

PROTEIN PATTERN CHANGES IN *POST-MORTEM* GOAT *SEMITENDINOSUS* MUSCLE: A CLUSTERING ANALYSIS TO REVEAL THE UNKNOWNNS IN MUSCLE TO MEAT CONVERSION

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I. INTRODUCTION

The conversion of muscle into meat is a complex process involving biochemical, energetic, and physical events occurring during cold storage of carcasses and during retail/display of meat cuts [1]. In particular, the changes during the *post-mortem* period in both the structure and status of muscle proteins along of their spatial arrangements are pivotal events defining the development of meat quality [2]. In the last decade, proteomics was intensively adopted in meat research to unravel the complexity of the muscle proteomes and to propose explanatory mechanisms at the origin of the variability of different eating qualities of meat from various species [2, 3]. However, at present, no study has examined the dynamic changes occurring in the early *post-mortem* muscle proteome of goat meat. Thus, this trial aimed to decipher such changes in *Semitendinosus* muscle of goats and to unveil, through label-free shotgun proteomics and bioinformatics, the molecular pathways underlying the conversion of muscle into meat.

II. MATERIALS AND METHODS

Eight male goats (Saanen x *Naine de Kabylie* crossbred) were used [4]. They were slaughtered the same day using similar handling practices in compliance with the Halal slaughter. *Semitendinosus* muscle biopsies were collected at different *post-mortem* times (1h, 8h, and 24h) and stored at -80°C until analysis [4]. For shotgun proteomics, total protein extracts were used for the preparation of the protein bands using one-dimensional SDS-PAGE [4] before LC-MS/MS analyses. For statistics, the full list of quantified proteins with > 2 peptides (n = 748) was subjected to cluster analysis of the time-of-day-dependent goat proteome after data normalization of the median value of logarithmized intensities (Log2) of each protein. For bioinformatics, the enriched Gene Ontology (GO) terms and pathways on the targeted clusters was investigated using Metascape® open-source tool.

III. RESULTS AND DISCUSSION

Six clusters based on the protein abundance changes have been built (Figure 1A). Among those, we selected for this paper cluster 1 (n = 155 proteins) with an upregulating trend from 1h to 24h *post-mortem*, whereas cluster 4 (n = 105 proteins) showed a continuous decrease in intensity from 1h to 8h, and remaining constant until 24h. The pathway enrichment analysis of the proteins of cluster 1 identified 20 significant GO terms (Figure 1B). The “generation of precursor metabolites and energy”, “actin filament-based process”, and “muscle system process” were the top enriched terms followed by others related to proteolysis, mitochondrial transport, and regulation of apoptosis. The increase in the expression of these pathways, during *post-mortem* time, agrees with the body of knowledge [2]. Indeed, apoptosis can affect skeletal muscle integrity during early *post-mortem* period through the modulation of calcium release with implications on endogenous proteases, hence affecting muscle structural changes [1]. Conversely, the pathway enrichment analysis of the cluster 4 evidenced as top enriched terms “energy derivation by oxidation of organic compounds” (Figure 1C). The comparison of the two clusters revealed 12 common enriched terms (Figure 1D). Furthermore, six terms were significantly and

exclusively specific to cluster 1, whereas “mitochondrial electron transport, ubiquinol to cytochrome c” and “reactive oxygen species metabolic process” were specific to cluster 4 thus evidencing their central role in driving the biochemical processes taking place during the first hours’ *post-mortem*.

