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Abstract

This study investigated the effect of hazelnut skin by-products supplementation on lamb myofibrillar proteome changes during post-mortem storage (0, 4, and 7 days). Gel-based proteomics and bioinformatics approaches were applied to reveal the underlying biochemical pathways and their importance in lamb meat texture. Twenty-two Valle del Belice male lambs were randomly assigned to two dietary treatments: control (C) for lambs fed with maize-barley diet, and hazelnut skin (H) for lambs fed hazelnut skin by-product as maize partial replacer in the concentrate diet (150 g/kg DM basis). A greater myofibrillar fragmentation index (MFI) was found in meat from the C group at day 0 of storage (91.33 *vs* 98.38 in H and C groups, respectively). Conversely, starting from 4 days of storage, higher MFI values were observed in meat from lambs fed hazelnut skin (113.74 and 116.1 *vs* 99.28 and 107.26 in H and C groups at 4 and 7 days, respectively). Myofibrillar proteome changes estimated by SDS-PAGE and immunoblotting revealed a degradation of desmin ($P<0.01$) and troponin T ($P<0.001$) intact proteins, and an increase in the abundance of the appearing 30 kDa fragment ($P<0.001$) after 4 days of storage in meat from H than the C group. In-depth proteomics and bioinformatics revealed 44 proteoforms (26 unique proteins), mainly involved in actin filament-based process/cytoskeleton organization, energy metabolism, and heat shock proteins, as the major interconnected pathways impacted by hazelnut by-product feeding strategy on lamb meat quality. Twelve proteins were proposed in this trial as potential biomarkers of lamb meat texture as a consequence of hazelnut skin by-products supplementation.

Keywords: Lamb meat; Feeding strategies; Proteomics; Biological mechanisms; Protein oxidation; Tannins; Storage.

1. Introduction

According to the Food and Agriculture Organization of the United Nations (FAO), one-third of food produced for human consumption is lost or wasted globally, which when combined with the future scarcity of foods, results in an unethical and unsustainable situation (FAO, 2011). In this regard and in order to explore the possibility of recovering highly-valuable compounds, several countries have introduced or implemented bio-based-economy regulations against traditional food waste processing. Among agro-industrial resource by-products, hazelnut (*Corylus avellana L.*) skins account for about 2.5 percent of the hazelnut processing by-products (Ivanović et al., 2020; Pelvan et al., 2018). Italy is the second largest country producing hazelnuts with 3,072 tons in 2021 (ISTAT, 2022; accessed on 02 August 2022). While being labeled as waste, hazelnuts skin stands out for their abundances of fiber and fat-soluble bioactive compounds, such as peculiar unsaturated fatty acids, phenolic compounds, polyphenols (mainly represented by condensed tannins), phytosterol, and vitamins (Del Rio et al., 2011; Ivanović et al., 2020; Pelvan et al., 2018). Due to the bioactive compounds content, hazelnut skin further possesses antioxidant, antimicrobial, anti-inflammatory, and immunomodulatory properties (Montella et al., 2013). In fact, there are many studies on the incorporation of this by-product in livestock diet, including small ruminants (Caccamo et al., 2019; Campione et al., 2020; Daghio et al., 2021; Priolo et al., 2021). In particular, Priolo et al. (2021) evidenced an impact on meat quality properties, but no biological mechanisms at the muscle level were detailed. In fact, it is known that changes in muscle during the post-mortem period might result in an unbalance of antioxidant and pro-oxidant capability, increasing the risk of oxidative damage. The oxidative deterioration of meat proteins could negatively affect its organoleptic quality by modifying the activation of the endogenous enzymes responsible for post-mortem tenderization (Rowe et al., 2004). In this context, dietary supplementation with antioxidant compounds in lambs demonstrated a positive effect in enhancing parameters related to tenderness development (Maggiolino et al., 2021; Mu et al., 2020; Muino et al., 2014). However, it is necessary to understand the role of antioxidants in the modulation of post-mortem biochemical mechanisms for better control of the final lamb meat quality. Recently, Priolo et al. (2021) found that the inclusion of 15% of hazelnut skin to replace an equal amount of maize in growing lambs is an effective strategy to reduce the cost of ruminant diet and the cost of disposal for industries. This dietary strategy had no detrimental effects on lamb growth, final body, and carcass weight, whereas improved the nutritional quality of lamb meat enriching the intramuscular meat fat with health-promoting fatty acids. Furthermore, feeding hazelnut skin led to a delay in lipid oxidation

processes, thus improving the lamb meat oxidative stability (Menci et al., 2023). Although the demonstrated impact of hazelnut skin by-products in delaying muscle oxidation processes, the biochemical pathways underlying their action and variations in lamb meat quality have yet to be understood. Thus, we retain that, in the objective of developing new feeding strategies in the context of a circular economy, there is a need to elucidate the role of hazelnut skin antioxidant molecules in driving the molecular mechanisms underlying lamb meat quality variation through the myofibrillar muscle proteome. Taking all together, the main objective of the present trial was to investigate for the first-time post-mortem variation induced in lamb meat by supplementation with hazelnut skins by-products during storage, with a focus on the myofibrillar proteome of *Longissimus thoracis et lumborum* muscle. In-depth characterization using bioinformatics analyses was further applied to reveal the main changing pathways and their importance in lamb meat quality determination.

2. Materials and Methods

2.1. Animals, dietary treatments, and meat sampling

The experimental protocol was designed according to the European Directive guidelines (2010/63/EU) on the protection of animals used for the scientific purpose and were conducted in compliance with the European Directive Council (98/58/EC) on the minimum standards for the protection of animals kept for farming purposes.

The experimental trial was carried out at the experimental farm of the University of Catania, in Sicily Region (latitude:37°24’35.3’’N; longitude: 15°03’34.9’’E). Details of the experimental design are previously described in Priolo et al. (2021). In brief, the experiment involved twenty-two Valle del Belice male lambs (60 ± 4 days of age) that were randomly assigned to two dietary treatments: control (C) -lambs fed with basal diet (maize-barley based concentrate) and hazelnut skin (H) -lambs fed with the same diet with 150 g kg^{-1} dry matter (DM) replacement of maize by hazelnut skin by-product. The ingredients, chemical composition and fatty acid profile of diets are given in **supplementary Table 1**. During the experimental period, animals were fed in individual pens (1.5 m^2) with straw litter equipped with clean water all the time. The effect of dietary treatment on growth performance and intakes were reported in the companion paper (Priolo et al., 2021).

After 56 days of experimental trial, animals were transported to a commercial abattoir (approximately 45 min of transport duration) in accordance with the Council Regulation EC No

1/2005 on the protection of animals during transport. Before slaughter, all lambs were kept in a lairage for 10-12 hours overnight without feed but with free access to water. Then, animals were slaughtered following industrial practices used in Italy and in line with European guidelines (EU rule n. 1099/2009). Each carcass was weighed and chilled at 2-4°C for 24 hours following the industrial routine conditions. After 24 h post-mortem, the carcasses were halved and the *Longissimus thoracis et lumborum* muscle (LTL) was excised from both sides. From the right LTL, three slices (2 cm thickness) were cut and placed in polystyrene trays, covered with oxygen-permeable PVC film, and stored in the dark at 4°C for 0 (24 hours post-mortem), 4 and 7 days (d). At the end of the respective storage times, myofibrillar fragmentation index (MFI), total collagen content and changes of myofibrillar proteome using SDS-PAGE, Western Blot and Two-Dimensional Gel Electrophoresis (2DE) coupled with mass spectrometry were performed.

2.2. Myofibrillar Fragmentation Index (MFI) determination

The myofibrillar fragmentation index (MFI) was determined by spectrophotometric assay according to the protocol of Culler et al. (1978) with some modifications. Briefly, 4 g of muscle sample, were homogenized with Ultra-Turrax homogenizer (IKA T18 basic, Germany) in 40 mL of cold buffer (100mM KCl, 20 mM KH_2PO_4 [pH 7.0], 1mM Ethylene Glycol Tetraacetic Acid, 1mM $MgCl_2$, and 1mM NaN_3) to extract the myofibrils. Then, each homogenate was centrifuged at 1,000 x g (Eppendorf 5810R, Eppendorf AG, Hamburg, Germany) for 15 min at 2°C and resuspended with 10 mL of cold buffer. After the determination of the protein concentrations with the biuret method, the suspensions were diluted with the same buffer to a final protein concentration of 0.5 mg mL^{-1}. The myofibrils suspension was measured spectrophotometrically by using a UV-Vis spectrophotometer (Biotek PowerWave XS2, Biotek Instruments, Inc. Highland Park, Winooski, Vermont, USA) at 540 nm. MFI was expressed as the absorbance at 540 nm multiplied by 150 conversion factors.

