



HAL
open science

In-depth characterization of myofibrillar muscle proteome changes in lambs fed hazelnut skin by-products

Antonella Della Malva, Mohammed Gagaoua, Antonella Santillo, Martina Di Corcia, Rosaria Marino, Antonio Natalello, Agostino Sevi, Marzia Albenzio

► **To cite this version:**

Antonella Della Malva, Mohammed Gagaoua, Antonella Santillo, Martina Di Corcia, Rosaria Marino, et al.. In-depth characterization of myofibrillar muscle proteome changes in lambs fed hazelnut skin by-products. *Food Bioscience*, 2023, 53, pp.102836. 10.1016/j.fbio.2023.102836 . hal-04126195

HAL Id: hal-04126195

<https://hal.inrae.fr/hal-04126195>

Submitted on 12 Sep 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **In-depth characterization of myofibrillar muscle proteome changes in lambs fed hazelnut**
2 **skin by-products**

3
4 Antonella della Malva^{a*}, Mohammed Gagaoua^{b**}, Antonella Santillo^a, Martina di Corcia^a,
5 Rosaria Marino^a, Antonio Natalello^c, Agostino Sevi^a, Marzia Albenzio^a

6
7 ^a Department of the Science of Agriculture, Food, Environment and Engineering (DAFNE),
8 University of Foggia, Via Napoli 25, 71121 Foggia, Italy

9 ^b PEGASE, INRAE, Institut Agro, 35590 Saint-Gilles, France

10 ^c Department of Agriculture, Food and Environment (Di3A), University of Catania, Via
11 Valdisavoia 5, 95123 Catania, Italy

12
13 * **Correspondence:** * Dr. Mohammed Gagaoua, mohammed.gagaoua@inrae.fr

14 Abstract

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

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

37

38 **1. Introduction**

39 According to the Food and Agriculture Organization of the United Nations (FAO), one-third
40 of food produced for human consumption is lost or wasted globally, which when combined with
41 the future scarcity of foods, results in an unethical and unsustainable situation (FAO, 2011). In
42 this regard and in order to explore the possibility of recovering highly-valuable compounds,
43 several countries have introduced or implemented bio-based-economy regulations against
44 traditional food waste processing. Among agro-industrial resource by-products, hazelnut
45 (*Corylus avellana L.*) skins account for about 2.5 percent of the hazelnut processing by-products
46 (Ivanović et al., 2020; Pelvan et al., 2018). Italy is the second largest country producing hazelnuts
47 with 3,072 tons in 2021 (ISTAT, 2022; accessed on 02 August 2022). While being labeled as
48 waste, hazelnuts skin stands out for their abundances of fiber and fat-soluble bioactive
49 compounds, such as peculiar unsaturated fatty acids, phenolic compounds, polyphenols (mainly
50 represented by condensed tannins), phytosterol, and vitamins (Del Rio et al., 2011; Ivanović et
51 al., 2020; Pelvan et al., 2018). Due to the bioactive compounds content, hazelnut skin further
52 possesses antioxidant, antimicrobial, anti-inflammatory, and immunomodulatory properties
53 (Montella et al., 2013). In fact, there are many studies on the incorporation of this by-product in
54 livestock diet, including small ruminants (Caccamo et al., 2019; Campione et al., 2020; Daghighi
55 et al., 2021; Priolo et al., 2021). In particular, Priolo et al. (2021) evidenced an impact on meat
56 quality properties, but no biological mechanisms at the muscle level were detailed. In fact, it is
57 known that changes in muscle during the post-mortem period might result in an unbalance of
58 antioxidant and pro-oxidant capability, increasing the risk of oxidative damage. The oxidative
59 deterioration of meat proteins could negatively affect its organoleptic quality by modifying the
60 activation of the endogenous enzymes responsible for post-mortem tenderization (Rowe et al.,
61 2004). In this context, dietary supplementation with antioxidant compounds in lambs
62 demonstrated a positive effect in enhancing parameters related to tenderness development
63 (Maggiolino et al., 2021; Mu et al., 2020; Muino et al., 2014). However, it is necessary to
64 understand the role of antioxidants in the modulation of post-mortem biochemical mechanisms
65 for better control of the final lamb meat quality. Recently, Priolo et al. (2021) found that the
66 inclusion of 15% of hazelnut skin to replace an equal amount of maize in growing lambs is an
67 effective strategy to reduce the cost of ruminant diet and the cost of disposal for industries. This
68 dietary strategy had no detrimental effects on lamb growth, final body, and carcass weight,
69 whereas improved the nutritional quality of lamb meat enriching the intramuscular meat fat with
70 health-promoting fatty acids. Furthermore, feeding hazelnut skin led to a delay in lipid oxidation

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

83 2. Materials and Methods

84 2.1. Animals, dietary treatments, and meat sampling

85 The experimental protocol was designed according to the European Directive guidelines
86 (2010/63/EU) on the protection of animals used for the scientific purpose and were conducted in
87 compliance with the European Directive Council (98/58/EC) on the minimum standards for the
88 protection of animals kept for farming purposes.

89 The experimental trial was carried out at the experimental farm of the University of Catania,
90 in Sicily Region (latitude:37°24'35.3''N; longitude: 15°03'34.9''E). Details of the experimental
91 design are previously described in Priolo et al. (2021). In brief, the experiment involved twenty-
92 two Valle del Belice male lambs (60 ± 4 days of age) that were randomly assigned to two dietary
93 treatments: control (C) -lambs fed with basal diet (maize-barley based concentrate) and hazelnut
94 skin (H) -lambs fed with the same diet with 150 g kg⁻¹ dry matter (DM) replacement of maize
95 by hazelnut skin by-product. The ingredients, chemical composition and fatty acid profile of
96 diets are given in **supplementary Table 1**. During the experimental period, animals were fed in
97 individual pens (1.5 m²) with straw litter equipped with clean water all the time. The effect of
98 dietary treatment on growth performance and intakes were reported in the companion paper
99 (Priolo et al., 2021).

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

102 1/2005 on the protection of animals during transport. Before slaughter, all lambs were kept in a
103 lairage for 10-12 hours overnight without feed but with free access to water. Then, animals were
104 slaughtered following industrial practices used in Italy and in line with European guidelines (EU
105 rule n. 1099/2009). Each carcass was weighed and chilled at 2-4°C for 24 hours following the
106 industrial routine conditions. After 24 h post-mortem, the carcasses were halved and the
107 *Longissimus thoracis et lumborum* muscle (LTL) was excised from both sides. From the right
108 LTL, three slices (2 cm thickness) were cut and placed in polystyrene trays, covered with
109 oxygen-permeable PVC film, and stored in the dark at 4°C for 0 (24 hours post-mortem), 4 and
110 7 days (d). At the end of the respective storage times, myofibrillar fragmentation index (MFI),
111 total collagen content and changes of myofibrillar proteome using SDS-PAGE, Western Blot
112 and Two-Dimensional Gel Electrophoresis (2DE) coupled with mass spectrometry were
113 performed.

