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ARE THE BIOCHEMICAL PATHWAYS UNDERLYING LAMB MEAT COLOUR AND TEXTURE DETERMINATION SIMILAR?

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I. INTRODUCTION

The *post-mortem* changes of muscle proteins, as a result of physicochemical and enzymatic reactions, are pivotal events defining the development of meat quality traits [1]. Historically, the role of sarcoplasmic proteins in meat colour has been identified [2], while changes in myofibrillar proteins have shown important role in meat tenderness determination [3]. Recently, proteomics combined with bioinformatics tools allowed deeper understanding of the biochemical pathways involved in the *post-mortem* processes and revealed that the development of meat colour and tenderness is the result of interconnected mechanisms [4]. However, to date and in lamb meat, there was no comparison of the mechanisms underlying multiple quality traits, for instance colour and texture. Therefore, this study aimed for the first time to compare the changing muscle proteins found to be associated with colour and texture quality traits of lamb meat that were fed differently. We further aim to compare the similarities and divergencies in the pathways underpinning these important meat quality traits.

II. MATERIALS AND METHODS

Twenty-two Valle del Belice male lambs (60 ± 4 days of age) were used in this trial, from which half were fed with maize-barley based diet and half were fed with the same diet but 150 g/kg dry matter of maize was replaced with hazelnut skin. After 56 days of the experimental trial, animals were slaughtered following industrial practices used in Italy and European guidelines (EU rule n. 1099/2009). Meat colour and texture (myofibrillar fragmentation index) were assessed on the *Longissimus thoracis et lumborum* muscle aged at 1, 4 and 7 days. Sarcoplasmic and myofibrillar proteins were analyzed using two-dimensional gel electrophoresis (2DE) coupled to LC-MS/MS [5]. The differentially abundant proteins (DAPs) identified in the sarcoplasmic (related to colour traits) and myofibrillar (related to texture) proteomes to study the impact of feeding strategy were analyzed by bioinformatics tools. First, pathway enrichment analysis to identify the enriched Gene Ontology (GO) terms was evaluated using Metascape®. Second, the same tool investigated the overlap in the GO terms between the protein lists. Finally, the DAPs proteins were visualised using Proteomaps open-source tool to consider all the proteoforms and their frequency of identification.

III. RESULTS AND DISCUSSION

Data revealed that hazelnut skin dietary supplementation impacted meat quality characteristics, with meat from the lamb of the hazelnut group displaying greater values of lightness, redness, and chroma colour parameters together with a greater myofibril fragmentation index (data not shown). A total of 10 protein spots, corresponding to 41 proteoforms (unique gene names), were found to be differentially abundant in the sarcoplasmic proteome of meat from lamb fed with hazelnut skin, whereas 13 DAPs (44 proteoforms) were found in the myofibrillar proteome. The comparison of the significantly enriched GO terms of the two protein lists (Figure 1A) revealed 20 enriched term clusters among which “negative regulation of cellular component organization”, “protein targeting”, “muscle contraction”, “response to

hormone", "retina homeostasis" and "regulation of supramolecular fiber organization" are common pathways to both colour and texture lamb meat determination. Several of the other pathways were specific to each quality trait, depicting disparity in the underlying pathways. The proteomaps (Figure 1B and 1C) further confirmed differences in the molecular pathways, but revealed that proteins from sarcoplasmic and myofibrillar proteomes of meat from the hazelnut group are involved in *post-mortem* glycolysis, central carbon metabolism, signal transduction, folding sorting and degradation, vesicular transport and cell growth and death. In line with previous studies [4], energy metabolism proteins, especially those related to mitochondria and involved in ATP metabolic processes, and heat shock proteins are strictly related to *post-mortem* processes linked with meat colour and tenderness variation. Comparative bioinformatics revealed also ATP5H and HSPB1, previously identified as biomarkers of beef tenderness and colour [2, 3], as common proteins, hence suggesting their potential use in monitoring lamb meat quality regardless of the two protein fractions.

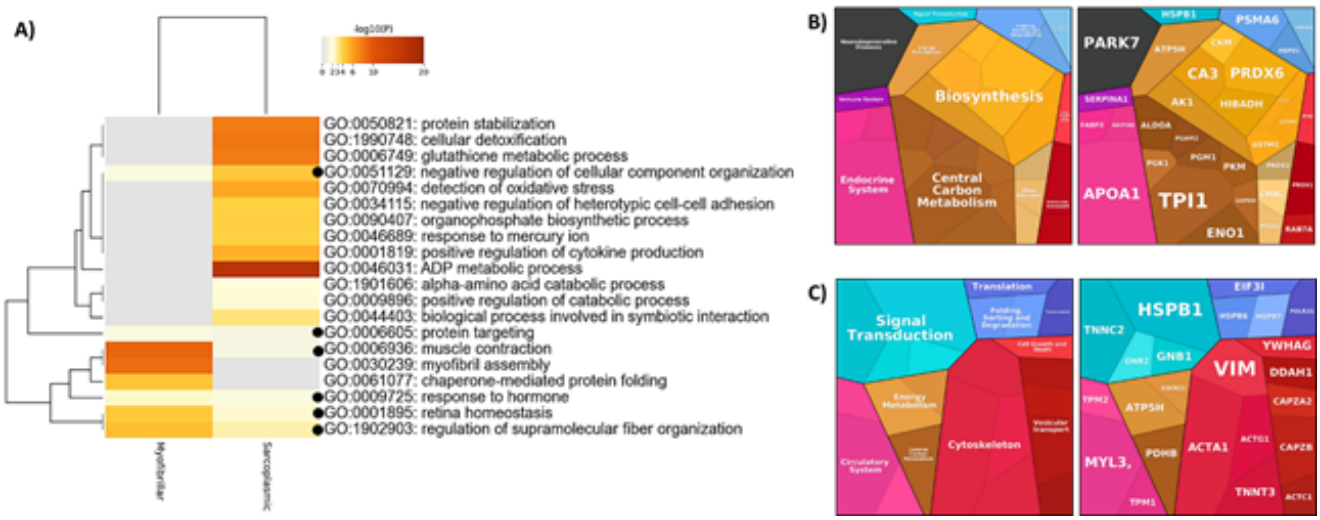

Figure 1. Biological pathways and process enrichment cluster analysis using Metascape®. **A)** Heatmap clustering performed on the proteins identified displaying the significant enriched Gene Ontology (GO) terms comparing the two dietary groups from both sarcoplasmic and myofibrillar proteins. The heat map is colored based on p-values colour scale representing the statistical significance and grey was used to indicate a lack of significance. Proteomaps analysis of the DAPs from the **B)** sarcoplasmic and **C)** myofibrillar proteome. Each polygon corresponds to a single KEGG pathway, and the size is proportional to its fraction in the total dataset.

IV. CONCLUSION

The findings revealed that meat colour and texture assessed by their sarcoplasmic and myofibrillar proteomes, respectively, share similar biochemical pathways. Two proteins (ATP5H and HSPB1) were revealed as common, and we suggest them as candidate biomarkers to monitor lamb meat colour changes and texture properties.

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