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A SECOND LOOK INTO THE UNDERLYING BIOCHEMICAL MECHANISMS OF ELECTRICALLY STIMULATED CARCASSES AS REVEALED BY PROTEOMICS: RESULTS OF AN INTEGRATIVE ANALYSIS ON CATTLE

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I. INTRODUCTION

Meat eating quality traits such as tenderness, flavor, and juiciness significantly influence beef palatability [1]. Research on meat consumption trends has revealed that beef tenderness is crucial for consumers' satisfaction, and they are willing to pay more if tender is guaranteed [1]. However, beef tenderness is variable due to multifaceted factors [2]. Several post-mortem interventions can be used to enhance beef tenderization, among which electrical stimulation (ES) has revolutionized meat processing [3]. Initially proposed to prevent cold shortening, ES accelerates proteolysis and myofibril degradation, thereby improving tenderness. However, the mechanisms behind ES were mostly described using traditional biochemistry methods, and very recently using proteomics. This paper aims to gather and reuse the published ES bovine proteomics studies to provide novel insights and a second look into the molecular pathways using integrative proteomics and in-depth bioinformatics.

II. MATERIALS AND METHODS

Through an integromics approach [4] on published ES proteomics studies, 6 papers that all applied low-voltage ES (LVES) to investigate the proteome changes triggered by stimulation in post-mortem bovine muscle have been gathered. The inclusion/exclusion criteria were based on i) proteomics on *Longissimus* muscle, ii) only proteins that were changing in response to LVES, and iii) exclusion of papers not in the frame of MeatOmics. Subsequently, we created the first compendium of *Longissimus* bovine muscle proteins that change in abundance due to LVES. The compendium was subjected to in- depth bioinformatics for i) manual annotation into pathways of the proteins using the gene ontology (GO) of UniProt KB (https://www.uniprot.org/), ii) protein-protein interactions using STRING database (https://string-db.org/), and GO enrichment analysis using Metascape® (https://metascape.org/).

III. RESULTS AND DISCUSSION

The compendium gathered 67 interconnected proteins belonging to 7 biological pathways (Figure 1A), from which 14 were consistently identified across the 6 studies. Shared molecular pathways features emerged (Figure 1B). First, in line with the purpose of ES, the most enriched GO term was "ATP metabolic process" (Figure 1C), common to 4 studies (Figure 1B). Interestingly, half of the common proteins were glycolytic enzymes and all were from the payoff phase of glycolysis (Figure 1D). Creatine kinase M-type (CKM) is the 7th and top protein that did not belong to glycolysis, but was found down-regulated in all ES studies (Figure 1D). Second, the "muscle system process" constituted the 2nd major pathway with 3 consistently identified proteins (ACTA1, MYL2 and MYLPF). The enhanced tenderization ascribed to ES was concomitant with the expression level of small heat shock proteins (CRYAB, HSPB1 and HSPB6, Figure 1D) and their enrichment across multiple studies (Figure 1E). The early post-mortem involvement of both energy metabolic pathways and cytoskeletal proteins as a result of ES may support apoptosis onset. In fact, two GO terms related to apoptosis regulation were significantly enriched (Figure 1B). Further, the results evidenced a sophisticated interplay among proteolysis, muscle structure, and responses to cellular and oxidative stress in ES muscle. As a proof- of-concept within the realm of data reuse, we demonstrated the first interconnectedness in the molecular signatures triggered by LVES. It further elucidated the intricate biochemical mechanisms underlying ES, likely amplifying apoptosis onset and its consequences on beef tenderization.