2.3. Total collagen content estimation

Collagen content was determined from the hydroxyproline concentration using the method outlined by Hutson et al. (2003) with slight modifications. Briefly, 0.1 g of sample was homogenized with 6M HCl using the Ultra-Turrax homogenizer (IKA T18 basic, Germany). Then, the homogenate was placed in a ventilated oven at 160°C for 75 min for hydrolysis. The tubes were cooled to room temperature, filtered with syringe filters (0.45 um), diluted 1:10 with ultrapure water. Then 1 mL of this solution was placed into a chromatographic vial for the

separations using the HPLC system Agilent Technologies 1260 Infinity. The analysis was performed in duplicate for each sample, and the mean values of all replicates were considered for statistical analysis. The retention times of the hydroxyproline peak were compared to those of the standard. A conversion factor of 7.25 was used to determine the quantity of total collagen from that of hydroxyproline content and reported as μg mg^{-1} of fresh meat.

2.4. Proteome analysis

2.4.1. Protein extraction, SDS-PAGE analysis and immunoblotting analyses

The myofibrillar proteins fraction was extracted according to Marino et al. (2013). The protein concentration of each muscle extract was determined in duplicate employing the 2-D Quant kit (GE Healthcare) using bovine serum albumin (BSA) as standard.

Denatured samples were separated with an 8-18% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gradient gel using a Protean II xi system (Bio-Rad Laboratories, Hercules, CA) in presence of a known molecular weight standard (Precision Plus protein standard-broad range, Bio-Rad Laboratories). Gels were analyzed with the Image Lab software (version 5.2.1, Bio-Rad Laboratory) to determine the percentage of the signal intensity of the defined bands in a lane.

Western blots for troponin-T and desmin were performed on 10% polyacrylamide separating gel as previously reported (Marino et al., 2015). Gels were loaded with 40 μg of protein and run was performed at 100 V for 3 h with Mini-Protean Tetra cell (Bio-Rad Laboratories). After SDS-PAGE, proteins were transferred into a nitrocellulose membrane then incubated with primary antibodies: monoclonal anti-troponin-T (JLT-12; Sigma-Aldrich, St Louis, MO; diluted 1:40000) and monoclonal anti-desmin (D1033, Sigma-Aldrich; diluted 1:5000). After washing, membranes were incubated with secondary goat antibody anti-mouse-HRP (No 2554; Sigma-Aldrich, St Louis, MO) and diluted 1:30000 and 1:5000 for troponin-T and desmin, respectively. Bands were visualized using Clarity Western ECC kit (Bio-Rad Laboratories). Images were acquired by the Chemi Doc EQ system (Bio-Rad Laboratories) and then analyzed with the Image lab software (version 5.2.1, Bio-Rad Laboratories).

2.4.2. Two-Dimensional Gel Electrophoresis (2DE)

Two-dimensional gel electrophoresis (2DE) was conducted according to Marino et al. (2015). Myofibrillar proteins, after rehydration (ready-Prep Rehydration/Sample Buffer, Bio-Rad

Laboratories), were separated in the first dimension with immobilized pH gradient (IPG) dry strips pH 4-7 (17 cm IPG strips, Bio-Rad Laboratories) loaded with 300 μg of protein, using the IPG Protean IEF Cell (Bio-Rad Laboratories). After the isoelectric focusing (IEF), IPG strips were equilibrated at room temperature for 15 min in equilibration buffers I and II (Bio-Rad Laboratories). Two-dimensional separation was performed on a Protean II xi system (Bio-Rad Laboratories) using 8–18% SDS-PAGE. Then, the destained gels were acquired using Chemi Doc EQ system (Bio-Rad Laboratories) and analyzed with the PDQuest 7.4.0 software (Bio-Rad Laboratories) to obtain information on the number of spots per gel, the isoelectric point (pI), molecular weight (MW), volume, area and intensity of each spot. The protein spots detect to differ between the two experimental groups were automatically matched among the gels using the spots of a master gel (virtual gel comprehensive of all matched and unmatched spots of all 2DE images) used as a reference. Landmark spots were used to confirm spot matching across all gels and manual verification was used to screen out any dust artefacts or incorrectly identified spots. The relative volume of each spot in a gel was normalized as a percentage of the total volume of all spots detected on the gel.

2.4.3. In-gel digestion of the protein spots and identification by LC-MS/MS

Spots displaying statistically significant differences among dietary treatments and storage time were carefully excised from the preparative gels and then destained for 30 min with 50 mM NH_4HCO_3. Samples were digested overnight at 37°C with trypsin (12.5 ng mL^{-1}, Promega). Then, peptides were extracted, dried under vacuum, and suspended in 15 μL of 0.1% formic acid for LC-MS/MS analysis. Separations were conducted using an LTQ-Orbitrap XL mass spectrometer (Thermo Fisher Scientific) coupled online with a nano-HPLC Ultimate 3000 (Dionex – Thermo Fisher Scientific) and equipped with a homemade pico-frit column (75 μm I.D., 15 μm Tip, 100 mm, New Objective) packed with C18 material (Aeris peptide 3.6 μm XB-C18, Phenomenex) where peptides were eluted using a linear gradient of ACN/0.1% FA (from 3% to 40% in 19 min), at a flow rate of 250 nL min^{-1}.

Protein identification was performed using Proteome Discoverer 1.4 (Thermo Fisher Scientific) aligned against the *Ovis aries* database (version Nov 2021, 23,110 entries) with Mascot Search Engine server (version 2.2.4, Matrix Science). Cys residue carbamidomethylation was defined as a static change, whereas Met oxidation was defined as a variable modification. The tolerances for the precursor and fragment were established at 10 ppm and 0.6 Da, respectively. Finally, only proteins identified with at least three distinct peptides with high

196 confidence (False Discovery Rate 0.01) and a coverage rate ≥20% were considered (high
197 confidence criteria).

198 **2.5. Bioinformatics analysis**

199 A total of 44 proteoforms corresponding to 26 unique proteins (gene names) were gathered
200 from the 13 different abundant protein spots and analyzed using different bioinformatics. To take
201 benefit of the most comprehensive annotation available, since the Gene Ontology (GO)
202 annotation in ovine databases is limited, the ovine gene Uniprot IDs were converted into the
203 human orthologs EntrezGene ID using the Uniprot Retrieve/ID Mapping as reported by Gagaoua
204 et al. (2021).

205 The web-based search STRING database (https://string-db.org) was applied to study the
206 Protein-Protein Interactions (PPI) between the 26 unique proteins based on their molecular
207 function. Confidence intervals was set to 0.400 and false discovery rate (FRD) stringency of 1.0
208 to obtain as many significant features as possible while incurring a relatively low proportion of
209 false positives. Subsequently, the human Uniprot IDs were uploaded on Metascape web tool
210 (https://metascape.org/; accessed on 16 May 2022) and combined with the information provided
211 by the Gene Ontology (GO) Biological Processes to develop the process enrichment clustering.
212 The statistically enriched terms (Zhou et al., 2019) from the submitted gene lists were clustered
213 considering terms with a similarity score >0.3, a *P*-value <0.01, a minimum count of 3, and an
214 enrichment factor >1.5. The results of the enrichment analysis were displayed in cluster
215 containing the most enriched terms hierarchically emphasized in significant clusters.
216 Subsequently, representative terms were visualized in enrichment network based on the
217 membership similarities of clustered proteins. A subset of representative terms from the full
218 cluster was selected and converted into a network layout. More specifically, each term is
219 represented by a circle node, where its size is proportional to the number of input genes fall under
220 that term, and its color represent its cluster identity (i.e., nodes of the same color belong to the
221 same cluster). Terms with a similarity score >0.3 are linked by an edge (the thickness of the edge
222 represents the similarity score).

223 Proteomaps open-source tool was used to show an in-depth overview of the different abundant
224 protein function using the polygon module based on the KEGG (Kyoto Encyclopedia of Genes
225 and Genomes) pathways gene classification. Individual proteins are shown as a polygon, whereas
226 polygon sizes reflect the fold of changes in abundances. Functionally related proteins are
227 arranged in proximity and with a similar color.

2.6. Statistical analysis

Myofibrillar fragmentation index, densitometry data of SDS-PAGE and immunoblotting protein bands, and the 2DE volume spots were analyzed using the mixed GLM procedure of the SAS statistical software 9.3 (SAS Institute, 2013). The model included the dietary treatment (C: control and H: hazelnut skin) and the days of storage (0, 4 and 7 days) and their interaction (diet x storage) as fixed effects, while the individual lamb was included in the model as random effect. All effects were tested for statistical significance ($P<0.05$). When significant effects were observed ($P<0.05$), the Tukey test was used to locate significant differences between groups. The main tendencies in variation between the two dietary groups during storage time were determined with a Principal Component Analysis (PCA) performed using XLSTAT 2021, 1.2.2 software. The PCA was executed on 11 variables matrix (MFI, myosin heavy chain, α-actinin, desmin, actin, troponin T, 30 kDa fragments, troponin I, troponin C, tropomyosin, myosin light chain 1, and myosin light chain 2) to study the relationships among the meat quality traits and the differential proteins along the post-mortem storage within the two dietary treatments.