114 **2.2. Myofibrillar Fragmentation Index (MFI) determination**

115 The myofibrillar fragmentation index (MFI) was determined by spectrophotometric assay
116 according to the protocol of Culler et al. (1978) with some modifications. Briefly, 4 g of muscle
117 sample, were homogenized with Ultra-Turrax homogenizer (IKA T18 basic, Germany) in 40 mL
118 of cold buffer (100mM KCl, 20 mM KH₂PO₄ [pH 7.0], 1mM Ethylene Glycol Tetraacetic Acid,
119 1mM MgCl₂, and 1mM NaN₃) to extract the myofibrils. Then, each homogenate was centrifuged
120 at 1,000 x g (Eppendorf 5810R, Eppendorf AG, Hamburg, Germany) for 15 min at 2°C and
121 resuspended with 10 mL of cold buffer. After the determination of the protein concentrations
122 with the biuret method, the suspensions were diluted with the same buffer to a final protein
123 concentration of 0.5 mg mL⁻¹. The myofibrils suspension was measured spectrophotometrically
124 by using a UV-Vis spectrophotometer (Biotek PowerWave XS2, Biotek Instruments, Inc.
125 Highland Park, Winooski, Vermont, USA) at 540 nm. MFI was expressed as the absorbance at
126 540 nm multiplied by 150 conversion factors.

127 **2.3. Total collagen content estimation**

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

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

139 **2.4. Proteome analysis**

140 **2.4.1. Protein extraction, SDS-PAGE analysis and immunoblotting analyses**

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

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

150 Western blots for troponin-T and desmin were performed on 10% polyacrylamide separating
151 gel as previously reported (Marino et al., 2015). Gels were loaded with 40 μg of protein and run
152 was performed at 100 V for 3 h with Mini-Protean Tetra cell (Bio-Rad Laboratories). After SDS-
153 PAGE, proteins were transferred into a nitrocellulose membrane then incubated with primary
154 antibodies: monoclonal anti-troponin-T (JLT-12; Sigma-Aldrich, St Louis, MO; diluted
155 1:40000) and monoclonal anti-desmin (D1033, Sigma-Aldrich; diluted 1:5000). After washing,
156 membranes were incubated with secondary goat antibody anti-mouse-HRP (No 2554; Sigma-
157 Aldrich, St Louis, MO) and diluted 1:30000 and 1:5000 for troponin-T and desmin, respectively.
158 Bands were visualized using Clarity Western ECC kit (Bio-Rad Laboratories). Images were
159 acquired by the Chemi Doc EQ system (Bio-Rad Laboratories) and then analyzed with the Image
160 lab software (version 5.2.1, Bio-Rad Laboratories).

161 **2.4.2. Two-Dimensional Gel Electrophoresis (2DE)**

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

164 Laboratories), were separated in the first dimension with immobilized pH gradient (IPG) dry
165 strips pH 4-7 (17 cm IPG strips, Bio-Rad Laboratories) loaded with 300 µg of protein, using the
166 IPG Protean IEF Cell (Bio-Rad Laboratories). After the isoelectric focusing (IEF), IPG strips
167 were equilibrated at room temperature for 15 min in equilibration buffers I and II (Bio-Rad
168 Laboratories). Two-dimensional separation was performed on a Protean II xi system (Bio-Rad
169 Laboratories) using 8–18% SDS-PAGE. Then, the destained gels were acquired using Chemi
170 Doc EQ system (Bio-Rad Laboratories) and analyzed with the PDQuest 7.4.0 software (Bio-
171 Rad Laboratories) to obtain information on the number of spots per gel, the isoelectric point (pI),
172 molecular weight (MW), volume, area and intensity of each spot. The protein spots detect to
173 differ between the two experimental groups were automatically matched among the gels using
174 the spots of a master gel (virtual gel comprehensive of all matched and unmatched spots of all
175 2DE images) used as a reference. Landmark spots were used to confirm spot matching across all
176 gels and manual verification was used to screen out any dust artefacts or incorrectly identified
177 spots. The relative volume of each spot in a gel was normalized as a percentage of the total
178 volume of all spots detected on the gel.

179 **2.4.3. In-gel digestion of the protein spots and identification by LC-MS/MS**

180 Spots displaying statistically significant differences among dietary treatments and storage
181 time were carefully excised from the preparative gels and then destained for 30 min with 50 mM
182 NH_4HCO_3 . Samples were digested overnight at 37°C with trypsin (12.5 ng mL⁻¹, Promega).
183 Then, peptides were extracted, dried under vacuum, and suspended in 15 µL of 0.1% formic acid
184 for LC-MS/MS analysis. Separations were conducted using an LTQ-Orbitrap XL mass
185 spectrometer (Thermo Fisher Scientific) coupled online with a nano-HPLC Ultimate 3000
186 (Dionex – Thermo Fisher Scientific) and equipped with a homemade pico-frit column (75 µm
187 I.D., 15 µm Tip, 100 mm, New Objective) packed with C18 material (Aeris peptide 3.6 µm XB-
188 C18, Phenomenex) where peptides were eluted using a linear gradient of ACN/0.1% FA (from
189 3% to 40% in 19 min), at a flow rate of 250 nL min⁻¹.

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

196 confidence (False Discovery Rate 0.01) and a coverage rate $\geq 20\%$ 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.

228 2.6. Statistical analysis

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

242 3. Results and Discussions

243 3.1. Effects of dietary treatment and storage time on lamb meat myofibrillar fragmentation 244 (MFI) and total collagen content

245 In the present study, myofibrillar fragmentation index was significantly affected by dietary
246 treatment ($P < 0.01$) and storage time ($P < 0.001$) as depicted in **Figure 1**. The two experimental
247 groups revealed opposite tendencies in myofibrillar degradation rate. At day 0 of storage, the
248 meat of the control group had higher MFI (98.38 vs 91.33 in C and H group, respectively;
249 $P < 0.05$) compared to meat from lambs fed hazelnut skins. Conversely, both at days 4 and 7, the
250 highest values of MFI were detected in meat of the H group (113.74 and 116.1 vs 99.28 and
251 107.26 in H and C group at 4 and 7 days, respectively). MFI increased during storage time in
252 both groups but with different behavior and rate; more important in meat from lambs fed hazelnut
253 skin by-products (+24% after 4 days). These changes are in line to the body of knowledge stating
254 that the major changes in the myofibrillar proteins concur in the tenderization period of raw meat
255 (Lonergan et al., 2010). In this study, meat from lambs fed hazelnut skins evidenced a rapid
256 increase of MFI during storage compared to the control, supporting the functional role of feeding
257 supplementation, including natural antioxidants, on the endogenous enzymatic activity which in
258 turn led to a major breakdown of the muscle protein structures. Indeed, it is known that
259 polyphenols, due to their ability to chelate transition metal ions, scavenge free radicals, and

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

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

273 3.2. Changes in myofibrillar proteins profile of lamb's meat

274 In **Fig. 2** are shown the results of 1D SDS-PAGE of the myofibrillar proteins from
275 *Longissimus thoracis et lumborum* muscle of lambs as affected by dietary treatment and storage
276 time (0, 4, and 7 d). Variations in myofibrillar protein profiles with different trends in the
277 experimental groups due to the dietary treatment and storage time were observed. The meat of
278 lambs fed hazelnut skin showed lower abundances of structural proteins (**Fig. 2**) known as
279 indicators of meat tenderization (Gagaoua et al., 2020) such as myosin heavy chains (MYH;
280 $P < 0.001$; **Fig. 2a**), desmin (DES; $P < 0.01$; **Fig. 2b**) and troponin T (TNNT3; $P < 0.001$; **Fig. 2c**).
281 Storage time affected the relative intensity of the same myofibrillar protein patterns. Meat from
282 H group showed a decrease of MYH ($P < 0.05$; **Fig. 2a**) at 7 d of storage compared to the controls.
283 In meat of H group, desmin ($P < 0.05$; **Fig. 2b**) and TNNT3 ($P < 0.01$; **Fig. 2c**) had lowest values
284 after 4 d of storage and then remained constant. It is well established that variations in the degree
285 of post-mortem improvement in texture can be reflected by changes in the amounts of the major
286 structural proteins (Gagaoua et al., 2021). Particularly, MYH, ACTA1, and DES, as significant
287 components of myofibril thick filaments, were crucial in defining the texture of muscle,
288 especially because they are the first proteins proposed to be degraded by muscle proteases (Ouali
289 et al., 2013). The percentage of 30 kDa fragments (**Fig. 2d**) was differently influenced by the
290 dietary treatment and storage time in the experimental groups. Meat from lambs fed hazelnut
291 skin showed the highest amount of 30 kDa fragments. Regarding storage, the H group showed