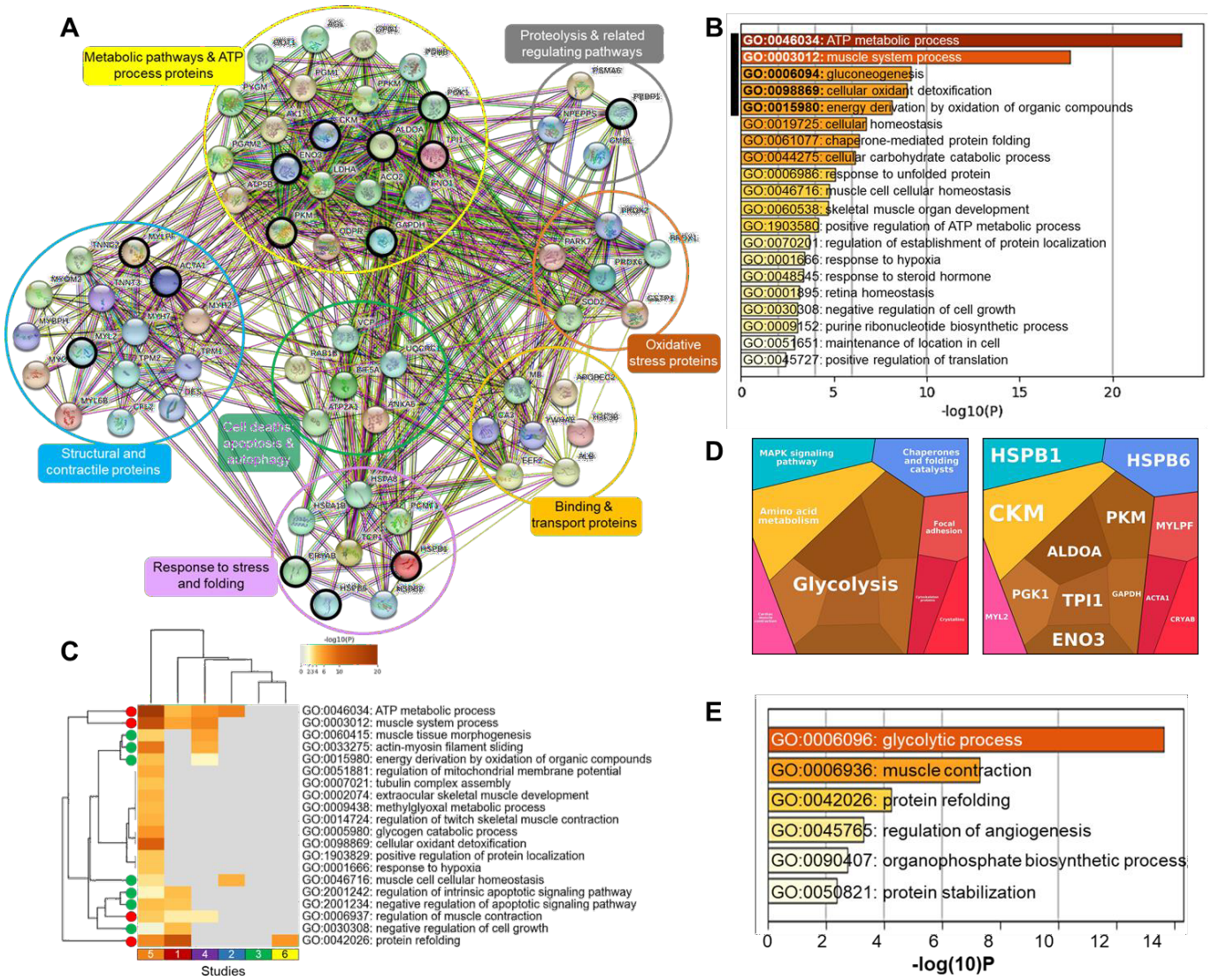

Figure 1. **A)** String protein-protein interaction (PPI) network (n=67 proteins). Proteins are clustered into 7 annotated biological pathways. Proteins (n=14) identified ≥2 times are indicated with black circles. **B)** GO enrichment analysis using Metascape®. **C)** Heatmap clustering comparing the enriched GO terms across studies. GO terms common to ≥ 3 studies are in red solid circles and those common to 2 studies are in green. **D)** Proteomaps analysis highlighting the enriched KEGG pathways and proteins consistently identified. **E)** GO enrichment based on the common 14 proteins using Metascape®.

IV. CONCLUSION

This study is the first to reveal, using in-depth bioinformatics, the molecular pathways and signatures behind LVES applied to bovine carcasses. The findings contribute to a better understanding of the complex biochemical processes involved in post-mortem muscle metabolism and their impact on meat tenderization, promoting evidence-informed strategies for optimizing meat processing techniques. The application of metabolomics and shotgun proteomics would allow decipher further mechanisms, including differences, behind ES systems and extend our understanding of the factors at interplay.

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