pH 24h	0.64
L* 24h	0.84
a* 24h	0.58
b* 24h	0.23
WHC 24h	0.85
WHC 48h	0.81
Drip loss (%) 24h	0.76
Drip loss (%) 48h	0.81
Cook loss (%) 24h	0.65
Cook loss (%) 48h	0.62
MFI 6h	0.73
MFI 24h	0.67
MFI 48h	0.70
Overall KMO	**0.71**

Figure 2.

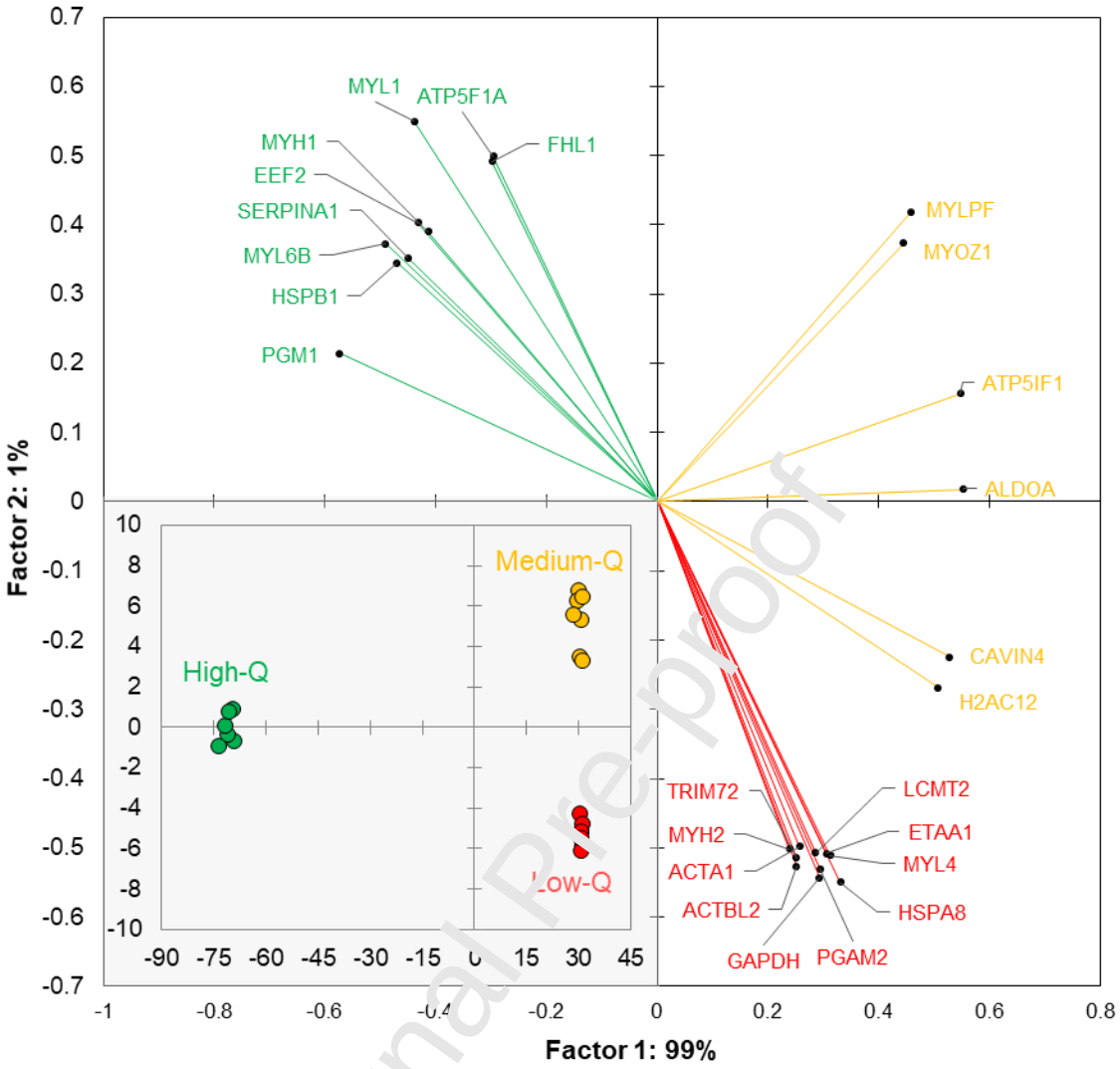


Figure 3.