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

301 To further elucidate the observed changes in desmin and troponin T proteins, immunoblotting
302 was performed (**Fig. 3** and **Fig. 4**). Desmin intact immunoreactive bands were detected at 54 kDa
303 whereas degraded forms were found at 46, 45, 40, and 37 kDa (**Fig. 3a**). Image analysis (**Fig.**
304 **3b**) revealed significant differences due to dietary treatment and storage time. Starting from 24
305 h post-mortem, a progressive decrease of 54 kDa band ($P<0.01$) was observed evidencing a
306 different degradation extent of the intact form of desmin. In particular, at 4 days of storage a
307 reduction of about 10% and 3% was found in lambs fed hazelnut skin and control diet,
308 respectively; whereas at 7 days of storage the reduction of 54 kDa band was about 28% and 5%
309 in H and C groups, respectively. Desmin is one of the most susceptible substrates of calpains
310 (Huff-Lonergan et al., 1996). It is also one of the first proteins to be degraded 24 hours post-
311 mortem (Huff-Lonergan & Lonergan, 1999; Koohmaraie, 1992). Accordingly, Starkey et al.
312 (2015) observed an improvement in shear force as a result of desmin degradation starting from
313 0 day in lamb meat. Rowe et al. (2004) found an early production of protein breakdown products
314 in meat from steers receiving Vitamin E dietary supplementation compared to the control
315 demonstrating that post-mortem oxidation could interfere with the tenderization process by
316 reducing calpain activity and delaying the rate of proteolysis in meat. In this study, the rapid
317 decline of the intact form of desmin in the meat of the H group suggested that hazelnut skins
318 might have a role in delaying muscle oxidation process thus influencing the rate of autolysis and
319 subsequent activation of μ -calpain. In agreement with our hypothesis, Soldado et al. (2021)
320 evidenced that the incorporation of condensed tannins rich sources in ruminant diets can reduce
321 the reactive oxygen species (ROS), the lipid peroxidation derivatives, and the toxic compounds
322 with an improvement of the antioxidant status in living animals and their products.

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

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

328 Immunoblot of troponin T (**Fig. 4a**) showed the presence of 8 immunoreactive bands (37, 36,
329 and 34 kDa, isoforms of the intact protein; 32, 31, 30, 28, and 25 kDa bands, degradation
330 products of TNNT3). The results (**Fig. 4b**) revealed and confirmed the impact of dietary
331 treatment and storage time on the rate of troponin T breakdown. Particularly, meat from lambs
332 fed hazelnut skin showed a degradation of the intact isoforms band at 37 kDa ($P<0.001$), 36 kDa
333 ($P<0.001$), and 34 kDa ($P<0.01$) after 4 days of storage, remaining constant thereafter.
334 Conversely, in meat from the control group, the percentage of the intact band at 37 kDa decreased
335 progressively during storage whereas a reduction of 36 and 34 kDa band was detected only after
336 7 and 4 days of storage, respectively. However, it is important to note that meat from the H group
337 showed lower values in the 37 kDa intact band starting from 0 day of storage, although after 7
338 days no differences were detected between the meat of the two experimental groups. A more
339 rapid and greater increase of troponin T fragments (sum of all degraded isoforms) was found in
340 meat from the hazelnut group after 4 days of storage (66.09 vs 81.19 in C and H, respectively)
341 confirming that dietary tannins supplementations can also affect the rate of the tenderization
342 process by modulating the endogenous enzymatic activity as previously reported (Zhao et al.,
343 2018). However, at the end of storage time, meat from both groups displayed comparable results
344 in terms of the amount of degradation products of TNNT3 (92.00 vs 88.60 in C and H,
345 respectively). Overall, these findings supported the greater myofibrillar fragmentation found in
346 the H group at 4 days and confirmed that the inclusion of tannins in the lamb diet can influence
347 the post-mortem processes through a protective impact on the development of oxidative
348 phenomena as also supported by the meat antioxidant capacity results reported in the companion
349 paper (Menci et al., 2023). Indeed, the oxidative conditions are responsible for physical and
350 chemical changes in muscle protein properties, including conformation, aggregation, and
351 solubility, that can reduce the protein susceptibility to proteolysis (Lonergan et al., 2010; Zhang
352 et al., 2013). Collectively, data on desmin and TNNT3 fractions together with levels of 30 kDa
353 fragments suggest the role of antioxidant compounds present in hazelnut skin in enhancing
354 tenderization processes in lamb meat during storage.

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

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

369 **3.3. Two-dimensional electrophoresis (2DE) of myofibrillar proteome and bioinformatics** 370 **analysis**

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

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

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

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

410 Among the dominating proteins (**Fig. 6B**), actin (ACTA1) was identified as the most
411 influenced proteins by dietary treatment due to the presence of 5 proteoforms. Actin is the main
412 constituent of the sarcomere thin filaments, and the first protein targeted by caspases in the post-
413 mortem period with the breakdown of the actomyosin complex (Du et al., 2004; Ouali et al.,
414 2013). Indeed, the integromics study of Gagaoua et al. (2021) pointed out that ACTA1 is the
415 most frequently identified protein related to meat tenderization processes. Conversely, among
416 the other proteins of actin filaments, γ - actin (ACTG1) has been recently found to be associated
417 with meat tenderization (Malheiros et al., 2021).

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

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

432 The myofibrillar proteome of meat from the hazelnut group was characterized also by the
433 presence of three proteins belonging to the F-actin capping protein family (CAPZB and
434 CAPZA2), involved in the cell signaling and regulation of actin in myofilament contractility.
435 Previous studies on beef (Guillemin et al., 2011) and pork (Lametsch et al., 2003; Wang et al.,
436 2014) meat, revealed a link between F-actin capping proteins expression and meat tenderness
437 highlighting that these protein patterns could be interesting biomarkers of post-mortem meat
438 tenderization. Among the other myofibrillar proteins that constitute the principal sub-network, 4
439 proteoforms of MYL3 characterized the myofibrillar proteome of meat from the H group after 7
440 days of storage time showing a greater abundance than control meat.

441 Pathways related to energy metabolism, especially those involved in the mitochondrial and
442 ATP metabolic processes are strictly linked with post-mortem underlying pathways of meat
443 texture determination (Gagaoua et al., 2021; Ouali et al., 2013; Purslow et al., 2021; Rosa et al.,
444 2018; Wang et al., 2016). Dietary treatment with hazelnut skin significantly affected the
445 expression of ATP5PD, UQCRC1 and PDHB in lamb meat after 7 days of storage time.
446 Cytochrome b-c1 complex subunit 1 (UQCRC1) is involved in the oxidation/reduction process
447 playing an important role in the cell electron transport and respiratory chain (Kunej et al., 2007).
448 Mitochondrial apoptosis and release of cytochrome C have been shown to play a fundamental
449 role in the breakdown of myofibrillar protein during post-mortem period (Zhang et al., 2017). In
450 particular, Wang et al. (2018) demonstrated that the release of cytochrome C induce caspase-9
451 activation, which may greatly influence meat tenderness. In this study, the greater abundance of
452 spots ascribed to mitochondrial electron transport chain proteins in the meat from the hazelnut
453 group confirms the high tenderization rate observed in H group. These proteins can be proposed

454 as biomarkers of lamb meat tenderization in line with previous studies on beef (Gagaoua et al.,
455 2021).