3. Results and Discussions

3.1. Effects of dietary treatment and storage time on lamb meat myofibrillar fragmentation (MFI) and total collagen content

In the present study, myofibrillar fragmentation index was significantly affected by dietary treatment ($P<0.01$) and storage time ($P<0.001$) as depicted in **Figure 1**. The two experimental groups revealed opposite tendencies in myofibrillar degradation rate. At day 0 of storage, the meat of the control group had higher MFI (98.38 vs 91.33 in C and H group, respectively; $P<0.05$) compared to meat from lambs fed hazelnut skins. Conversely, both at days 4 and 7, the highest values of MFI were detected in meat of the H group (113.74 and 116.1 *vs* 99.28 and 107.26 in H and C group at 4 and 7 days, respectively). MFI increased during storage time in both groups but with different behavior and rate; more important in meat from lambs fed hazelnut skin by-products (+24% after 4 days). These changes are in line to the body of knowledge stating that the major changes in the myofibrillar proteins concur in the tenderization period of raw meat (Lonergan et al., 2010). In this study, meat from lambs fed hazelnut skins evidenced a rapid increase of MFI during storage compared to the control, supporting the functional role of feeding supplementation, including natural antioxidants, on the endogenous enzymatic activity which in turn led to a major breakdown of the muscle protein structures. Indeed, it is known that polyphenols, due to their ability to chelate transition metal ions, scavenge free radicals, and

remove carbonyl compounds, can prevent or delay oxidation processes in meat, thus increasing the proteolytic activity of endogenous enzymes. Recently, Zhao et al. (2018) reported an improvement of tenderness of lamb meat due to dietary supplementation with wine grape pomace, a rich source of polyphenols. We can assume that the protection of the proteolytic enzymes μ-calpain and m-calpain from the oxidative processes, increases their functionality, hence enhancing meat texture (Huff-Lonergan & Lonergan, 2005).

Refers to collagen content, no significant differences were found as a consequence of dietary treatment with hazelnut skin by-product (at the amount we used) and storage time (data not shown). Although collagen content varies due to several factors mainly animal age and muscle type, it was reported that collagen has minor significance in determining the texture of the *Longissimus thoracis* muscle of young animals due to its high solubility after cooking and reduced amounts in this muscle compared for instance to *Semitendinosus* muscle (Listrat, Gagaoua, Andueza, et al., 2020; Listrat, Gagaoua, Normand, et al., 2020; Taylor, 2004).

3.2. Changes in myofibrillar proteins profile of lamb's meat

In **Fig. 2** are shown the results of 1D SDS-PAGE of the myofibrillar proteins from *Longissimus thoracis et lumborum* muscle of lambs as affected by dietary treatment and storage time (0, 4, and 7 d). Variations in myofibrillar protein profiles with different trends in the experimental groups due to the dietary treatment and storage time were observed. The meat of lambs fed hazelnut skin showed lower abundances of structural proteins (**Fig. 2**) known as indicators of meat tenderization (Gagaoua et al., 2020) such as myosin heavy chains (MYH; $P<0.001$; **Fig. 2a**), desmin (DES; $P<0.01$; **Fig. 2b**) and troponin T (TNNT3; $P<0.001$; **Fig. 2c**). Storage time affected the relative intensity of the same myofibrillar protein patterns. Meat from H group showed a decrease of MYH ($P<0.05$; **Fig. 2a**) at 7 d of storage compared to the controls. In meat of H group, desmin ($P<0.05$; **Fig. 2b**) and TNNT3 ($P<0.01$; **Fig. 2c**) had lowest values after 4 d of storage and then remained constant. It is well established that variations in the degree of post-mortem improvement in texture can be reflected by changes in the amounts of the major structural proteins (Gagaoua et al., 2021). Particularly, MYH, ACTA1, and DES, as significant components of myofibril thick filaments, were crucial in defining the texture of muscle, especially because they are the first proteins proposed to be degraded by muscle proteases (Ouali et al., 2013). The percentage of 30 kDa fragments (**Fig. 2d**) was differently influenced by the dietary treatment and storage time in the experimental groups. Meat from lambs fed hazelnut skin showed the highest amount of 30 kDa fragments. Regarding storage, the H group showed

an increase in the amount of 30 kDa fragments after 4 d of storage remaining constant thereafter ($P<0.05$) whereas, in the control group an increase was observed only at 7 d of storage. The 30 kDa fragment has long been identified as a meat tenderization marker and it was commonly considered to be a troponin T breakdown product. Recently, Gagaoua et al. (2020) demonstrated that the content of this fragment contains several other proteins fragments, such as actin (ACTA1), fast troponin T (TNNT1), and myosin light chain 1 (MYL1) among others. Overall, the greater degradation of myofibril proteins together with the major presence of the 30 kDa band fragments in meat from the H group evidenced the possible role of diet in post-mortem degradation of myofibrillar proteins.

To further elucidate the observed changes in desmin and troponin T proteins, immunoblotting was performed (**Fig. 3** and **Fig. 4**). Desmin intact immunoreactive bands were detected at 54 kDa whereas degraded forms were found at 46, 45, 40, and 37 kDa (**Fig. 3a**). Image analysis (**Fig. 3b**) revealed significant differences due to dietary treatment and storage time. Starting from 24 h post-mortem, a progressive decrease of 54 kDa band ($P<0.01$) was observed evidencing a different degradation extent of the intact form of desmin. In particular, at 4 days of storage a reduction of about 10% and 3% was found in lambs fed hazelnut skin and control diet, respectively; whereas at 7 days of storage the reduction of 54 kDa band was about 28% and 5% in H and C groups, respectively. Desmin is one of the most susceptible substrates of calpains (Huff-Lonergan et al., 1996). It is also one of the first proteins to be degraded 24 hours post-mortem (Huff-Lonergan & Lonergan, 1999; Koohmaraie, 1992). Accordingly, Starkey et al. (2015) observed an improvement in shear force as a result of desmin degradation starting from 0 day in lamb meat. Rowe et al. (2004) found an early production of protein breakdown products in meat from steers receiving Vitamin E dietary supplementation compared to the control demonstrating that post-mortem oxidation could interfere with the tenderization process by reducing calpain activity and delaying the rate of proteolysis in meat. In this study, the rapid decline of the intact form of desmin in the meat of the H group suggested that hazelnut skins might have a role in delaying muscle oxidation process thus influencing the rate of autolysis and subsequent activation of μ-calpain. In agreement with our hypothesis, Soldado et al. (2021) evidenced that the incorporation of condensed tannins rich sources in ruminant diets can reduce the reactive oxygen species (ROS), the lipid peroxidation derivates, and the toxic compounds with an improvement of the antioxidant status in living animals and their products.

Refers to the 46 and 45 kDa desmin degraded forms, meat from the H group showed their significant increase ($P<0.01$) after 4 and 7 days of storage, respectively. However, no significant

differences were observed during storage in the control group. A minor increase was observed for the 40 kDa band ($P<0.01$) after 4 days in meat from control group, whereas in both groups the 37 kDa band increased significantly ($P<0.001$) after 7 days of storage.