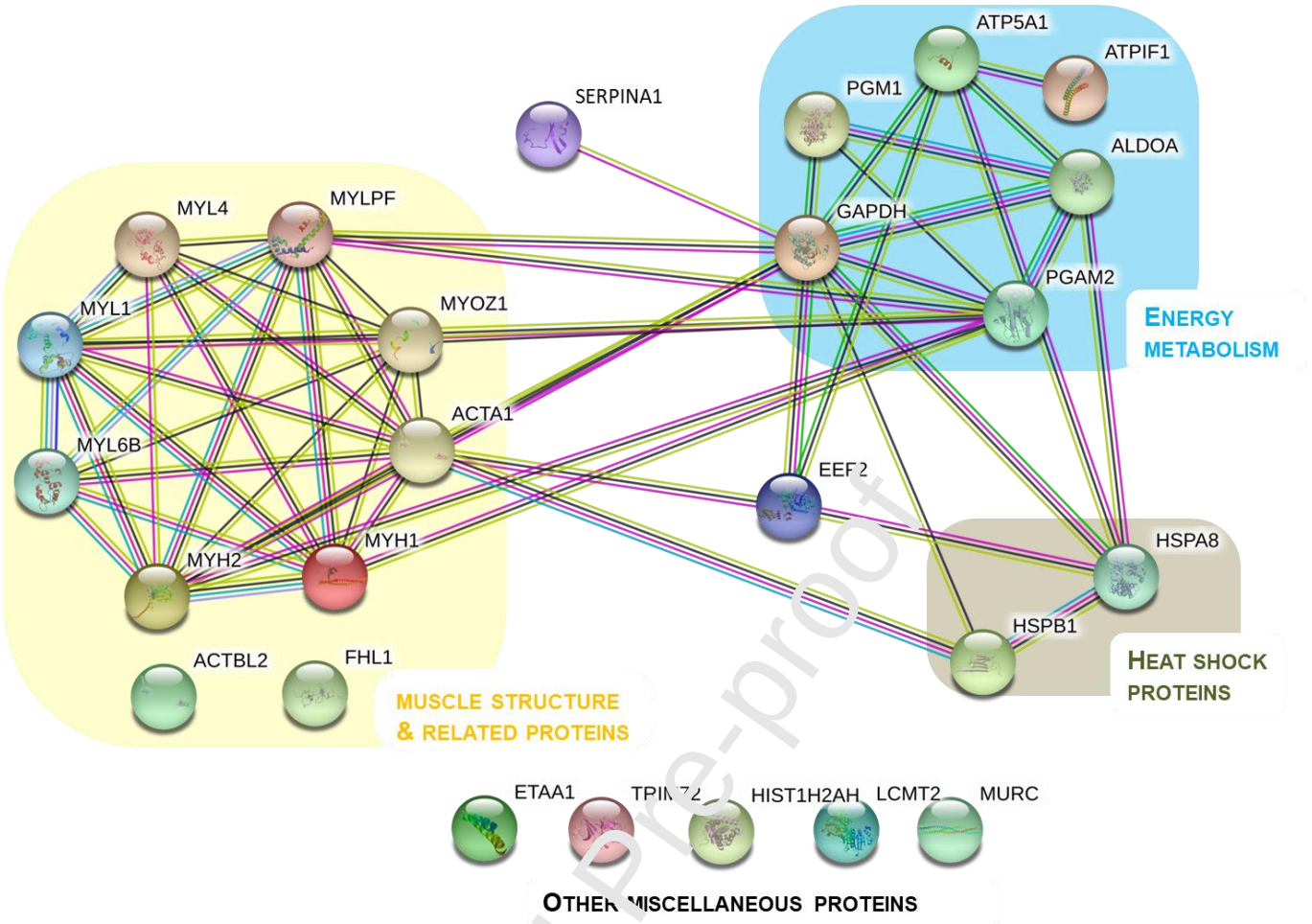