456 Among the changing pathways as a consequence of hazelnut skin by-products
457 supplementation, heat shock proteins (HSPB1, HSP70, HSPB6, HSPB7), usually associated with
458 the response to stress processes, also impacted the lamb myofibrillar proteome. HSPs as
459 chaperones, play a role in stress resistance by restoring proteins altered by external stimulus thus
460 assuming a fundamental role in controlling the onset of apoptosis and post-mortem proteolytic
461 processes (Lomiwes et al., 2014; Ouali et al., 2013). Additionally, several studies (Balan et al.,
462 2014; Cramer et al., 2018) reported that an increase in degradation of small heat shock proteins,
463 like HSPB1 identified in this study, may indicate a loss of its anti-apoptotic function evidencing
464 their important role in the post-mortem processes related to meat quality. The integromics study
465 conducted by Gagaoua et al. (2021) evidenced HSPB1 as the top biomarker related to beef
466 tenderness. Several studies (Gagaoua et al., 2021; Kim et al., 2018) suggested that HSPB1 may
467 delay the activity of endogenous enzymes, and consequently their proteolytic activity. Higher
468 levels of HSPB1 were found to predict tenderness due to the prevention of aggregation processes
469 thus facilitating the action of proteolytic enzymes during the post-mortem period (Morzel et al.,
470 2008). Recently, an overabundance of HSP70 (Heat shock 70 kDa protein) was observed by Ma
471 et al. (2020) in callipyge lambs genotypes with tougher meat indicating delayed apoptosis and
472 proteolysis.

473 Heat shock protein beta-6 (HSPB6), due to the presence of a binding domain for troponin I,
474 play a key role in muscle contraction and metabolic processes (Rembold et al., 2000). In the
475 meta-proteomics study of Picard and Gagaoua (2020), HSPB6 was identified as a putative
476 biomarker of beef tenderness, based on 12 proteomic studies. Additionally, Ma and Kim (2020),
477 in an aging study, found a strong relationship between the greater abundance of HSPB6
478 fragments and the tenderization rate of different bovine muscles. About HSPB7, Li and Liu
479 (2022) and Dang et al. (2022) found an overabundance of this protein in bovine meat after 7 and
480 16 days of aging, respectively.

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

486 **4. Conclusion**

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

505 **Conflict of interest**

506 The authors declare no conflict of interest.

507 **Funding**

508 This research did not receive any specific grant from funding agencies in the public, commercial,
509 or not-for-profit sector.

510 **References**

- 511 Balan, P., Kim, Y. H. B., & Blijenburg, R. (2014). Small heat shock protein degradation could
512 be an indicator of the extent of myofibrillar protein degradation. *Meat science*, *97*(2),
513 220-222.
- 514 Caccamo, M., Valenti, B., Luciano, G., Priolo, A., Rapisarda, T., Belvedere, G., Marino, V. M.,
515 Esposto, S., Taticchi, A., & Servili, M. (2019). Hazelnut as ingredient in dairy sheep diet:
516 Effect on sensory and volatile profile of cheese. *Frontiers in Nutrition*, *6*, 125.
- 517 Campione, A., Natalello, A., Valenti, B., Luciano, G., Rufino-Moya, P. J., Avondo, M.,
518 Morbidini, L., Pomente, C., Krol, B., & Wilk, M. (2020). Effect of feeding hazelnut skin

- 519 on animal performance, milk quality, and rumen fatty acids in lactating ewes. *Animals*,
 520 10(4), 588.
- 521 Cramer, T., Penick, M. L., Waddell, J. N., Bidwell, C. A., & Kim, Y. H. B. (2018). A new insight
 522 into meat toughness of callipyge lamb loins-the relevance of anti-apoptotic systems to
 523 decreased proteolysis. *Meat science*, 140, 66-71.
- 524 Culler, R., Smith, G., & Cross, H. (1978). Relationship of myofibril fragmentation index to
 525 certain chemical, physical and sensory characteristics of bovine longissimus muscle.
 526 *Journal of food Science*, 43(4), 1177-1180.
- 527 Daghighi, M., Viti, C., Mannelli, F., Pauselli, M., Natalello, A., Luciano, G., Valenti, B., &
 528 Buccioni, A. (2021). A diet supplemented with hazelnut skin changes the microbial
 529 community composition and the biohydrogenation pattern of linoleic acid in the rumen
 530 of growing lambs. *Italian Journal of Animal Science*, 20(1), 1256-1263.
- 531 Dang, D. S., Zhai, C., Nair, M. N., Thornton, K. J., Sawalhah, M. N., & Matarneh, S. K. (2022).
 532 Tandem mass tag labeling to assess proteome differences between intermediate and very
 533 tender beef steaks. *Journal of Animal Science*, 100(8), skac042.
- 534 Del Rio, D., Calani, L., Dall'Asta, M., & Brighenti, F. (2011). Polyphenolic composition of
 535 hazelnut skin. *Journal of Agricultural and Food Chemistry*, 59(18), 9935-9941.
- 536 Della Malva, A., De Palo, P., Lorenzo, J. M., Maggiolino, A., Albenzio, M., & Marino, R.
 537 (2019). Application of proteomic to investigate the post-mortem tenderization rate of
 538 different horse muscles. *Meat science*, 157, 107885.
- 539 Della Malva, A., Marino, R., Santillo, A., Annicchiarico, G., Caroprese, M., Sevi, A., &
 540 Albenzio, M. (2017). Proteomic approach to investigate the impact of different dietary
 541 supplementation on lamb meat tenderness. *Meat science*, 131, 74-81.
- 542 Du, J., Wang, X., Miereles, C., Bailey, J. L., Debigare, R., Zheng, B., Price, S. R., & Mitch, W.
 543 E. (2004). Activation of caspase-3 is an initial step triggering accelerated muscle
 544 proteolysis in catabolic conditions. *The Journal of clinical investigation*, 113(1), 115-
 545 123.
- 546 FAO, G. (2011). Global Food Losses and Food Waste-Extent. *Causes and Prevention*, 1-37.
- 547 Gagaoua, M., Terlouw, E. C., Mullen, A. M., Franco, D., Warner, R. D., Lorenzo, J. M., Purslow,
 548 P. P., Gerrard, D., Hopkins, D. L., & Troy, D. (2021). Molecular signatures of beef
 549 tenderness: Underlying mechanisms based on integromics of protein biomarkers from
 550 multi-platform proteomics studies. *Meat science*, 172, 108311.
- 551 Gagaoua, M., Troy, D., & Mullen, A. M. (2020). The extent and rate of the appearance of the
 552 major 110 and 30 kDa proteolytic fragments during post-mortem aging of beef depend
 553 on the glycolysing rate of the muscle and aging time: An LC-MS/MS approach to
 554 decipher their proteome and associated pathways. *Journal of Agricultural and Food
 555 Chemistry*, 69(1), 602-614.
- 556 Gao, X., Wu, W., Ma, C., Li, X., & Dai, R. (2016). Postmortem changes in sarcoplasmic proteins
 557 associated with color stability in lamb muscle analyzed by proteomics. *European Food
 558 Research and Technology*, 242, 527-535.
- 559 Gomes, A. V., Potter, J. D., & Szczesna-Cordary, D. (2002). The role of troponins in muscle
 560 contraction. *IUBMB life*, 54(6), 323-333.
- 561 Guillemin, N., Bonnet, M., Jurie, C., & Picard, B. (2011). Functional analysis of beef tenderness.
 562 *Journal of Proteomics*, 75(2), 352-365.
- 563 Huff-Lonergan, E., & Lonergan, S. M. (1999). Postmortem mechanisms of meat tenderization:
 564 The roles of the structural proteins and the calpain system. *Quality attributes of muscle
 565 foods*, 229-251.
- 566 Huff-Lonergan, E., & Lonergan, S. M. (2005). Mechanisms of water-holding capacity of meat:
 567 The role of postmortem biochemical and structural changes. *Meat science*, 71(1), 194-
 568 204.

