MUSCLE PROTEOME CHANGES IN *LONGISSIMUS THORACIS ET LUMBORUM* MUSCLE AFTER 28 DAYS OF DRY-AGING

Sara Álvarez[1,2*], Mohammed Gagaoua[3], Carlos Álvarez[1], Ruth M. Hamill[1], Eileen O'Neill[2], Anne Maria Mullen[1]

1: Dept. of Food Quality and Sensory Science, Teagasc Food Research Centre Ashtown, Dublin, D15 DY05, Ireland

2: School of Food and Nutritional Sciences, University College, Cork, Western Road, Cork T12 YN60, Ireland

3: PEGASE, INRAE, Institut Agro, 35590 Saint-Gilles, France

*Corresponding author email: Sara.Alvarez@teagasc.ie

I. INTRODUCTION

Dry-aging is a *post-mortem* (p-m) intervention where meat is exposed to controlled refrigerated air conditions to produce tender and more flavoursome meat [1]. Elucidating changes occurring at the proteome level in dry-aged beef during aging would advance our knowledge on the underlying pathways and molecular processes at interplay. Therefore, the main objective of this work was to compare the proteome of an economically significant muscle before dry-aging (3 days p-m), and after dry-aging (31 days p-m), and to explore the underlying biochemical pathways. In addition, the relationships between various meat quality traits (pH, *L*, a*, b*,* Warner-Bratzler Shear Force (WBSF)) and the differentially abundant proteins (DAPs) were examined.

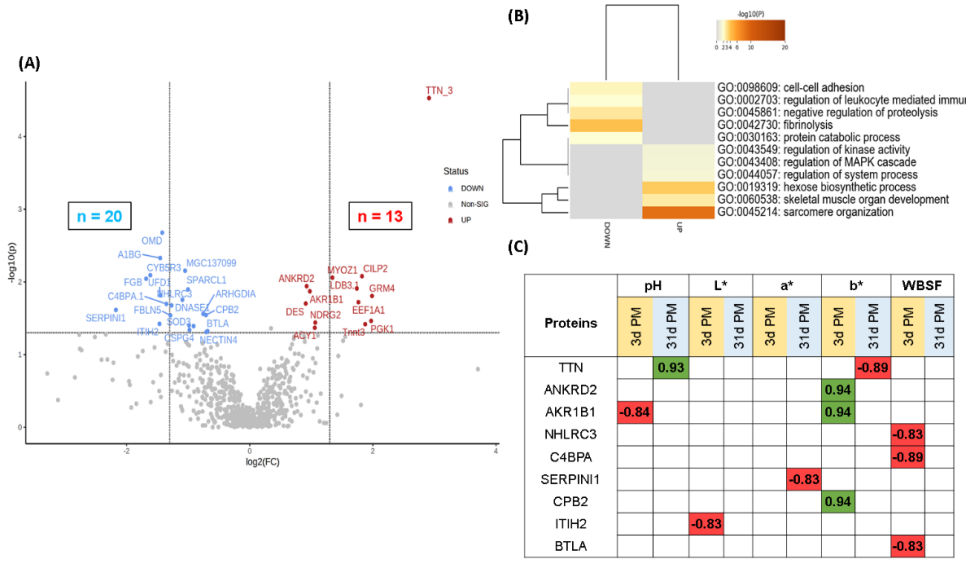
II. MATERIALS AND METHODS

Twelve *Longissimus thoracis et lumborum* (LTL) muscles, sampled from six beef carcasses, were divided into equal sections at 3 days p-m. Half of the sections were analysed immediately, and half were dry-aged until 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). Each section was cut into steaks at appropriate time points, sub-sampled for downstream analysis and stored at -80 °C (proteomics) or -20 °C (WBSF). pH and bloomed colour readings were recorded on the meat samples. Protein extraction and quantification, and preparation of proteins bands for SWATH-MS were performed according to Lamri *et al.* [2] and Chantada-Vázquez *et al.* [3], respectively. To identify the DAPs across aging days, a volcano plot analysis (fold change > 1.5; $P \leq 0.05$) was performed. Gene Ontology (GO) enrichment analysis (Metascape®) ($P \leq 0.05$, minimum overlap of 2, and enrichment factor > 1.5) was conducted on DAPs to decipher the underlying enriched pathways. Spearman correlation analysis was carried out to investigate the associations between meat quality traits (pH, *L*, a*, b**, WBSF) investigated at 3 and 31 days p-m, and the individual DAPs. Correlations were considered significant at $P \leq 0.05$.