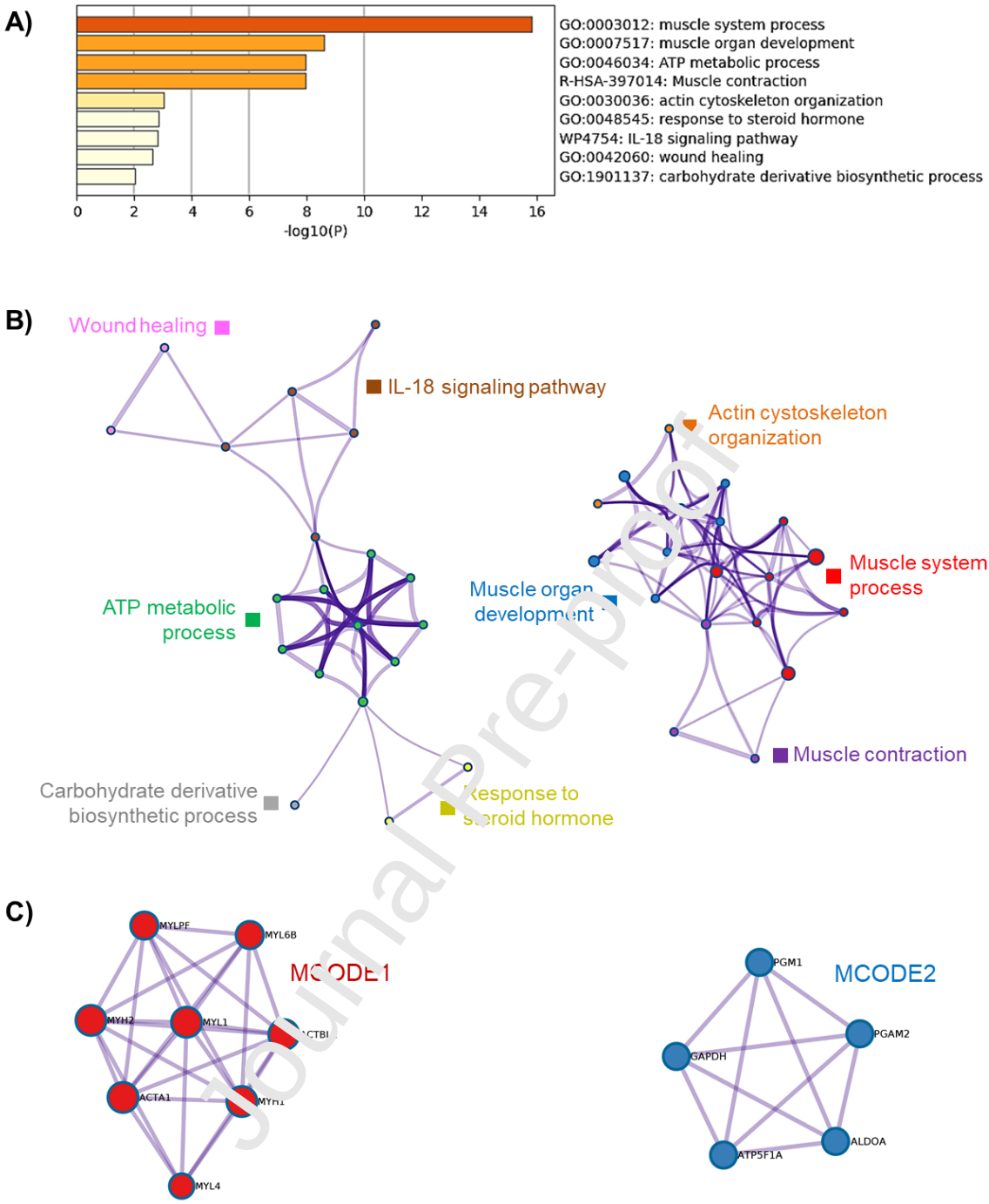