- 569 Huff-Lonergan, E., Mitsuhashi, T., Beekman, D. D., Parrish Jr, F. C., Olson, D. G., & Robson,
570 R. M. (1996). Proteolysis of specific muscle structural proteins by μ -calpain at low pH
571 and temperature is similar to degradation in postmortem bovine muscle. *Journal of*
572 *Animal Science*, 74(5), 993-1008.
- 573 Hutson, P. R., Crawford, M. E., & Sorkness, R. L. (2003). Liquid chromatographic determination
574 of hydroxyproline in tissue samples. *Journal of Chromatography B*, 791(1-2), 427-430.
- 575 Ivanović, S., Avramović, N., Dojčinović, B., Trifunović, S., Novaković, M., Tešević, V., &
576 Mandić, B. (2020). Chemical composition, total phenols and flavonoids contents and
577 antioxidant activity as nutritive potential of roasted hazelnut skins (*Corylus avellana* L.).
578 *Foods*, 9(4), 430.
- 579 Kim, Y. H. B., Ma, D., Setyabrata, D., Farouk, M. M., Lonergan, S. M., Huff-Lonergan, E., &
580 Hunt, M. C. (2018). Understanding postmortem biochemical processes and post-harvest
581 aging factors to develop novel smart-aging strategies. *Meat science*, 144, 74-90.
- 582 Koochmaraie, M. (1992). The role of Ca^{2+} -dependent proteases (calpains) in post mortem
583 proteolysis and meat tenderness. *Biochimie*, 74(3), 239-245.
- 584 Kunej, T., Wang, Z., Michal, J. J., Daniels, T. F., Magnuson, N. S., & Jiang, Z. (2007). Functional
585 UQCRC1 polymorphisms affect promoter activity and body lipid accumulation. *Obesity*,
586 15(12), 2896-2901.
- 587 Lametsch, R., Karlsson, A., Rosenvold, K., Andersen, H. J., Roepstorff, P., & Bendixen, E.
588 (2003). Postmortem proteome changes of porcine muscle related to tenderness. *Journal*
589 *of Agricultural and Food Chemistry*, 51(24), 6992-6997.
- 590 Laville, E., Sayd, T., Morzel, M., Blinet, S., Chambon, C., Lepetit, J., Renand, G., & Hocquette,
591 J. F. (2009). Proteome changes during meat aging in tough and tender beef suggest the
592 importance of apoptosis and protein solubility for beef aging and tenderization. *Journal*
593 *of Agricultural and Food Chemistry*, 57(22), 10755-10764.
- 594 Li, S., & Liu, S. (2022). Estimation of the proteome affecting changes in tenderness of yak meat
595 during storage by label-free mass spectrometry. *Veterinary Medicine and Science*, 8(4),
596 1640-1649.
- 597 Listrat, A., Gagaoua, M., Andueza, D., Gruffat, D., Normand, J., Mairesse, G., Picard, B., &
598 Hocquette, J.-F. (2020). What are the drivers of beef sensory quality using metadata of
599 intramuscular connective tissue, fatty acids and muscle fiber characteristics? *Livestock*
600 *Science*, 240, 104209. <https://doi.org/10.1016/j.livsci.2020.104209>
- 601 Listrat, A., Gagaoua, M., Normand, J., Gruffat, D., Andueza, D., Mairesse, G., Mourot, B. P.,
602 Chesneau, G., Gobert, C., & Picard, B. (2020). Contribution of connective tissue
603 components, muscle fibres and marbling to beef tenderness variability in longissimus
604 thoracis, rectus abdominis, semimembranosus and semitendinosus muscles. *Journal of*
605 *the Science of Food and Agriculture*, 100(6), 2502-2511.
- 606 Lomiwes, D., Farouk, M., Wiklund, E., & Young, O. (2014). Small heat shock proteins and their
607 role in meat tenderness: A review. *Meat science*, 96(1), 26-40.
- 608 Lonergan, E. H., Zhang, W., & Lonergan, S. M. (2010). Biochemistry of postmortem muscle—
609 Lessons on mechanisms of meat tenderization. *Meat science*, 86(1), 184-195.
- 610 Ma, D., & Kim, Y. H. B. (2020). Proteolytic changes of myofibrillar and small heat shock
611 proteins in different bovine muscles during aging: Their relevance to tenderness and
612 water-holding capacity. *Meat science*, 163, 108090.
- 613 Ma, D., Yu, Q., Hedrick, V. E., Cooper, B. R., Sobreira, T. J. P., Oh, J.-H., Chun, H., & Kim, Y.
614 H. B. (2020). Proteomic and metabolomic profiling reveals the involvement of apoptosis
615 in meat quality characteristics of ovine *M. longissimus* from different callipyge
616 genotypes. *Meat science*, 166, 108140.
- 617 Maggiolino, A., Bragaglio, A., Salzano, A., Rufrano, D., Claps, S., Sepe, L., Damiano, S.,
618 Ciarcia, R., Dinardo, F., & Hopkins, D. (2021). Dietary supplementation of suckling

