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SWATH-MS AS AN EMERGING TOOL FOR DARK-CUTTING BEEF BIOMARKER DISCOVERY

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I. INTRODUCTION

The appearance of quality defects such as dark, firm, and dry (DFD), known also as dark-cutting beef, causes economic losses and food waste and might lead to consumer rejection. DFD beef is characterised by high ultimate pH, unappealing dark colour, and shorter shelf-life [1]. Different pre-slaughter factors have been linked to high ultimate pH, but the underlying mechanisms explaining this quality defect are complex, multi-factorial and not fully understood. In fact, proteomics have been used to better address this quality defect and identify putative biomarkers [2]. However, most of the proteomics studies used traditional methods on very low number of samples. In this study, we applied for the first time SWATH-MS, a data-independent acquisition method, to deeply characterise and quantify the *post-mortem* (*p-m*) muscle proteome of representative DFD and normal pH beef samples from a larger database. We further aimed to decipher the underlying mechanisms and identify potential biomarkers of this quality defect.

II. MATERIALS AND METHODS

Fifty-two Asturiana de los Valles young bulls of similar characteristics (farm, transport, weight) were analysed. Half of the carcasses were classified as DFD (pH_{24}>6.2) and the rest as normal pH_{24} (5.4 to 5.6). Muscle samples were taken from the *Longissimus thoracis et lumborum* (LTL) at 24h *p-m* and stored at -80°C for proteomics analysis. Total muscle protein extracts were used to prepare the protein bands for shotgun proteomics [3], that were analysed by SWATH-MS [4]. Drip loss and colour (*L**, *a**, *b**, *C**, *h°*) quality traits were evaluated at 48h *p-m*. The muscle proteome database was analysed to detect the differentially abundant proteins (DAPs) between control and DFD samples at the significance level of 5%.

III. RESULTS AND DISCUSSION

Table 1 shows the differences in the meat quality traits evaluated, highlighting in DFD meat lower drip loss (P<0.001), and darker, brownish, and saturated colour (*L** and *a** (P<0.001), *b**(P<0.01), and *C** (P<0.001)). These results agree with previous studies that related these changes in DFD beef quality with modifications of the *p-m* muscle metabolism that affect the muscle-to-meat conversion process [1].

Table 1. Effect of pH_{24} sample type (Control vs DFD) on drip loss and colour parameters.

Variable	CONTROL (5.4 ≤ pH_{24} ≤ 5.6)	DFD (pH_{24} ≥ 6.2)	SEM	P-value
Drip loss (%)	31.0	21.9	0.73	0.000
Meat Colour				
*L**	36.2	29.4	1.05	0.000
*a**	11.0	7.5	0.46	0.000
*b**	13.3	8.1	0.87	0.003
*C**	16.8	10.1	0.85	0.000
h°	0.8	0.8	0.04	0.158

The SWATH proteomics allowed the quantification of 758 unique proteins in LTL muscle. Among them, 29 proteins were significantly different (P<0.05) in their abundance between control and DFD samples (Figure 1). The DAPs belong to 5 major and interconnected biochemical pathways: i) muscle contraction, structure and associated proteins (n=8; 28%); ii) energy metabolism (n=7; 24%); iii) binding, regulation of apoptosis and signalling (n=7; 24%); iv) calcium homeostasis (n=4; 14%); and v) proteolysis,

hydrolase and activity regulation (n=3; 10%). Only 4 DAPs, these being TTN, MYOM3, MYL3 and ALDH1A3, were previously proposed as biomarkers of DFD beef [2].

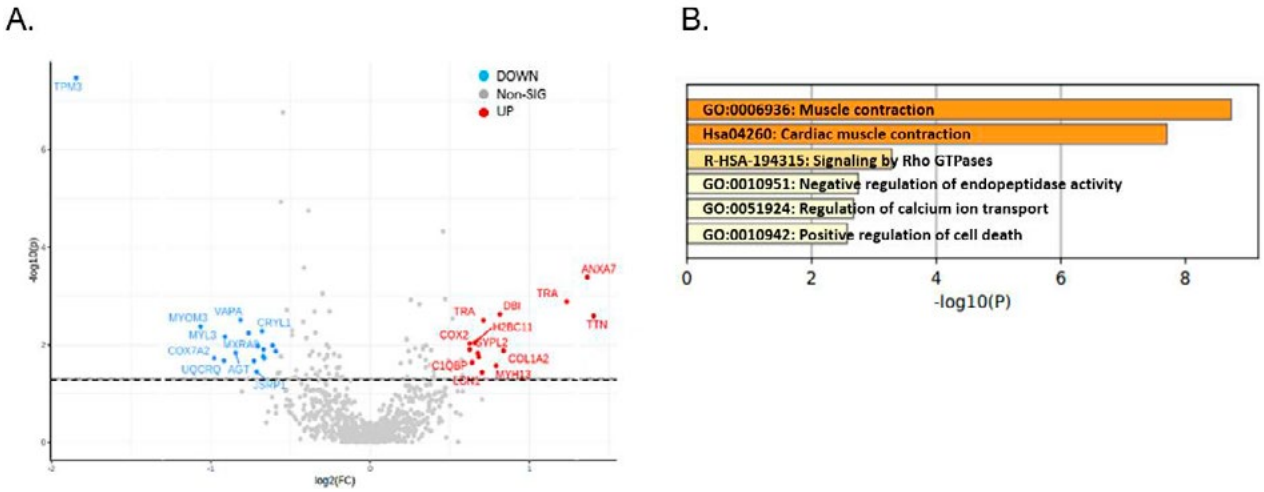

Figure 1. Statistical and bioinformatics analyses of the proteins identified to be differential between control and DFD samples. (A) Volcano plot highlighting the differentialy abundant proteins between control and DFD beef samples. Abundant proteins in DFD beef are shown in red and the abundant ones in control samples are shown in blue. (B) Significant enriched Gene Ontology (GO) terms obtained using Metascape tool.

Tropomyosin alpha-3 (TPM3), a structural protein with a key role in muscle contraction, was the protein with the highest abundance difference (P<0.000) being less abundant in DFD beef, probably due to higher *p-m* proteolysis and muscle destructuration, enhanced light-scattering, resulting in poorer final quality. The abundance of this protein at 24 h *p-m* can therefore serve as a candidate DFD beef biomarker, which is under evaluation using validation methods for its suitability.

CONCLUSION

In this study, SWATH-MS was used for the first time as a tool for the discovery of biomarkers in the largest database of DFD beef samples to date. Twenty-five novel putative protein biomarkers have been identified, which will feed the pipeline of biomarkers discovery, in the objective of their evaluation using appropriate targeted proteomics.

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