Figure 4.



Color	MCODE	GO	Description	Log10(P)
	MCODE_1	GO:0006936	muscle contraction	-14.0
	MCODE_1	GO:0003012	muscle system process	-13.4
	MCODE_1	GO:0033275	actin-myosin filament sliding	-11.8

Color	MCODE	GO	Description	Log10(P)
	MCODE_2	GO:0046034	ATP metabolic process	-11.0
	MCODE_2	GO:0006096	glycolytic process	-10.9
	MCODE_2	GO:0006757	ATP generation from ADP	-10.9

Figure 5.

	Proteins	pH 30min	pH 6h	pH 24h	L* 24h	a* 24h	b* 24h	WHC 24h	WHC 48h	Drip loss (%) 24h	Drip loss (%) 48h	Cook loss (%) 24h	Cook loss (%) 48h	MFI 6h	MFI 24h	MFI 48h	Total
Muscle structure	MYL1				0.69			0.52	0.52	-0.71	-0.65		-0.65	0.63	0.56	0.59	9
	MYL4				-0.47					0.54	0.52		0.49	-0.47	-0.48	-0.51	7
	MYLPF					0.52			-0.49								2
	MYL6B	-0.63	-0.45		0.61					-0.45	-0.52			0.57	0.62	0.64	8
	MYH1								0.45	-0.50	-0.55		-0.57			0.58	5
	MYH2										0.62		0.53			-0.45	3
	ACTA1									0.45	0.58		0.50			-0.45	4
	ACTBL2									0.48	0.6		0.50			-0.45	4
	FHL1										0.55				0.47	0.52	3
	MYOZ1	0.52													-0.42	-0.35	3
Energy metabolism	ALDOA								-0.52						-0.45	-0.48	3
	PGAM2									0.51	0.66		0.55	-0.45		-0.48	5
	ATP5F1A									-0.47	-0.64					0.50	3
	GAPDH				-0.45					0.56	0.63		0.54	-0.46	-0.45	-0.50	7
	PGM1				0.49	-0.46			0.51					0.50	0.59	0.67	6
	ATP5IF1				-0.45											-0.45	2
Heat shock proteins	HSPB1			-0.48	0.48			0.52	0.46	-0.57	-0.60		-0.52		0.45	0.49	9
	HSPA8				-0.45					0.60	0.56		0.47	-0.49	-0.50	-0.55	7
Miscellaneous proteins	EEF2	-0.51		-0.53	0.54					-0.65	-0.71		-0.45	0.48	0.48	0.48	9
	SERPINA1			-0.55				0.56		-0.49	-0.54				0.55	0.60	6
	ETAA1									0.62	0.60		0.68	-0.45		-0.52	5
	LCMT2									0.50	0.67		0.54			-0.47	4
	H2AC12				0.60				-0.47	0.56	0.51		0.47	-0.59	-0.50	-0.54	8
	CAVIN4				0.47							0.45	0.55		-0.49	-0.63	5
	TRIM72									0.56	0.60		0.46				3
	Total	3	1	3	11	2	0	3	7	17	19	1	16	10	14	23	
		pH			Color			WHC		Drip loss		Cook loss		MFI			

Figure 6.

Table 1. Descriptive statistics of the main meat quality traits evaluated on the 20 entire male Saanen x Naine de Kabylie crossbred goats.

Variables	Minimum	Maximum	Mean	Coefficient of variation (%)
Texture evaluated by Myofibrillar Fragmentation Index				
MFI 6h	23.56	61.87	39.99	24.7
MFI 24h	29.33	73.32	45.92	30.9
MFI 48h	30.45	80.89	50.43	29.3
pH traits				
pH 30min	6.58	6.90	6.78	1.2
pH 6h	6.05	6.65	6.39	2.3
pH 24h	5.58	6.11	5.78	2.8
Color traits				
*L** 24h	32.15	37.43	34.90	4.8
*a** 24h	10.11	12.32	11.28	5.1
*b** 24h	7.98	10.34	9.26	6.2
Water retention/loss: Water-holding capacity, drip and cook losses traits				
WHC 24h	26.00	39.40	31.12	11.5
WHC 48h	26.15	38.78	29.75	13.7
Drip loss (%) 24h	2.01	8.26	4.61	9.5
Drip loss (%) 48h	2.15	8.86	4.74	10.5
Cook loss (%) 24h	21.34	25.13	23.58	4.8
Cook loss (%) 48h	21.77	28.00	25.00	7.8

MFI: Myofibrillar fragmentation index; *L**: lightness; *a**: redness; *b**: yellowness; WHC: Water-holding capacity.