- 619 lambs with anthocyanins: Effects on growth, carcass, oxidative and meat quality traits.
620 *Animal Feed Science and Technology*, 276, 114925.
- 621 Malheiros, J., Enríquez-Valencia, C. E., Braga, C. P., Vieira, J. C. S., Vieira, D., Pereira, G. L.,
622 Curi, R. A., Neto, O. M., Oliveira, H., & Padilha, P. M. (2021). Application of proteomic
623 to investigate the different degrees of meat tenderness in Nellore breed. *Journal of*
624 *Proteomics*, 248, 104331.
- 625 Marino, R., Albenzio, M., Della Malva, A., Santillo, A., Loizzo, P., & Sevi, A. (2013).
626 Proteolytic pattern of myofibrillar protein and meat tenderness as affected by breed and
627 aging time. *Meat science*, 95(2), 281-287.
- 628 Marino, R., Della Malva, A., & Albenzio, M. (2015). Proteolytic changes of myofibrillar proteins
629 in Podolian meat during aging: Focusing on tenderness. *Journal of Animal Science*,
630 93(3), 1376-1387.
- 631 Menci, R., Biondi, L., Natalello, A., Lanza, M., Priolo, A., Valenti, B., Bertino, A., Scerra, M.,
632 & Luciano, G. (2023). Feeding hazelnut skin to lambs delays lipid oxidation in meat.
633 *Meat science*, 109218.
- 634 Montella, R., Coïsson, J. D., Travaglia, F., Locatelli, M., Bordiga, M., Meyrand, M., Barile, D.,
635 & Arlorio, M. (2013). Identification and characterisation of water and alkali soluble
636 oligosaccharides from hazelnut skin (*Corylus avellana* L.). *Food Chemistry*, 140(4), 717-
637 725.
- 638 Morzel, M., Terlouw, C., Chambon, C., Micol, D., & Picard, B. (2008). Muscle proteome and
639 meat eating qualities of *Longissimus thoracis* of “Blonde d’Aquitaine” young bulls: A
640 central role of HSP27 isoforms. *Meat science*, 78(3), 297-304.
- 641 Mu, C., Yang, W., Wang, P., Zhao, J., Hao, X., & Zhang, J. (2020). Effects of high-concentrate
642 diet supplemented with grape seed proanthocyanidins on growth performance, liver
643 function, meat quality, and antioxidant activity in finishing lambs. *Animal Feed Science*
644 *and Technology*, 266, 114518.
- 645 Muino, I., Apeleo, E., de la Fuente, J., Pérez-Santaescolástica, C., Rivas-Cañedo, A., Pérez, C.,
646 Díaz, M. T., Caneque, V., & Lauzurica, S. (2014). Effect of dietary supplementation with
647 red wine extract or vitamin E, in combination with linseed and fish oil, on lamb meat
648 quality. *Meat science*, 98(2), 116-123.
- 649 Ouali, A., Gagaoua, M., Boudida, Y., Becila, S., Boudjellal, A., Herrera-Mendez, C. H., &
650 Sentandreu, M. A. (2013). Biomarkers of meat tenderness: present knowledge and
651 perspectives in regards to our current understanding of the mechanisms involved. *Meat*
652 *science*, 95(4), 854-870.
- 653 Paim, T. d. P., Viana, P., Tilburg, M. F. v., Moura, A. d. A., Souza, J. R. d., McManus, C.,
654 Abdalla, A. L., & Louvandini, H. (2019). Feeding effects of cottonseed and its co-
655 products on the meat proteome from ram lambs. *Scientia Agricola*, 76, 463-472.
- 656 Pelvan, E., Olgun, E. Ö., Karadağ, A., & Alasalvar, C. (2018). Phenolic profiles and antioxidant
657 activity of Turkish Tombul hazelnut samples (natural, roasted, and roasted hazelnut skin).
658 *Food Chemistry*, 244, 102-108.
- 659 Picard, B., & Gagaoua, M. (2020). Meta-proteomics for the discovery of protein biomarkers of
660 beef tenderness: An overview of integrated studies. *Food Research International*, 127,
661 108739.
- 662 Priolo, A., Valenti, B., Natalello, A., Bella, M., Luciano, G., & Pauselli, M. (2021). Fatty acid
663 metabolism in lambs fed hazelnut skin as a partial replacer of maize. *Animal Feed Science*
664 *and Technology*, 272, 114794.
- 665 Purslow, P. P., Gagaoua, M., & Warner, R. D. (2021). Insights on meat quality from combining
666 traditional studies and proteomics. *Meat science*, 174, 108423.
- 667 Rembold, C. M., Foster, D. B., Strauss, J. D., Wingard, C. J., & Van Eyk, J. E. (2000). cGMP-
668 mediated phosphorylation of heat shock protein 20 may cause smooth muscle relaxation

- 669 without myosin light chain dephosphorylation in swine carotid artery. *The Journal of*
670 *physiology*, 524(3), 865-878.
- 671 Rosa, A. F., Moncau, C. T., Poleti, M. D., Fonseca, L. D., Balieiro, J. C., Silva, S. L., & Eler, J.
672 P. (2018). Proteome changes of beef in Nellore cattle with different genotypes for
673 tenderness. *Meat science*, 138, 1-9.
- 674 Rowe, L., Maddock, K., Lonergan, S. M., & Huff-Lonergan, E. (2004). Influence of early
675 postmortem protein oxidation on beef quality. *Journal of Animal Science*, 82(3), 785-
676 793.
- 677 Soldado, D., Bessa, R. J., & Jerónimo, E. (2021). Condensed tannins as antioxidants in
678 ruminants—Effectiveness and action mechanisms to improve animal antioxidant status
679 and oxidative stability of products. *Animals*, 11(11), 3243.
- 680 Starkey, C. P., Geesink, G. H., Oddy, V. H., & Hopkins, D. L. (2015). Explaining the variation
681 in lamb longissimus shear force across and within ageing periods using protein
682 degradation, sarcomere length and collagen characteristics. *Meat science*, 105, 32-37.
- 683 Taylor, R. (2004). Connective tissue structure, function and influence on meat quality In: Jensen
684 WK, Devine C., Dikeman M.(eds). Encyclopedia of Meat Science. In: Elsevier Academic
685 Press, Amsterdam.
- 686 Wang, L.-L., Yu, Q.-L., Han, L., Ma, X.-L., Song, R.-D., Zhao, S.-N., & Zhang, W.-H. (2018).
687 Study on the effect of reactive oxygen species-mediated oxidative stress on the activation
688 of mitochondrial apoptosis and the tenderness of yak meat. *Food Chemistry*, 244, 394-
689 402.
- 690 Wang, L., Xu, Y., Wang, Y., Zhong, T., Tang, G., Li, L., Zhang, H., & Xiong, Y. (2014).
691 Identification and characterization of a differentially expressed protein (CAPZB) in
692 skeletal muscle between Meishan and Large White pigs. *Gene*, 544(2), 107-113.
- 693 Wang, Z., He, F., Rao, W., Ni, N., Shen, Q., & Zhang, D. (2016). Proteomic analysis of goat
694 Longissimus dorsi muscles with different drip loss values related to meat quality traits.
695 *Food Science and Biotechnology*, 25, 425-431.
- 696 Xie, Y., Rile, N., Li, X., Li, H., Zhao, M., Che, T., Cai, T., Liu, Z., & Li, J. (2022). Analysis of
697 cashmere goat meat by label-free proteomics shows that MYL3 is a potential molecular
698 marker of meat toughness. *Czech Journal of Animal Science*, 67(4), 137-146.
- 699 Zhang, J., Yu, Q., Han, L., Chen, C., Li, H., & Han, G. (2017). Study on the apoptosis mediated
700 by cytochrome c and factors that affect the activation of bovine longissimus muscle
701 during postmortem aging. *Apoptosis*, 22, 777-785.
- 702 Zhang, W., Xiao, S., & Ahn, D. U. (2013). Protein oxidation: basic principles and implications
703 for meat quality. *Critical Reviews in Food Science and Nutrition*, 53(11), 1191-1201.
- 704 Zhao, J., Li, Q., Zhang, R., Liu, W., Ren, Y., Zhang, C., & Zhang, J. (2018). Effect of dietary
705 grape pomace on growth performance, meat quality and antioxidant activity in ram
706 lambs. *Animal Feed Science and Technology*, 236, 76-85.
- 707 Zhou, Y., Zhou, B., Pache, L., Chang, M., Khodabakhshi, A. H., Tanaseichuk, O., Benner, C.,
708 & Chanda, S. K. (2019). Metascape provides a biologist-oriented resource for the
709 analysis of systems-level datasets. *Nature communications*, 10(1), 1523.

710

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, TNNTI= troponin I, TNNTC= 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.