Immunoblot of troponin T (**Fig. 4a**) showed the presence of 8 immunoreactive bands (37, 36, and 34 kDa, isoforms of the intact protein; 32, 31, 30, 28, and 25 kDa bands, degradation products of TNNT3). The results (**Fig. 4b**) revealed and confirmed the impact of dietary treatment and storage time on the rate of troponin T breakdown. Particularly, meat from lambs fed hazelnut skin showed a degradation of the intact isoforms band at 37 kDa ($P<0.001$), 36 kDa ($P<0.001$), and 34 kDa ($P<0.01$) after 4 days of storage, remaining constant thereafter. Conversely, in meat from the control group, the percentage of the intact band at 37 kDa decreased progressively during storage whereas a reduction of 36 and 34 kDa band was detected only after 7 and 4 days of storage, respectively. However, it is important to note that meat from the H group showed lower values in the 37 kDa intact band starting from 0 day of storage, although after 7 days no differences were detected between the meat of the two experimental groups. A more rapid and greater increase of troponin T fragments (sum of all degraded isoforms) was found in meat from the hazelnut group after 4 days of storage (66.09 *vs* 81.19 in C and H, respectively) confirming that dietary tannins supplementations can also affect the rate of the tenderization process by modulating the endogenous enzymatic activity as previously reported (Zhao et al., 2018). However, at the end of storage time, meat from both groups displayed comparable results in terms of the amount of degradation products of TNNT3 (92.00 *vs* 88.60 in C and H, respectively). Overall, these findings supported the greater myofibrillar fragmentation found in the H group at 4 days and confirmed that the inclusion of tannins in the lamb diet can influence the post-mortem processes through a protective impact on the development of oxidative phenomena as also supported by the meat antioxidant capacity results reported in the companion paper (Menci et al., 2023). Indeed, the oxidative conditions are responsible for physical and chemical changes in muscle protein properties, including conformation, aggregation, and solubility, that can reduce the protein susceptibility to proteolysis (Lonergan et al., 2010; Zhang et al., 2013). Collectively, data on desmin and TNNT3 fractions together with levels of 30 kDa fragments suggest the role of antioxidant compounds present in hazelnut skin in enhancing tenderization processes in lamb meat during storage.

Finally, for a better understanding of the above results, a PCA was performed using myofibrils fragmentation index and the changes of myofibrillar muscle proteome (**Fig. 2e**). The bi-plot accounted for 42.81% of the total variance, with 27.04% of the total variability explained by PC1

and 15.77% explained by PC2. MFI and 30 kDa fragment were the factors positively related to the PC1, whereas myosin heavy chain (MYH) and troponin T (TNNT3) were the factors negatively related to the first principal component. Desmin (DES), troponin I (TNNI), and actin (ACTA1) were the main contributing variables along with PC2, with actin negatively related to the principal component. The score plot differentiated the dietary treatment in different clusters that moved along the first principal component across storage time. The PCA and the used protein biomarkers confirmed the results discussed above, showing a better improvement in the texture of meat from lambs fed hazelnut skin by-product compared to the controls. Therefore, our evidence highlights that the impact of hazelnut skin by-products on the myofibrillar proteome is extremely complex, and there is a need to elucidate, with further studies, the possible mechanisms by which tannin compounds can delay muscle oxidation processes.

3.3. Two-dimensional electrophoresis (2DE) of myofibrillar proteome and bioinformatics analysis

The 2DE gel maps of myofibrillar protein fraction from LT muscle of lambs as affected by different dietary treatments after 0, 4, and 7 days of storage are depicted in **Fig. 5**. Results revealed a significant effect of dietary treatment and storage time in the variation of the myofibrillar muscle proteome. The 2DE allowed us to further visualize the impact of dietary treatment on the abundances of the different myosin light chain isoforms (**Fig. 5**; MYL area). Meat from lambs fed hazelnut skins displayed more MYL spot isoforms compared to the control at 0 and 4 days of storage, whereas, after 7 days of storage, both groups showed a similar profile. The presence of several spot isoforms ascribed to MYL starting from 0 day observed in the H group confirmed the greater proteolysis in line with the myofibrillar fragmentation data and degradation of key structural proteins discussed above. In particular, it could be hypothesized that the weakening of the actomyosin complex by muscle proteases may result in greater extractability and release of MYL isoforms.

The overall comparison of the 2DE gels revealed that 13 protein spots, corresponding to 44 proteoforms (26 unique proteins), were differently expressed as a consequence of dietary treatment with hazelnut skin after 7 days of storage (**Table 1** and annotation of **Fig. 6A**). Certain isoforms were identified more than one time (likely ACTA1, 5 times; HSPB1 and MYL3, 4 times; and TNNC2, 3 times) hence dominating the proteomap polygon given in **Fig. 6B, 6C**. The protein-protein interaction network on the 26 unique proteins evidenced three major sub-networks (**Fig. 6D** and details on the proteins names in **Table 1**). These being a sub-network of

i) actin filament-based process/cytoskeleton organization (*n* = 11 proteins; ACTC1, ACTA1, CAPZA2, CAPZB, ACTG1, TMP2, TMP1, DES, TNNC2, TNNT3 and MYL3); of ii) heat shock proteins (*n* = 4 proteins; HSPB1, HSPA4, HSPB6 and HSPB7) and of iii) energy metabolism (*n* = 4 proteins; ATP5H, PDHB, UQCRC1 and MTX2). Interestingly, a huge number (n = 18) of these proteins (ACTA1, ACTC1, ACTG1, CAPZA2, CAPZB, DES, TNNC2, TNNT3, TPM1, TPM2, MYL3, HSPB1, HSP70, HSPB6, HSPB7, UQCRC1, ATP5H, and PDHB) have been previously identified as biomarkers of beef tenderness (Gagaoua et al., 2021). Refers to small ruminant proteomic studies, 6 proteins were identified as linked with different meat quality traits such as drip loss (Wang et al. (2016); ACTA1, MYL3, UQCRC1, HSPB1), tenderness (Della Malva et al. (2017); (Paim et al., 2019; Xie et al., 2022); MYL3, TNNT3) and color stability (Gao et al. (2016); HSP70, ACTA1).

The enrichment and cluster process analysis of the identified proteins based on gene ontology (GO) allowed the identification of six significant enriched terms (**Fig. 6E**): Muscle Contraction (GO:0006936), Myofibril assembly (GO:0030239), Tissue homeostasis (GO:0001894), Response to unfolded protein (GO:0006986), Aerobic respiration (GO:0009060), and Positive regulation of angiogenesis (GO:0045766). The interconnectedness of these enriched terms and their functional enrichment was evidenced by three sub-networks given in **Fig. 6F**, highlighting the extent of enrichment of the clusters contributing to each GO term. Overall, the results depicted that muscle contraction and myofibril assembly were the most important pathways related to the supplementation of lamb diets by hazelnut skins.

Among the dominating proteins (**Fig. 6B**), actin (ACTA1) was identified as the most influenced proteins by dietary treatment due to the presence of 5 proteoforms. Actin is the main constituent of the sarcomere thin filaments, and the first protein targeted by caspases in the post-mortem period with the breakdown of the actomyosin complex (Du et al., 2004; Ouali et al., 2013). Indeed, the integromics study of Gagaoua et al. (2021) pointed out that ACTA1 is the most frequently identified protein related to meat tenderization processes. Conversely, among the other proteins of actin filaments, γ- actin (ACTG1) has been recently found to be associated with meat tenderization (Malheiros et al., 2021).

Regarding the contractile and associated proteins, the dietary treatment applied in this study allowed to find the appearance of 5 fragments ascribed to proteins of troponin complex (TNNC2 and TNNT3) in meat from lambs fed hazelnut skin by-product. The subunits of the troponin complex play a pivotal role in displaying myofilament changes as they are anchored to the

tropomyosin-actin filaments and enhanced actomyosin bonds (Gomes et al., 2002). Consequently, the degradation of troponin proteins highlights that the interacting bonds are impacted and the thin filaments in the sarcomeric I band could be broken. Furthermore, the appearance of the different fragments ascribed to TNNC2 and TNNT3 in meat from the H group, starting from 4 days of storage, confirmed the greater degradation and fragmentation of myofibrils, hence validating the data discussed above using mono-dimensional electrophoresis and immunoblotting. In the context of tenderness, several studies on different species (Della Malva et al., 2019; Della Malva et al., 2017; Gagaoua et al., 2021; Laville et al., 2009) found a strictly link between the degradation of troponin complex proteins and the tenderization rate confirming that troponin-derived fragments are reliable biomarkers of meat tenderization.

The myofibrillar proteome of meat from the hazelnut group was characterized also by the presence of three proteins belonging to the F-actin capping protein family (CAPZB and CAPZA2), involved in the cell signaling and regulation of actin in myofilament contractility. Previous studies on beef (Guillemin et al., 2011) and pork (Lametsch et al., 2003; Wang et al., 2014) meat, revealed a link between F-actin capping proteins expression and meat tenderness highlighting that these protein patterns could be interesting biomarkers of post-mortem meat tenderization. Among the other myofibrillar proteins that constitute the principal sub-network, 4 proteoforms of MYL3 characterized the myofibrillar proteome of meat from the H group after 7 days of storage time showing a greater abundance than control meat.