Table 2. Analysis of variance comparing the quality traits parameters among the three texture quality clusters identified based on myofibrillar fragmentation index.

Variables	Low-Q (n = 7)[1]	Medium-Q (n = 7)	High-Q (n = 6)	SEM	P-value[2]
Texture evaluated by Myofibrillar Fragmentation Index					
MFI 6h	30.10^{c}	39.48^{b}	52.12^{a}	2.27	***
MFI 24h	31.35^{c}	44.96^{b}	64.05^{a}	3.25	***
MFI 48h	34.62^{c}	49.86^{b}	69.53^{a}	3.39	***
pH traits					
pH 30min	6.81^{a}	6.80^{a}	6.70^{b}	0.02	*
pH 6h	6.48^{a}	6.41^{a,b}	6.27^{b}	0.04	*
pH 24h	5.83^{a}	5.85^{a}	5.65^{b}	0.04	*
Color traits					
*L** 24h	33.37^{c}	34.80^{b}	36.81^{a}	0.30	***
*a** 24h	11.43	11.48	10.87	0.13	ns
*b** 24h	9.32	9.33	9.11	0.13	ns
Water retention/loss: Water-holding capacity, drip and cook losses traits					
WHC 24h	27.91^{b}	31.29^{a,b}	34.60^{a}	0.82	**
WHC 48h	27.54^{b}	27.54^{b}	34.84^{a}	0.93	***
Drip loss (%) 24h	7.75^{a}	3.30^{b}	2.15^{c}	0.76	***
Drip loss (%) 48h	8.21^{a}	3.40^{b}	2.25^{c}	0.87	***
Cook loss (%) 24h	24.12	23.58	22.94	0.26	ns
Cook loss (%) 48h	26.99^{a}	24.70^{a}	23.04^{b}	0.45	***

[1] Least square means in the same row not followed by a common letter (a-c) differ significantly: $P < 0.05$.

[2] Significances: ns: not significant; * p< 0.05; ** p < 0.01; *** p< 0.001

Abbreviations: MFI: Myofibrillar fragmentation index; *L**: lightness; *a**: redness; *b**: yellowness; WHC: Water-holding capacity; SEM: standard error of mean.

Table 3. Correlation matrix (Pearson) between the 15 meat quality traits evaluated on the 20 entire male Saanen x Naine de Kabylie crossbred goats.