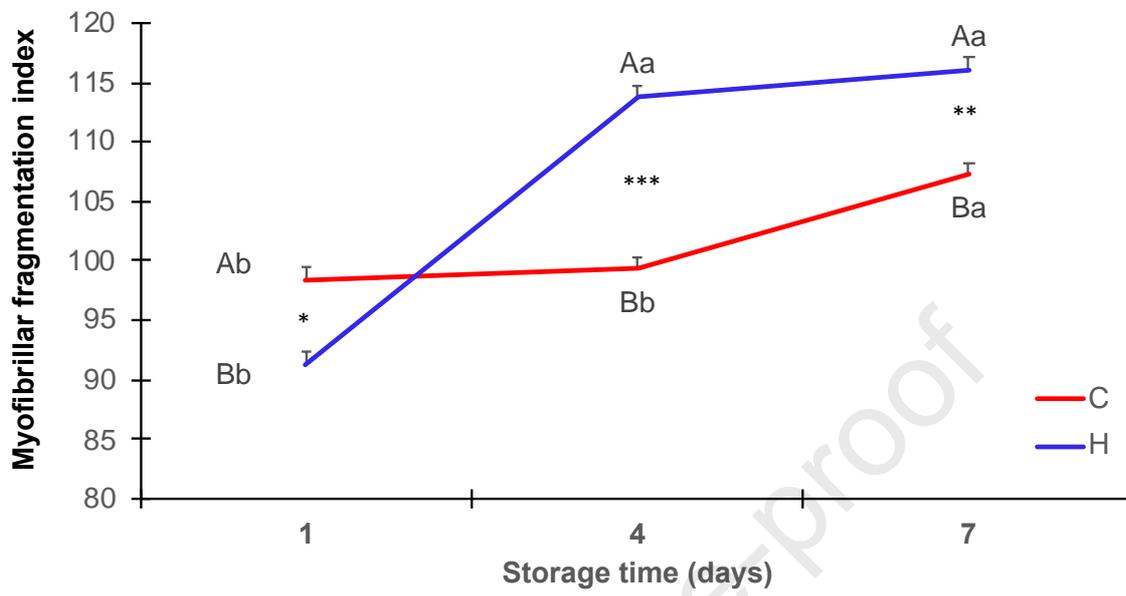


Figure 1.

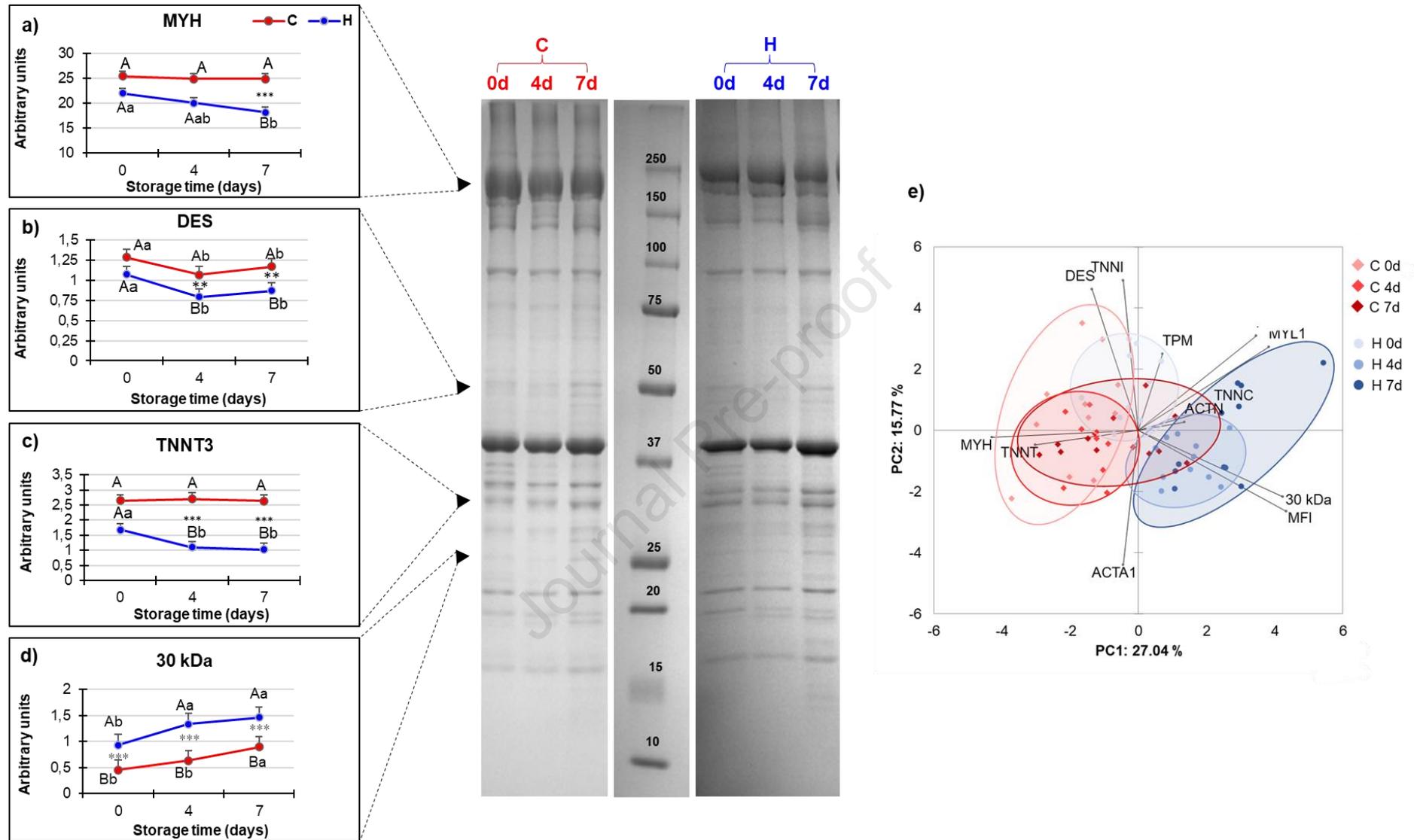
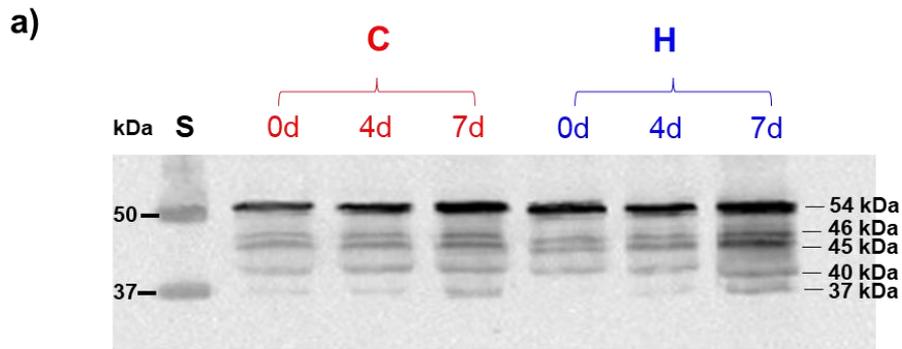


Figure 2.

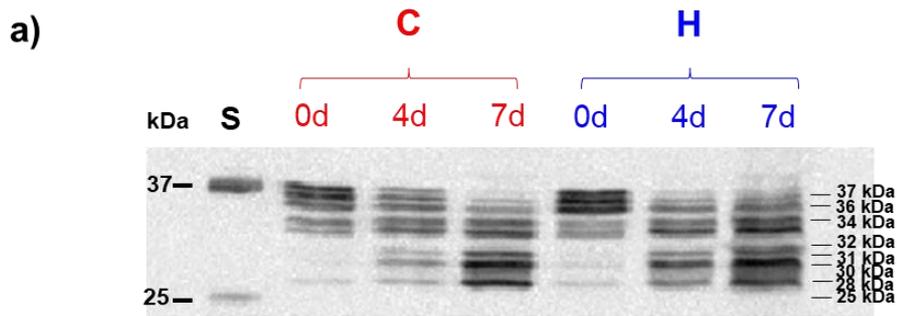


b)

	C			H			SEM	Effect, <i>P</i>	
	0d	4d	7d	0d	4d	7d		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.



b)

	C			H			SEM	Effect, <i>P</i>	
	0d	4d	7d	0d	4d	7d		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.