Pathways related to energy metabolism, especially those involved in the mitochondrial and ATP metabolic processes are strictly linked with post-mortem underlying pathways of meat texture determination (Gagaoua et al., 2021; Ouali et al., 2013; Purslow et al., 2021; Rosa et al., 2018; Wang et al., 2016). Dietary treatment with hazelnut skin significantly affected the expression of ATP5PD, UQCRC1 and PDHB in lamb meat after 7 days of storage time. Cytochrome b-c1 complex subunit 1 (UQCRC1) is involved in the oxidation/reduction process playing an important role in the cell electron transport and respiratory chain (Kunej et al., 2007). Mitochondrial apoptosis and release of cytochrome C have been shown to play a fundamental role in the breakdown of myofibrillar protein during post-mortem period (Zhang et al., 2017). In particular, Wang et al. (2018) demonstrated that the release of cytochrome C induce caspase-9 activation, which may greatly influence meat tenderness. In this study, the greater abundance of spots ascribed to mitochondrial electron transport chain proteins in the meat from the hazelnut group confirms the high tenderization rate observed in H group. These proteins can be proposed

as biomarkers of lamb meat tenderization in line with previous studies on beef (Gagaoua et al., 2021).

Among the changing pathways as a consequence of hazelnut skin by-products supplementation, heat shock proteins (HSPB1, HSP70, HSPB6, HSPB7), usually associated with the response to stress processes, also impacted the lamb myofibrillar proteome. HSPs as chaperones, play a role in stress resistance by restoring proteins altered by external stimulus thus assuming a fundamental role in controlling the onset of apoptosis and post-mortem proteolytic processes (Lomiwes et al., 2014; Ouali et al., 2013). Additionally, several studies (Balan et al., 2014; Cramer et al., 2018) reported that an increase in degradation of small heat shock proteins, like HSPB1 identified in this study, may indicate a loss of its anti-apoptotic function evidencing their important role in the post-mortem processes related to meat quality. The integromics study conducted by Gagaoua et al. (2021) evidenced HSPB1 as the top biomarker related to beef tenderness. Several studies (Gagaoua et al., 2021; Kim et al., 2018) suggested that HSPB1 may delay the activity of endogenous enzymes, and consequently their proteolytic activity. Higher levels of HSPB1 were found to predict tenderness due to the prevention of aggregation processes thus facilitating the action of proteolytic enzymes during the post-mortem period (Morzel et al., 2008). Recently, an overabundance of HSP70 (Heat shock 70 kDa protein) was observed by Ma et al. (2020) in callipyge lambs genotypes with tougher meat indicating delayed apoptosis and proteolysis.

Heat shock protein beta-6 (HSPB6), due to the presence of a binding domain for troponin I, play a key role in muscle contraction and metabolic processes (Rembold et al., 2000). In the meta-proteomics study of Picard and Gagaoua (2020), HSPB6 was identified as a putative biomarker of beef tenderness, based on 12 proteomic studies. Additionally, Ma and Kim (2020), in an aging study, found a strong relationship between the greater abundance of HSPB6 fragments and the tenderization rate of different bovine muscles. About HSPB7, Li and Liu (2022) and Dang et al. (2022) found an overabundance of this protein in bovine meat after 7 and 16 days of aging, respectively.

The current study also found an overabundance of the above-mentioned HSPs in meat from the H group confirming the role of feeding as an important modulator of post-mortem processes linked with meat tenderness. Our emerging evidence underlines the protective effect of molecules contained in hazelnut skin against oxidative damage thus modulating the trend of post-mortem processes that affect meat tenderness.

4. Conclusion

The results of this study evidenced that the supplementation of hazelnut skin by-products in the diet of lambs was an effective strategy to improve lamb meat quality characteristics during storage, in terms of better meat texture. Specifically, compared to lambs fed the control diet, meat from lambs-fed hazelnut skins showed a higher and more rapid increase of MFI during storage, supporting the functional role of feeding supplementation. Immunoblotting results revealed a greater degradation of desmin and troponin T in meat from the hazelnut group together with an increase in the accumulation of the 30 kDa polypeptide fragment. These results evidenced the positive effect of tannins-based dietary treatment in modulating the proteolytic processes in lamb muscle in to meat conversion during storage. The proteomic approach combined with bioinformatic analysis clarified the impact of this feeding strategy on lamb muscle proteome during post-mortem storage and revealed the underlying biochemical processes. Twelve putative biomarkers (ACTA1, MYL3, TNNT3, TNNC2, CAPZB, CAPZA2, UQCRC1, ATP5PD, HSPB1, HSP70, HSPB6, HSPB7), belonging to actin filament-based process/cytoskeleton organization, energy metabolism, and heat shock proteins, were the major underlying pathways. Our findings underline that the identification of protein biomarkers represents a tool to evaluate the effect of feeding strategies on the final meat quality. Further studies are needed to consider multiple lamb meat quality traits for the validation of the proposed candidate biomarkers.

Conflict of interest

The authors declare no conflict of interest.

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Table 1. List of the identified proteins from 2DE of myofibrillar fraction of *Longissimus thoracis et lumborum* muscle as affected by dietary treatment and storage time.

Spot number	Identified proteins	Gene names	Uniprot ID	MW [kDa]	calc. pI	Score	Seq. Coverage	Matched peptides
1307	Actin, alpha cardiac muscle 1	ACTC1	A0A6P3E6H9	42.0	5.39	2020.96	35.28	11
	Desmin	DES	W5QG29	53.5	5.27	239.71	21.49	9
	Troponin T, fast skeletal muscle	TNNT3	W5NRC7	25.6	10.14	234.85	23.32	5
	Myosin light chain 3	MYL3	A0A0U1Z4T4	21.9	5.07	227.31	26.63	5
	14-3-3 protein gamma	YWHAG	A0A6P7DFS2	28.3	4.89	147.55	20.65	5
	ATP synthase subunit d, mitochondrial	ATP5PD	W5PP37	18.7	6.24	90.06	24.22	3
2205	Heat shock 27 kDa protein	HSPB1	A0A6P7DEW2	22.3	6.70	1075.41	33.83	10
	Actin, alpha skeletal muscle isoform X4	ACTA1	A0A6P3ERW2	42.0	5.39	373.86	32.10	10
	Desmin	DES	W5QG29	53.5	5.27	352.97	20.21	8
	F-actin-capping protein subunit beta	CAPZB	A0A6P7DRJ9	31.3	5.58	282.77	28.52	7
	Myosin light chain 3	MYL3	A0A0U1Z4T4	21.9	5.07	230.00	28.14	6
	Troponin C, skeletal muscle	TNNC2	A0A6P7ELW5	18.1	4.20	139.78	28.13	3
2402	F-actin-capping protein subunit beta	CAPZB	A0A6P7DRJ9	31.3	5.58	813.53	39.35	10
3101	Troponin C, skeletal muscle	TNNC2	A0A6P7ELW5	18.1	4.20	319.90	49.38	6
3204	Heat shock 27 kDa protein	HSPB1	A0A6P7DEW2	22.3	6.70	2138.36	33.83	9
	Actin, alpha skeletal muscle isoform X4	ACTA1	A0A6P3ERW2	42.0	5.39	493.32	25.73	8
	Myosin light chain 3	MYL3	A0A0U1Z4T4	21.9	5.07	226.74	33.17	6
3301	Actin, alpha skeletal muscle isoform X4	ACTA1	A0A6P3ERW2	42.0	5.39	1218.74	61.27	9
	Actg1	ACTG1	A0A3R5SS76	41.8	5.48	496.89	33.33	4
3401	actin, alpha skeletal muscle isoform X4	ACTA1	A0A6P3ERW2	42.0	5.39	2537.57	55.97	10
	Actg1	ACTG1	A0A3R5SS76	41.8	5.48	1143.25	35.20	4
	Eukaryotic translation initiation factor 3 subunit I	EIF3I	A0A6P3E6N2	36.4	5.64	530.79	37.23	12
	Pyruvate dehydrogenase E1 component subunit beta	PDHB	A0A6P7D5G1	39.1	6.44	305.51	20.89	6
	Tropomyosin 2	TPM2	W5PQL7	32.8	4.70	267.68	25.70	7