	pH			Color			Water holding capacity		Drip loss		Cook loss		Myofibrillar fragmentation index		
Variables	**pH 30 min**	**pH 6h**	**pH 24h**	***L**** **24h**	***a**** **24h**	***b**** **24h**	**WHC 24h**	**WHC 48h**	**Drip loss (%) 24h**	**Drip loss (%) 48h**	**Cook loss (%) 24h**	**Cook loss (%) 48h**	**MFI 6h**	**MFI 24h**	**MFI 48h**
Correlation coefficients															
pH 30 min															
pH 6h	**0.66**														
pH 24h	**0.54**	**0.48**													
*L** 24h	-0.32	-0.31	-0.20												
*a** 24h	0.33	**0.45**	**0.45**	-0.18											
*b** 24h	0.03	-0.10	-0.24	-0.24	-0.03										
WHC 24h	**-0.44**	**-0.52**	**-0.45**	**0.60**	-0.38	-0.32									
WHC 48h	**-0.45**	**-0.51**	-0.44	**0.60**	-0.42	-0.21	**0.77**								
Drip loss (%) 24h	0.43	**0.46**	0.33	**-0.75**	0.21	0.08	**-0.71**	**-0.52**							
Drip loss (%) 48h	0.44	**0.46**	0.35	**-0.73**	0.27	0.11	**-0.72**	**-0.52**	**0.99**						
Cook loss (%) 24h	0.21	-0.04	-0.02	-0.40	-0.03	0.44	-0.23	-0.06	0.38	0.37					
Cook loss (%) 48h	0.25	0.16	0.20	**-0.68**	0.12	0.40	**-0.59**	**-0.49**	**0.81**	**0.80**	**0.56**				
MFI 6h	**-0.48**	**-0.58**	-0.34	**0.81**	-0.27	-0.09	**0.72**	**0.79**	**-0.82**	**-0.80**	-0.15	**-0.64**			
MFI 24h	**-0.51**	**-0.59**	-0.41	**0.85**	-0.43	-0.15	**0.75**	**0.73**	**-0.83**	**-0.82**	-0.26	**-0.62**	**0.94**		
MFI 48h	**-0.51**	**-0.48**	-0.40	**0.80**	-0.43	-0.11	**0.70**	**0.65**	**-0.87**	**-0.85**	-0.39	**-0.75**	**0.85**	**0.93**	
P-values															
pH30 min															
pH 6h	**0.002**														
pH 24h	**0.014**	**0.031**													
*L** 24h	0.170	0.186	0.386												
*a** 24h	0.158	**0.047**	**0.049**	0.440											
*b** 24h	0.895	0.676	0.305	0.313	0.891										
WHC 24h	**0.049**	**0.018**	**0.047**	**0.005**	0.094	0.170									
WHC 48h	**0.047**	**0.021**	0.055	**0.005**	0.062	0.381	**< 0,0001**								
Drip loss (%) 24h	0.058	**0.043**	0.161	**0.000**	0.367	0.732	**0.000**	**0.018**							
Drip loss (%) 48h	0.055	**0.042**	0.134	**0.000**	0.243	0.633	**0.000**	**0.019**	**< 0,0001**						
Cook loss (%) 24h	0.381	0.855	0.945	0.081	0.900	0.054	0.338	0.788	0.100	0.105					
Cook loss (%) 48h	0.292	0.492	0.410	**0.001**	0.608	0.082	**0.006**	**0.030**	**< 0,0001**	**< 0,0001**	**0.010**				
MFI 6h	**0.031**	**0.007**	0.138	**< 0,0001**	0.241	0.705	**0.000**	**< 0,0001**	**< 0,0001**	**< 0,0001**	0.528	**0.002**			
MFI 24h	**0.020**	**0.006**	0.076	**< 0,0001**	0.061	0.537	**0.000**	**0.000**	**< 0,0001**	**< 0,0001**	0.268	**0.004**	**< 0,0001**		
MFI 48h	**0.021**	**0.033**	0.078	**< 0,0001**	0.061	0.646	**0.001**	**0.002**	**< 0,0001**	**< 0,0001**	0.092	**0.000**	**< 0,0001**	**< 0,0001**	

the bottom part of the table.

Table 4. List of the 25 differentially abundant proteins (DAPs), organized by biological family, identified by Analysis of Variance at a level of 5% to differ among the three texture quality clusters built using myofibrillar fragmentation index.

