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SAMPLING LOCATION MATTERS: DIFFERENCES IN THE PROTEOME CHANGES AND BIOCHEMICAL PATHWAYS BETWEEN INTERIOR AND SURFACE TISSUES OF BOVINE STRIPLOINS DURING DRY-AGING

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I. INTRODUCTION

During the dry-aging process in meat, water migrates to the outer surface of the muscle and evaporates, hence leaving a drier crust on the surface of the end product. Understanding the molecular processes at play during dry-aging, both in the interior of the meat and at the surface, can contribute to optimising the quality of the dry-aged meat. While some researchers have examined, using metabolomics, the metabolic changes of the external (crust) and internal portion of dry-aged beef [1], to the best of our knowledge, there are no studies on proteomics that explored the differences in the muscle proteome between external and internal locations of dry-aged beef. Therefore, the aim of this work was to investigate the differentially abundant proteins (DAPs) between these two locations of dry-aged beef at two aging times, and explore differences in the underlying biological pathways.

II. MATERIALS AND METHODS

Longissimus thoracis et lumborum muscle (LTL, striploin) was dissected from both sides of six beef carcasses at 3 days *post-mortem* (p-m). They were subsequently divided into equal sections, and assigned to one treatment: 0 days dry-aged (i.e. 3 days p-m) and 28 days dry-aged (i.e. 31 days p-m). Dry-aging conditions were set at 2.0 °C, 75% relative humidity, and air flow of 0.5-2.0 m/s on a DRY AGER DX 1000® (DRY AGER®, Germany). Sections were cut into steaks at each time point, sub-sampled (external, internal) for downstream analysis, and stored at -80 °C for proteomics analysis. Protein extraction, quantification and, preparation and analysis of protein bands for SWATH-MS, were performed as Lamri *et al.* [2] and Chantada-Vázquez *et al.* [3]. The quantitative proteomics data were analysed by means of volcano plots (fold change > 1.5; $P \leq 0.05$) to identify the DAPs between the two locations at each aging time. Gene Ontology (GO) enrichment analysis (Metascape®) ($P \leq 0.05$, minimum overlap of 2, and enrichment factor > 1.5) was further carried out.

III. RESULTS AND DISCUSSION

At 3 days p-m, 18 DAPs were identified across the surface and interior locations (Figure 1A), from which 11 proteins were more abundant in the external and 7 were more abundant in the internal location. The bioinformatics enrichment analysis identified 5 GO terms specific for external location, from which the two most significant were "defence response to Gram-negative bacterium (GO: 0050829)" and "regulation of viral entry into host cell (GO: 0046596)". One GO term, "muscle structure development GO: 0061061" was specific for internal location. At 31 days p-m (28 days of dry-aging), 23 DAPs were identified across both locations (Figure 1C), from which 10 proteins were more abundant in the external location and 13 in the internal location. The two most significant GO terms specific for external location (Figure 1D) were "ATP metabolic process (GO: 0046034)" and "chaperone-mediated protein folding (GO: 0061077); while 2 GO terms were specific for the internal

location: “regulation of T cell proliferation (GO: 0042129)” and “extracellular matrix organisation (GO: 0030198)”. Overall, results demonstrate that the proteome profile of the external location underwent a distinct physiological evolution during dry-aging, compared to the internal location of the dry-aged beef. At 3 days p-m, the changing proteins in the external location seem to be related to biological pathways associated with response to external stimuli, which may be explained by the oxidative conditions and microbial activity to which the muscle is exposed either, during the dressing of the carcass, during the rigor onset, or later on, during the quartering and cutting. These outcomes highlight the relevance of sampling from the internal location (avoiding external surfaces) when investigating the proteome of meat for the identification of quality biomarkers. This is particularly important for dry-aging, where meat is in direct contact with the external atmosphere throughout the process. Further, oxidative conditions, dehydration and microbial activity during dry-aging may have also contributed to the differences in the proteome profiles.

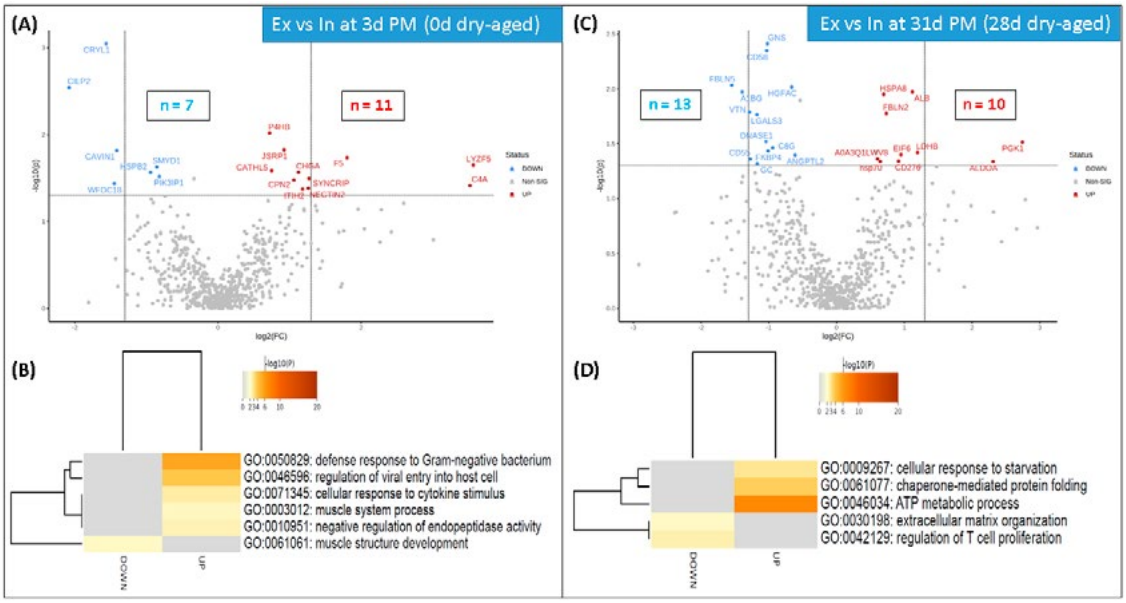

Figure 1. (A,C) Volcano plots showing the DAPs between external (crust) and internal location at 3- and 31-days p-m. (B,D) Bioinformatics heatmaps based on significantly enriched GO terms, comparing the molecular pathways at interplay.

IV. CONCLUSION

Proteomic data revealed divergent physiological evolution and distinct molecular mechanisms in the different locations of dry-aged beef. Understanding how the dry-aging atmosphere, including oxidative conditions, may contribute to this difference may help in producing high-value dry-aged meat.

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