3402	Pyruvate dehydrogenase E1 component subunit beta	PDHB	A0A6P7D5G1	39.1	6.44	1259.40	21.73	7
	F-actin-capping protein subunit alpha	CAPZA2	A0A6P3EBW2	33.0	5.85	651.52	44.41	8
	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-2	GNB2	A0A6P7DFT3	37.3	6.00	621.63	32.06	3
	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1	GNB1	A0A6P7EKA8	37.4	6.00	557.22	32.35	4
	Troponin T, fast skeletal muscle	TNNT3	W5NRC7	25.6	10.14	519.86	32.74	9
	Heat shock 27 kDa protein	HSPB1	A0A6P7DEW2	22.3	6.70	490.75	25.37	6
	Tropomyosin alpha-1 chain	TPM1	A0A6P9FRC8	32.7	4.74	342.44	23.59	8
	Dimethylargininase	DDAH1	A0A6P3E5A7	31.2	6.01	250.79	25.96	6
3702	Cytochrome b-c1 complex subunit 1, mitochondrial	UQCRC1	A0A6P3THK9	52.8	6.32	1660.49	27.29	13
	Actin, alpha skeletal muscle isoform X4	ACTA1	A0A6P3ERW2	42.0	5.39	1166.32	48.54	17
3902	Heat shock 70 kDa protein	HSP70	A0A5A4U680	70.2	5.92	4375.53	53.67	29
	Albumin	ALB	W5PWE9	69.3	6.15	2486.52	58.95	38
4101	ATP synthase subunit d, mitochondrial	ATP5PD	W5PP37	18.7	6.24	314.64	24.22	3
	DNA-directed RNA polymerase II subunit RPB7	POLR2G	A0A6P9FRD6	19.2	5.54	204.98	29.65	4
4102	Heat shock protein beta-7	HSPB7	A0A6P7DS42	18.8	5.96	208.79	22.54	3
	Troponin C2, fast skeletal type	TNNC2	W5P9C1	16.2	4.30	153.03	26.35	3
	Heat shock protein beta-6	HSPB6	A0A6P7ESN6	17.5	6.40	87.08	45.73	4
5202	Heat shock 27 kDa protein	HSPB1	A0A6P7DEW2	22.3	6.70	2261.60	33.83	9
	Metaxin-2 isoform X2	MTX2	A0A6P3T8X3	24.4	7.40	158.20	20.47	4
	Myosin light chain 3	MYL3	A0A0U1Z4T4	21.9	5.07	156.34	24.12	4

Figure captions

Fig. 1. Myofibrillar fragmentation index (MFI) of *Longissimus thoracis et lumborum* muscle of lambs as affected by dietary treatment (H= hazelnut; C= control) and storage time (0, 4 and 7 days). Different letters: A, B= P< 0.05 (diet effect), a, b, c= P<0.05 (storage effect). *=P<0.05; **=P<0.01; ***=P<0.001.

Fig. 2. Representative SDS-PAGE gels and densitometry analyses of changing myofibrillar proteins. (a) MYH= Myosin heavy chain, (b) DES= desmin, (c) TNNT3= Troponin T and (d) the 30 kDa fragment of *Longissimus thoracis et lumborum* muscle from lambs as affected by dietary treatment (H= hazelnut; C= control) and storage time (0, 4 and 7 days). *=P<0.05; **=P<0.01; ***=P<0.001. (e) Principal component analysis (PCA) of myofibrillar fragmentation index (MFI) and protein band percentage (MYH = myosin heavy chain, ACTN= α-actinin, DES = desmin ACTA1 = actin, TNNT = troponin T, TPM = tropomyosin, 30 kDa= 30 kDa fragments, MYL1 = myosin light chain 1, TNNI= troponin I, TNNC= troponin C, MYL2 = myosin light chain 2) of *Longissimus thoracis et lumborum* lamb muscles as affected by different dietary treatment (H= hazelnut; C= control) and storage time (0, 4 and 7 days).

Fig. 3. Representative Western blot of (a) Desmin, and (b) variance analysis on the relative abundances of the intact fragments and degraded forms of the myofibrillar proteins of *Longissimus thoracis et lumborum* muscle of lambs fed with different dietary treatment (H= hazelnut; C= control) after 0, 4 and 7 days of storage.

Fig. 4. Representative Western blot of (a) Troponin-T degradation, and (b) variance analysis on the relative abundances of the intact fragments and degraded forms of the myofibrillar proteins fractions of *Longissimus thoracis et lumborum* muscle of lambs fed with different dietary treatment (H= hazelnut; C= control) after 0, 4 and 7 days of storage.

Fig. 5. Representative 2DE gels of myofibrillar fractions and details of myosin light chains isoforms (myosin light chain 1, fast skeletal myosin light chain 2, myosin light chain 1/3, skeletal muscle) of *Longissimus thoracis et lumborum* muscle from lambs fed with different dietary treatment (H= hazelnut; C= control) after 0, 4, and 7 days of storage.

Fig. 6. Differentially expressed proteins identified by mass spectrometry and bioinformatics analyses. (A) Representative 2DE gel map of the myofibrillar fraction highlighting the identified protein spots in Table 1; (B) Proteomaps pathway analysis of the myofibrillar proteins showing by a polygon arranged in common regions gene classification and (C) related biological function; (D) Protein-protein interaction network of the differentially expressed proteins using STRING database; (E) Significant enriched ontology clusters and molecular pathways based on Gene Ontology (GO) from the differentially expressed proteins. The bar graphs highlight all the enriched terms across the protein lists coloured according to Log *P*-values: terms with a P-value <0.01, a minimum count of 3, and an enrichment factor >1.5; (F) Network of pathways and process enrichment cluster analysis.

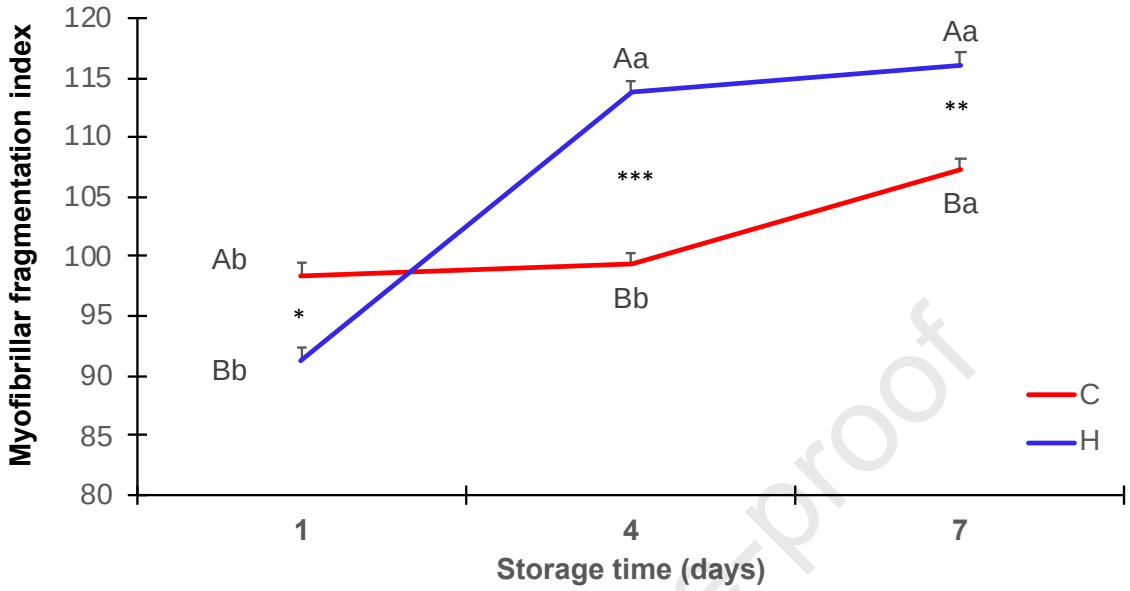


Figure 1.