Full protein name	Gene name	Uniprot ID	Low-Q (n = 7)[1]	Medium-Q (n = 7)	High-Q (n = 6)	P-value[2]
Muscle structure (n = 10)						
Myosin light chain 1/3	MYL1	A0A452EUN1	5.34[b]	6.65[a]	6.95[a]	0.009
Myosin light chain 4	MYL4	A0A452EQ73	-1.19[a]	-1.65[b]	-1.76[b]	0.026
Myosin regulatory light chain 2	MYLPF	A0A452DPJ6	-0.50[b]	0.85[a]	-0.28[a,b]	0.018
Myosin light chain 6B	MYL6B	A0A452DSU5	-0.80[c]	0.76[b]	1.24[a]	0.015
Myosin-1	MYH1	A0A452G9P5	11.53[b]	12.79[b]	13.13[a]	0.004
Myosin-2	MYH2	A0A452G8R7	-2.33[a]	-3.49[b]	-4.16[b]	0.009
Actin, alpha skeletal muscle	ACTA1	A0A452EFE6	-3.09[a]	-6.06[b]	-5.41[c]	0.006
Beta-actin-like protein 2	ACTBL2	A0A452DT40	-2.94[a]	-5.02[b]	-4.41[b]	0.034
Four and a half LIM domains protein 1	FHL1	Q13642	0.70[b]	2.37[a]	2.62[a]	0.008
Myozenin-1	MYOZ1	A0A452DLN0	0.26[b]	1.63[a]	-0.08[c]	0.036
Energy metabolism (n = 6)						
Fructose-bisphosphate aldolase A	ALDOA	A0A452FG79	-0.37[c]	0.44[a]	0.03[b]	0.009
Phosphoglycerate mutase 2	PGAM2	A0A452DL91	-1.74[a]	-4.33[b]	-3.71[b]	0.024
ATP synthase subunit alpha, mitochondrial	ATP5F1A	A0A452ZN82	0.18[c]	1.71[b]	1.92[a]	0.005
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	A0A452FNW6	-1.39[a]	-4.77[b]	-4.08[b]	0.010
Phosphoglucomutase-1	PGM1	A0A452F6J5	-3.29[c]	-1.79[b]	-0.82[a]	0.001
ATPase inhibitor, mitochondrial	ATP5I1	A0A452DUN3	5.03[b]	5.94[a]	5.55[a,b]	0.037
Heat Shock Proteins (n = 2)						
Heat shock protein beta-1	HSPB1	A0A452ESU7	-0.34[c]	1.00[b]	1.40[a]	0.001
Heat shock cognate 71 kDa protein	HSPA8	I6ZQY0	-1.06[a]	-1.65[b]	-1.76[b]	0.013
Miscellaneous proteins (n = 7)						
Elongation factor 2	EEF2	A0A452EIR1	-1.77[c]	-0.39[b]	0.06[a]	0.002
Serpin family A member 1	SERPINA1	A0A452FJ07	-1.84[c]	-0.52[b]	-0.04[a]	0.007
Ewing's tumor-associated antigen 1 homolog	ETAA1	A0A452G9X0	-1.06[a]	-1.40[b]	-1.59[b]	0.026
Leucine carboxyl methyltransferase 2	LCMT2	A0A452DQ16	-0.78[a]	-5.24[b]	-6.70[b]	0.030
Histone H2A type 1-H	H2AC12	Q96KK5	0.03[b]	0.49[a]	-0.84[c]	0.005
Caveolae-associated protein 4	CAVIN4	A0A452E015	-1.51[b]	-0.90[a]	-1.41[b]	0.035
Tripartite motif containing 72	TRIM72	A0A452FH94	2.92[b]	3.24[a]	3.47[a]	0.039

[1] Arbitrary units for each protein in the same row not followed by a common letter (a-c) differ significantly: $P < 0.05$.

Table 5. TOP 9 Gene Ontology (GO) clusters related to **Figure 5A,B** with their representative enriched terms (one per cluster) using the 25 differentially abundant proteins (DAPs) among the three texture quality clusters of goat males.

Gene Ontology (GO)	Category[a]	Description	Count	Proteins	%	Log10(P)[b]	Log10(q)[c]
GO:0003012	GO Biological Processes	Muscle system process	11	ACTA1, ALDOA, MYH1, MYH2, MYL1, MYL4, PGAM2, MYLPF, MYOZ1, MYL6B, TRIM72	44	-15.83	-11.49
GO:0007517	GO Biological Processes	Muscle organ development	7	ACTA1, FHL1, MYLPF, MYOZ1, MYL6B, CAVIN4, TRIM72	28	-8.59	-4.85
GO:0046034	GO Biological Processes	ATP metabolic process	6	ALDOA, ATP5F1A, GAPDH, HSPA8, PGAM2, PGM1	24	-7.97	-4.38
R-HSA-397014	Reactome Gene Sets	Muscle contraction	6	ACTA1, MYL1, MYL4, MYLPF, MYL6B, TRIM72	24	-7.94	-4.38
GO:0030036	GO Biological Processes	Actin cytoskeleton organization	4	ACTA1, ALDOA, MYOZ1, ACTBL2	16	-3.02	-0.42
GO:0048545	GO Biological Processes	Response to steroid hormone	3	ACTA1, ATP5F1A, HSPA8	12	-2.84	-0.25
WP4754	WikiPathways	IL-18 signaling pathway	4	ACTA1, EEF2, HSPB1, SERPINA1	12	-2.81	-0.23
GO:0042060	GO Biological Processes	Wound healing	3	HSPB1, MYOZ1, TRIM72	12	-2.62	-0.04
GO:1901137	GO Biological Processes	Carbohydrate derivative biosynthetic process	3	ALDOA, ATP5F1A, LCMT2	12	-2.03	0

[a] The GO analysis was performed using Metascape® (https://metascape.org/) based on Biological Processes, Reactome gene sets and WikiPathways.

