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EXUDATE MEAT PROTEOME ANALYSIS USING LABEL-FREE PROTEOMICS: A NOVEL WAY TO DECIPHER THE COMPLEX UNDERLYING MECHANISMS OF MEAT AGING

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I. INTRODUCTION

Meat aging is a complex process involving various biochemical, energetic and physical changes, resulting in the determination of the final meat quality. While meat aging is widely recognized as a crucial step in meat processing, the underlying biochemical mechanisms are not fully understood. Recent advances in proteomics offer new opportunities for exploring the changes in muscle/meat proteins, allowing better understanding of the underlying molecular events [1,2]. However, the use of proteomics in a temporal time on aged meat is understudied. Thus, we suggest exudate meat proteome analysis as a novel approach for investigating the complex mechanisms of meat aging. Exudate is a protein-rich drip that accumulates on the surface of meat during storage. It can provide valuable insights about the protein changes occurring *post-mortem*. Exudate meat proteome analysis using label-free proteomics would allow a better understanding of the *post-mortem* dynamic changes of muscle proteins and discover meat quality biomarkers. Therefore, we aim to investigate for the first-time goat meat exudate proteome using shotgun proteomics to shed light on the biochemical mechanisms of meat aging.

II. MATERIALS AND METHODS

Eight male goats of the Saanen x *Naine de Kabylie* crossbred reared under traditional and extensive production systems in the Kabylia Mountains (North of Algeria) were used [2]. To avoid season effect, the animals were from the batch slaughtered in March, that were all slaughtered on the same day. The exudates (drip) from vacuum packaged *Longissimus thoracis* muscle cuts in sterile bags were collected at 24, 48 and 72h *post-mortem*. The drip samples were stored at -80°C for proteomics analyses. Protein extraction, quantification, and preparation of proteins bands for shotgun proteomics were performed according to Lamri *et al.* [2]. LC-MS/MS analysis on individual exudate samples at the three sampling times (n = 24) was performed by means of a nanoelectrospray ion source column coupled to Q Exactive™ HF-X hybrid Quadrupole-Orbitrap mass spectrometer. The proteome database was subjected to i) bioinformatics analysis [3] using Metascape® to identify the enriched Gene Ontology (GO) pathways, and ii) statistical analysis using partial least square discriminant analysis (PLS-DA) to evaluate the potential of the exudate proteome to discriminate the aging times.

III. RESULTS AND DISCUSSION

We successfully quantified with high confidence 823 proteins from the goat meat exudate samples. The proteins have broad molecular weights distribution (Figure 1A), with a significant number below 60 kDa. The PLS-DA allowed a clear separation of the three aging times (Figure 1B), hence evidencing the potential of meat exudate as a promising biofluid for the discovery of meat quality biomarkers. GO analysis revealed 20 enriched terms (Figure 1C,D), from which 5 were related to muscle structure (1, 2, 4, 9 & 11, see Figure 1C), followed by 7 belonging to energy metabolism and related pathways (3, 8, 10, 14, 15, 17 & 20). Other pathways were related to response to protein folding (5), proteolysis, protein catabolic process and their regulation (6, 12 & 19), response to inorganic substance and detoxification (7 & 13), positive regulation of protein localization (16) and response to wounding (18). The results clearly evidenced the potential of meat exudate proteome as a novel way to decipher the complex underlying mechanisms of meat aging. In line with the existing knowledge, the main molecular signatures characterizing meat aging from meat exudate are related to muscle structure breakdown [4].