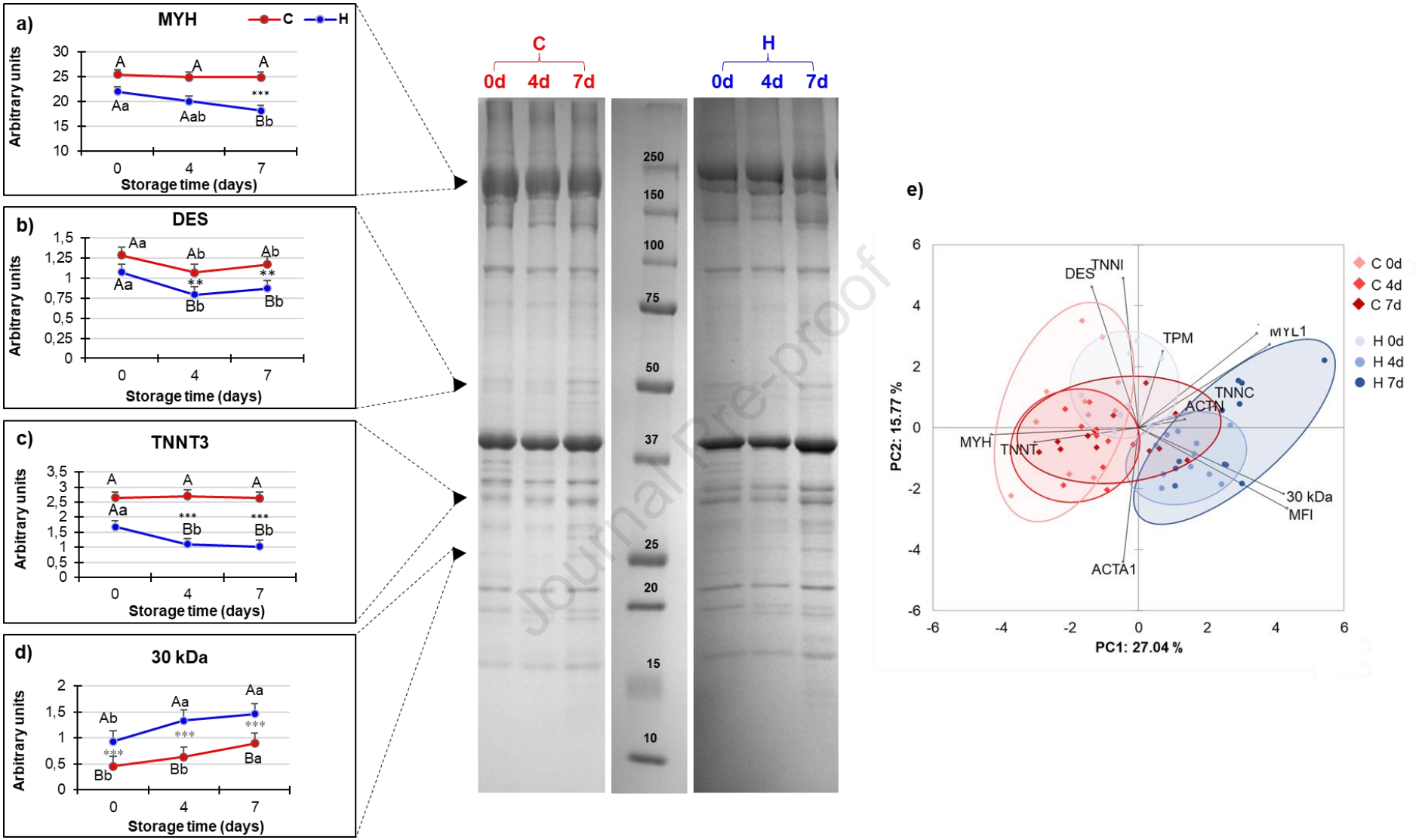
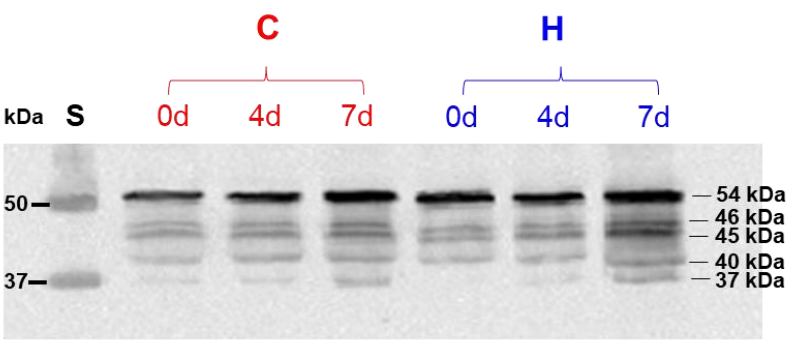

a)
MYH
C
H
Arbitrary units
Storage time (days)
b)
DES
c)
TNNT3
d)
30 kDa
C
H
0d
4d
7d
250
150
100
75
50
37
25
20
15
10
e)
C 0d
C 4d
C 7d
H 0d
H 4d
H 7d
DES
TNNI
TPM
MYL1
TNNC
ACTN
MYH
TNNT
30 kDa
MFI
ACTA1
PC1: 27.04 %
PC2: 15.77 %

Figure 2.

a)



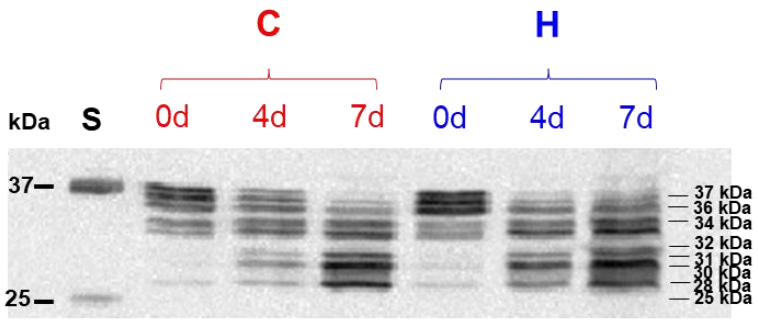
b)

	C			H				Effect, *P*	
	0d	**4d**	**7d**	**0d**	**4d**	**7d**	SEM	Diet	Storage
54 kDa	87.73 a	85.05 Ab	83.58 Ab	85.51 a	77.60 Bb	61.93 Bc	0.82	**	***
Degraded forms									
46 kDa	2.59 B	3.35 B	3.09 B	8.37 Ab	12.22 Aa	12.49 Aa	0.53	***	*
45 kDa	5.49	5.23	5.62 B	3.49 c	6.76 b	20.20 Aa	0.97	***	**
40 kDa	3.33 b	5.51 Aa	5.63 Aa	2.63	2.96 B	3.38 B	0.59	**	*
37 kDa	0.86 b	0.86 b	2.08 a	ND	0.46 b	2.00 a	0.36	NS	**

NS = not significant; * = P < 0.05; **=P < 0.01; ***= P < 0.001. ND = not detected. a, b, c= P< 0.05, storage effect; A, B= P< 0.05, diet effect.

Figure 3.

a)



b)

	C			H				Effect, *P*	
	0d	**4d**	**7d**	**0d**	**4d**	**7d**	SEM	Diet	Storage
Intact forms									
37 kDa	27.50 Aa	10.03 Ab	0.89 c	16.92 Ba	2.73 Bb	0.90 b	2.27	***	***
36 kDa	18.24 Ba	15.28 Aa	1.54 b	28.38 Aa	4.52 Bb	2.34 b	1.02	***	***
34 kDa	15.93 Ba	8.60 b	5.57 b	20.46 Aa	11.55 b	8.16 b	1.23	***	**
Degraded forms									
32 kDa	16.63A	17.53 A	17.96 A	10.97 Bb	15.03 Ba	14.17 Ba	0.86	**	*
31 kDa	10.80 Ab	16.07 Aa	15.75 a	8.36 Bc	12.34 Bb	14.90 a	0.73	**	**
30 kDa	1.15 c	6.46 Bb	12.11 a	1.35 b	13.09 Aa	13.74 a	0.91	***	***
28 kDa	1.57 c	14.94 Bb	24.36 a	3.91 b	24.77 Aa	26.26 a	1.18	***	**
25 kDa	8.18 b	11.20 Bb	21.82 a	9.65 b	15.96 Aa	19.53 a	1.27	**	**
Sum	38.32 Ac	66.09 Bb	91.99 a	34.24 Bc	81.19 Ab	88.59 a	1.18	***	**

NS = not significant; * = P < 0.05; **=P < 0.01; ***= P < 0.001. a, b, c= P< 0.05, storage effect; A, B= P< 0.05, diet effect.

Figure 4.