[b] The p-value is log base 10

[c] Adjusted Benjamini−Hochberg p-value correction.

Table 6. Regression (best) models of the different 15 meat quality traits using the 25 differentially abundant proteins (DAPs) following a parsimony rule of two proteins maximum to consider in the model.

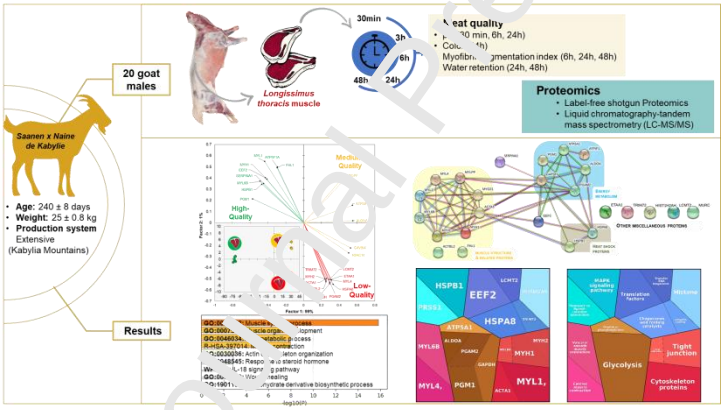
Meat quality trait	R-Squared	Entered proteins	Regression Coefficient	t-Value	p-Value
pH traits					
pH30 min	0.57***	MYOZ1	0.56	3.48	**
		EEF2	-0.54	-3.40	**
pH 6h[a]	-	-	-	-	-
pH 24h	0.42**	EEF2	-0.37	-1.86	t
		SERPINA1	-0.41	-2.04	*
Color traits					
L* 24h	0.62***	MYL1	0.75	4.97	***
		MYOZ1	-0.39	-2.57	*
a* 24h	0.39*	MYLPF	0.56	2.95	**
		PGAM2	0.36	1.89	t
b* 24h[a]	-	-	-	-	-
Water retention/loss: Water-holding capacity, drip and cook losses traits					
WHC 24h	0.45**	MYL1	0.39	2.05	*
		SERPINA1	0.45	2.40	*
WHC 48h	0.54**	HSPB1	0.56	3.35	**
		MYLPF	-0.58	-3.49	**
Drip loss (%) 24h	0.66***	EEF2	-0.54	-3.67	**
		ETAA1	0.49	3.37	**
Drip loss (%) 48h	0.70***	EEF2	-0.61	-4.44	***
		ETAA1	0.45	3.34	**
Cook loss (%) 24h[a]	-	-	-	-	-
Cook loss (%) 48h	0.57**	ETAA1	0.57	3.37	**
		HSPB1	-0.33	-1.96	*
Texture evaluated by Myofibrillar Fragmentation Index					
MFI 6h	0.57***	MYL1	0.69	4.30	***
		MYOZ1	-0.43	-2.66	*
MFI 24h	0.57***	MYOZ1	-0.58	-3.53	**
		HSPA8	-0.65	-3.94	**
MFI 48h	0.62***	SERPINA1	0.42	2.67	**
		PGM1	0.54	3.40	**

[a] No variable could be entered in the model given the input criteria and thresholds.

Abbreviations: MFI: Myofibrillar fragmentation index; *L**: lightness; *a**: redness; *b**: yellowness; WHC: Water-holding capacity. The full names of the proteins are given in **Table 4**.

Significances: t: tendency $p < 0.1$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Graphical abstract



Highlights

- Shotgun proteomics of goat *Longissimus thoracis* muscle for the discovery of protein biomarkers
- Twenty-five differentially abundant proteins (DAPs) discriminate goat meat texture clusters
- Muscle structure, energy metabolism and response to stress are major biological pathways underlying goat meat quality
- pH, color, water-holding capacity, drip and cook losses and texture traits were correlated with the DAPs
- First study to propose candidate protein biomarkers of multiple goat meat quality traits