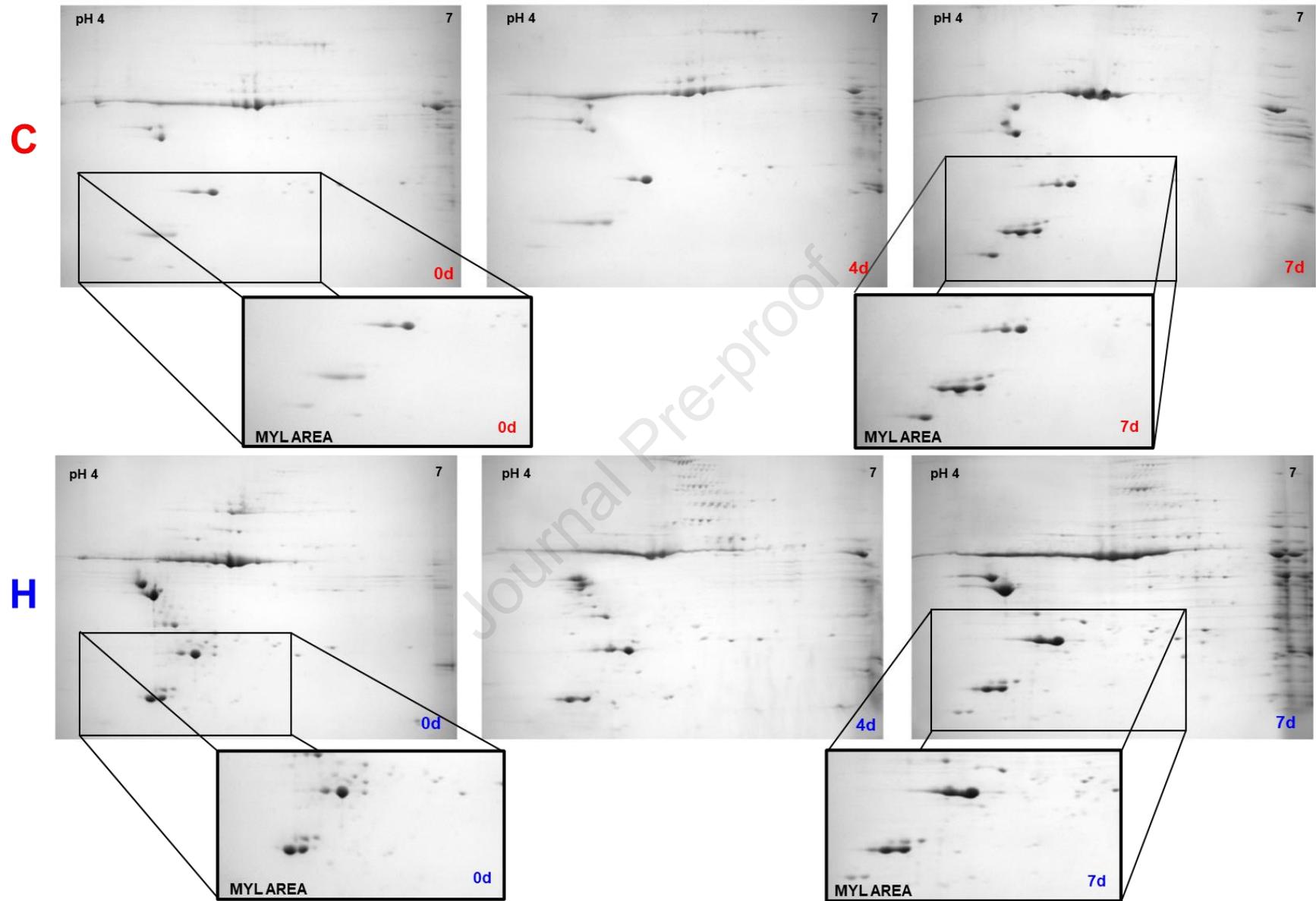


Figure 5.

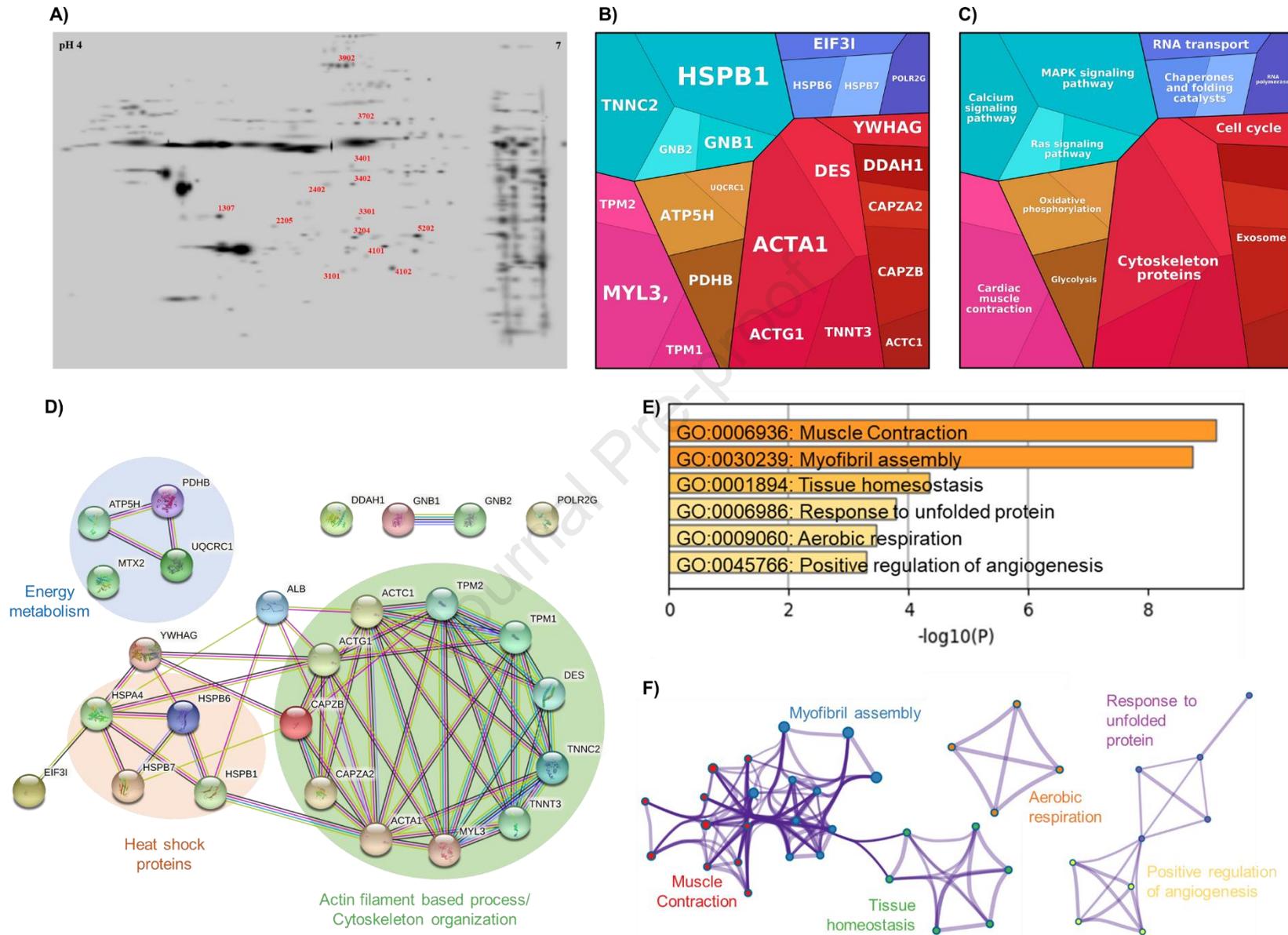


Figure 6.

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.

Journal Pre-proof

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.