III. RESULTS AND DISCUSSION

The comparison of pre- and post- dry-aging samples (Figure 1A) revealed 33 unique DAPs, from which 13 were more abundant at 3 days p-m, and 20 after 31 days p-m. The enrichment analysis (Figure 1B), showed that 6 and 5 GO terms were specific at 3 and 31 days p-m, respectively. The GO term "sarcomere organisation (GO: 0045214)" was the most enriched, and also, specific to 3 days p-m. Proteins like TTN (Titin), TNNT3 (Troponin T, fast skeletal muscle), and ANKRD2 (Ankyrin repeat domain-containing protein 2) were more abundant at 3 days p-m before decreasing with dry-aging, possibly due to proteolysis. Breakdown of cytoskeletal structure proteins during aging are involved in tenderness development [4]. Extent of this breakdown is influenced by rigor temperature, and the rate and extent of pH decline, which in turn, can influence colour traits due to light scattering [5]. This may partly explain the associations (Figure 1C): TTN, pH and *b*;* and ANKRD2 and *b**. "Hexose biosynthetic

process (GO: 0019319)" was the other significantly enriched term, and grouped proteins involved in catalytic, metabolism and ATP metabolic processes (AKR1B1 (Aldo-keto reductase family 1 member B1) and PGK1(Phosphoglycerate kinase 1)), which decreased with dry-aging time. AKR1B1 correlated with pH and *b*.* Energy metabolism is one of the major pathways related to colour and pH. Overall, proteins in Figure 1C can be suggested as candidate biomarkers [4] of dry-aged beef tenderisation.



Proteins	pH		L*		a*		b*		WBSF	
	3d PM	31d PM	3d PM	31d PM	3d PM	31d PM	3d PM	31d PM	3d PM	31d PM
TTN		0.93						-0.89		
ANKRD2							0.94			
AKR1B1	-0.84						0.94			
NHLRC3									-0.83	
C4BPA									-0.89	
SERPINI1					-0.83					
CPB2							0.94			
ITIH2			-0.83							
BTLA									-0.83	

Figure 1. (A) Volcano plot showing the differentially abundant proteins (DAPs) between 3 and 31 days p-m . (B) Gene Ontology (GO) heatmap comparing the significantly enriched GO terms (potential functions and molecular pathways related to the DAPs). (C) Correlation matrix (Spearman) between the quality traits at two aging times and the DAPs. Only those signficant correlations at P ≤ 0.05 are shown.

IV. CONCLUSION

Proteins related to muscle structure and metabolism pathways were affected by the dry-aging process. This trial provides a better understanding of the factors contributing to delivering the desired dry-aged product, which ultimately, can lead to identification of quality biomarkers for dry-aging optimisation.

ACKNOWLEDGEMENTS

This work was funded as part of the Strategic Partnership Agreement co-funded by INIA and the Walsh Scholarship program (2019041) supported by Teagasc - project number 0821.The authors acknowledge Dr. Susana B. Bravo for performing the Sequential Window Acquisition of all Theoretical Mass Spectra (SWATH-MS) analysis.

REFERENCES

1. Zhang, R., Yoo, M. J. Y., Ross, A. B., & Farouk, M. M. (2022). Mechanisms and strategies to tailor dry-aged meat flavour. Trends in Food Science & Technology 119: 400-411.
2. Lamri, M., della Malva, A., Djenane, D., López-Pedrouso, M., Franco, D., Albenzio, M., Lorenzo, J. M., & Gagaoua, M. (2023). Towards the discovery of goat meat quality biomarkers using label-free proteomics. Journal of Proteomics 278: 104868.
3. Chantada-Vázquez, M. d. P., García Vence, M., Serna, A., Núñez, C., & Bravo, S. B. (2021). SWATH-MS Protocols in Human Diseases. In M. Carrera & J. Mateos (Eds.), Shotgun Proteomics: Methods and Protocols (pp. 105-141). Springer US.
4. Gagaoua M., Terlouw E.M.C., Mullen A.M., Franco D., Warner R.D., Lorenzo J.M., Purslow P.P., Gerrard D., Hopkins D.L., Troy D. & Picard B. (2021). Molecular signatures of beef tenderness: Underlying mechanisms based on integromics of protein biomarkers from multi-platform proteomics studies. Meat Science 172: 108311.
5. Hughes, J., Oiseth, S., Purslow, P., & Warner, R. (2014). A structural approach to understanding the interactions between colour, water-holding capacity and tenderness. Meat Science 98: 520-532.