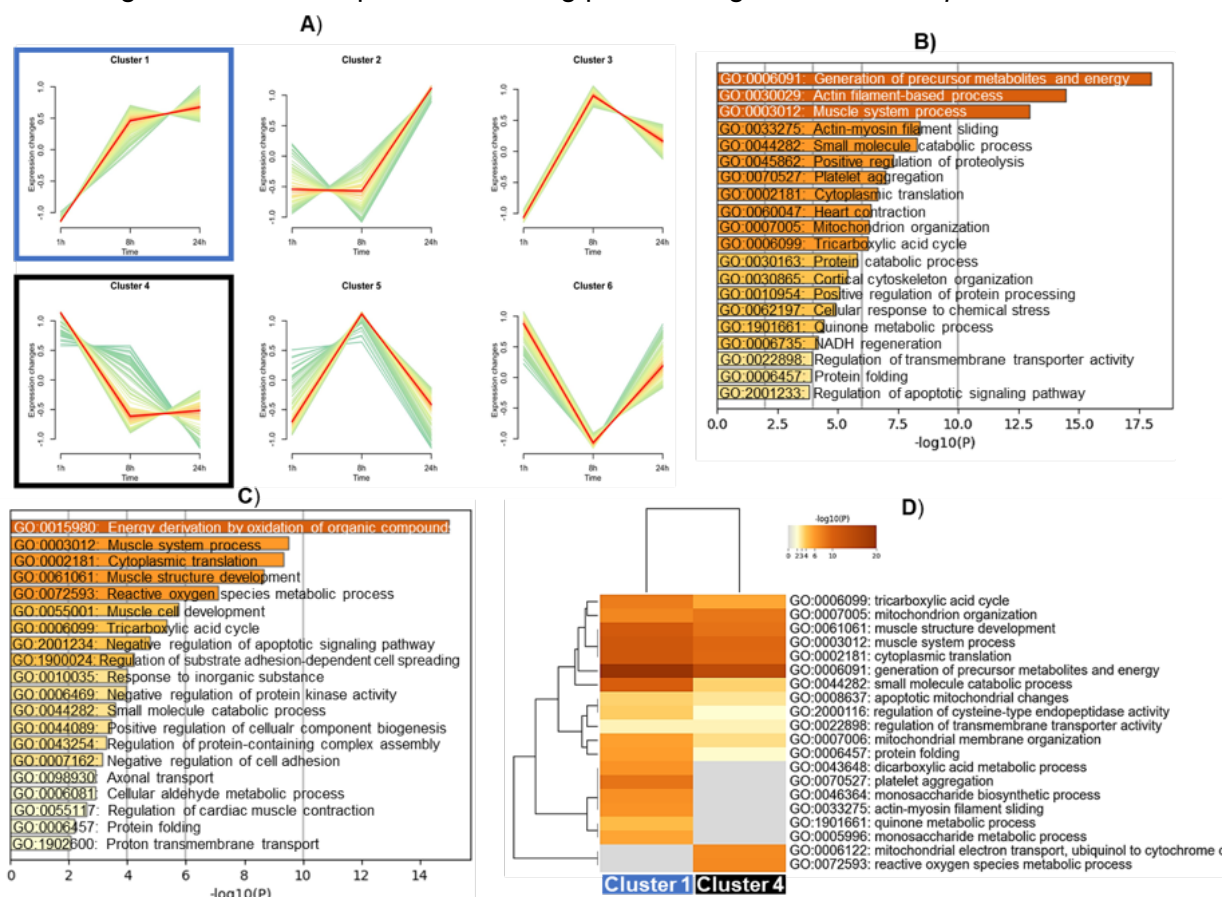


Figure 1. Statistical and bioinformatics analyses. **A)** Statistical clustering analysis (cluster 1, blue square; cluster 4, black square). The y-axis of each cluster represents the *post-mortem* time, while the x-axis represents the rich factor. Functional GO enrichment analysis of the **B)** cluster 1 and **C)** cluster 4 proteins. The top 20 enriched and significant GO terms ranked by their P -value are given. **D)** Heatmap performed on the proteins identified on the targeted clusters (cluster 1, blue; cluster 4, black) displaying the significantly enriched GO terms.

IV. CONCLUSION

First study that applied shotgun proteomics, clustering and bioinformatics analyses to shed light on the complex dynamic changes behind early *post-mortem* muscle samples taken at 1, 8 and 24h. The analyses and comparisons of all the clusters identified are under consideration for in-depth characterization of the multiple mechanisms at interplay in the conversion of muscle into meat.

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