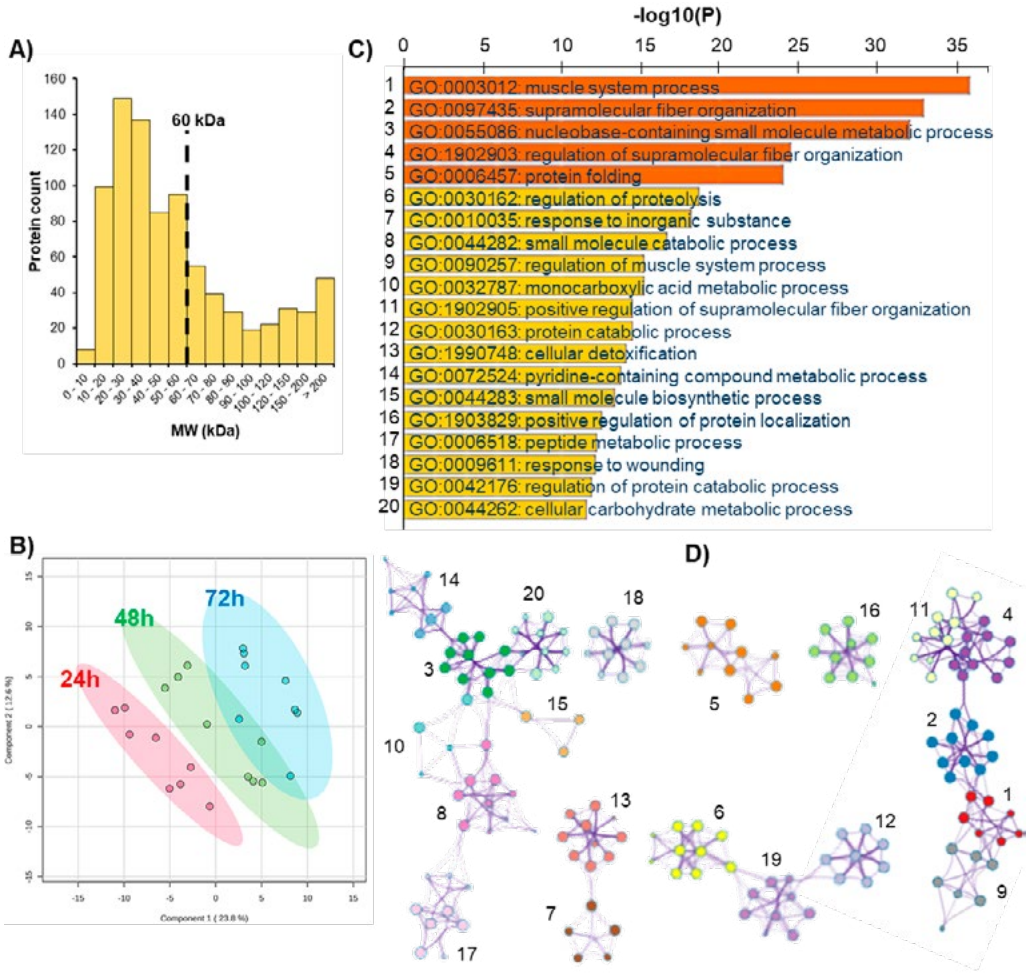

Figure 1. Summary of the results characterizing the 823 proteins quantified in the goat meat exudate samples during aging. **A)** Distribution of the proteins based on their molecular weight (MW). The black discontinuous line highlights the proteins <60 kDa. **B)** PLS-DA score plot. **C)** Significant enriched GO cluster terms. The bar graphs are coloured according to *P*-values (from high to low). **D)** Enriched ontology network where each GO term is shown with the corresponding colour. The sizes of the nodes reflect the enrichment significance of the terms.

IV. CONCLUSION

Our findings evidenced the potential of meat exudate profiling using label-free proteomics to discover biomarkers of meat quality. The next steps of this project are ii) the evaluation of the highly contributing proteins in the PLS-DA as biomarkers of goat meat quality, and ii) in-depth characterization of the protein changes over aging/storage to capture the molecular signatures at interplay. By understanding the molecular events that underlie meat aging, we aim to develop more effective strategies for optimizing meat quality, ultimately benefiting both consumers and meat industry stakeholders.

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