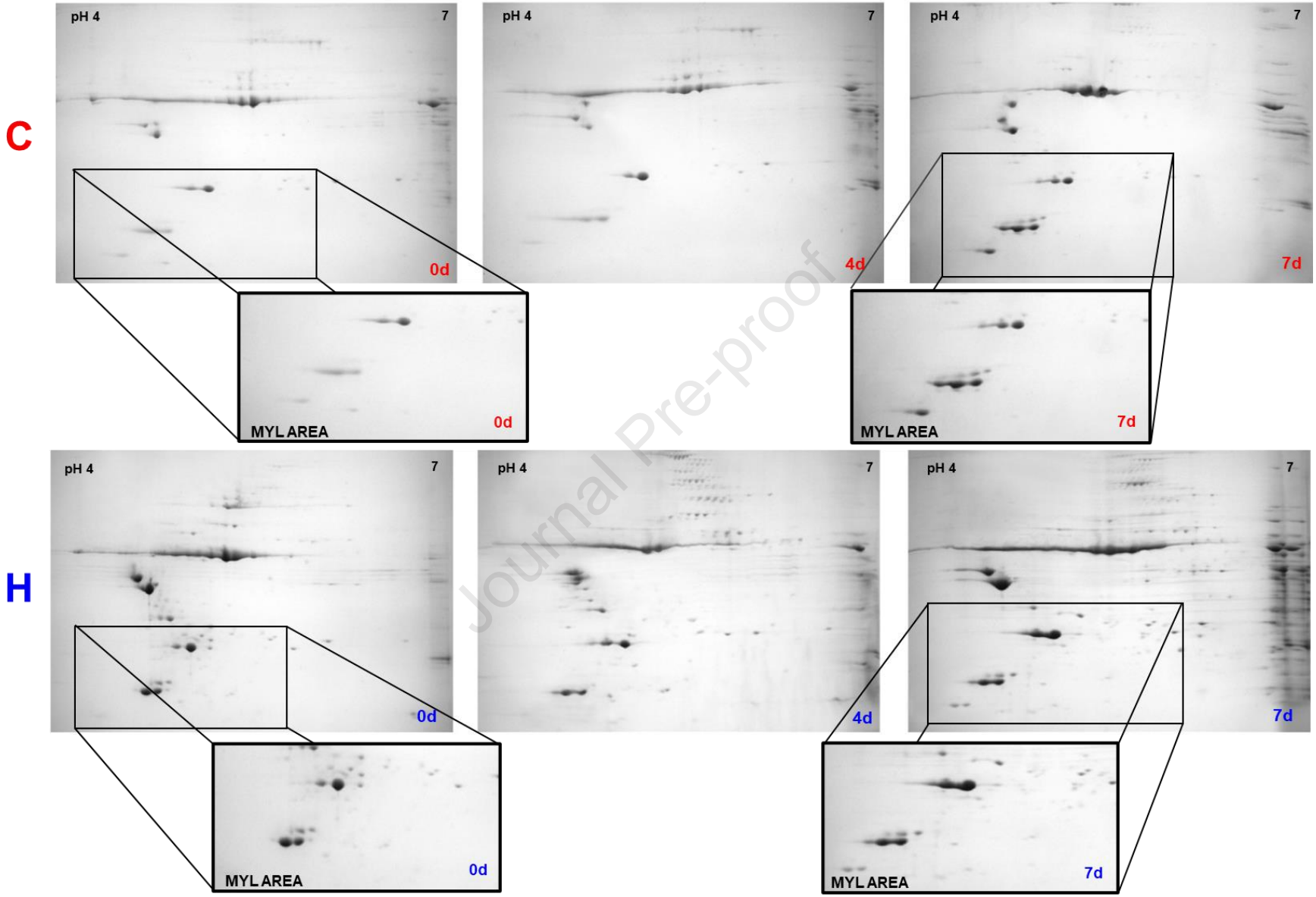
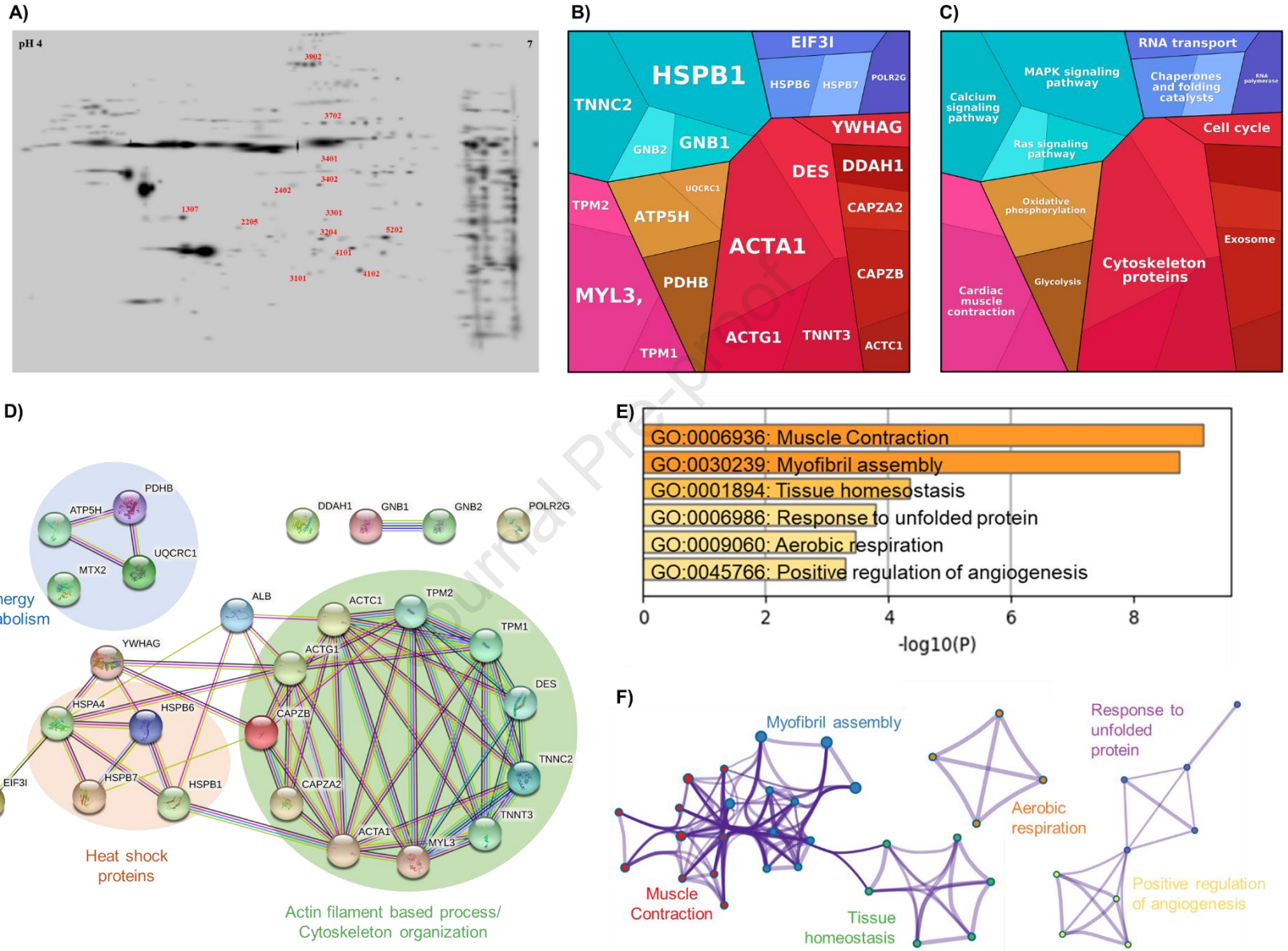


Figure 5.


A)
pH 4
7
3902
3702
3401
3402
2402
1307
2205
3301
3204
5202
4101
3101
4102
B)
HSPB1
EIF3I
HSPB6
HSPB7
POLR2G
TNNC2
GNB2
GNB1
YWHAG
DES
DDAH1
UQCRC1
TPM2
ATP5H
CAPZA2
ACTA1
PDHB
CAPZB
MYL3,
TPM1
ACTG1
TNNT3
ACTC1
C)
RNA transport
MAPK signaling pathway
Chaperones and folding catalysts
RNA polymerase
Calcium signaling pathway
Ras signaling pathway
Cell cycle
Oxidative phosphorylation
Exosome
Cytoskeleton proteins
Cardiac muscle contraction
Glycolysis
D)
PDHB
ATP5H
UQCRC1
MTX2
Energy metabolism
DDAH1
GNB1
GNB2
POLR2G
ALB
ACTC1
TPM2
TPM1
YWHAG
ACTG1
DES
HSPA4
HSPB6
CAPZB
TNNC2
EIF3I
HSPB7
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CAPZA2
TNNT3
ACTA1
MYL3
Heat shock proteins
Actin filament based process/ Cytoskeleton organization
E)
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GO:0030239: Myofibril assembly
GO:0001894: Tissue homeostasis
GO:0006986: Response to unfolded protein
GO:0009060: Aerobic respiration
GO:0045766: Positive regulation of angiogenesis
0
2
4
6
8
-log10(P)
F)
Myofibril assembly
Response to unfolded protein
Aerobic respiration
Positive regulation of angiogenesis
Muscle Contraction
Tissue homeostasis

Figure 6.

Highlights

- Hazelnut skin supplementation is an effective strategy to improve lamb meat texture
- Meat from lambs fed hazelnut skin had major and higher protein degradation
- Intense degradation of desmin and troponin T in meat from lambs fed hazelnut skin
- Twenty-six proteins suggested to monitor the impact of hazelnut skin supplementation

Conflict of interest

The authors declare no conflict of interest.

CRediT authorship contribution statement

Antonella della Malva: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Mohammed Gagaoua**: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Antonella Santillo:** Conceptualization, Funding acquisition, Investigation, Methodology, Writing – review & editing. **Martina Di Corcia:** Formal analysis, Investigation, Methodology, Writing – review & editing. **Rosaria Marino:** Conceptualization, Funding acquisition, Investigation, Methodology, Writing – review & editing. **Natalello Antonio:** Conceptualization, Funding acquisition, Investigation, Methodology, Writing – review & editing. **Agostino Sevi:** Conceptualization, Funding acquisition, Investigation, Methodology, Writing – review & editing. **Marzia Albenzio**: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Writing – review & editing.