Improving knowledge on the fresh pork colour variations and underlying mechanisms through integrated data mining of proteomics studies

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IMPROVING KNOWLEDGE ON FRESH PORK COLOUR VARIATIONS AND UNDERLYING MECHANISMS THROUGH INTEGRATED DATA MINING OF PROTEOMICS STUDIES

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I. INTRODUCTION

Colour is a significant indicator of pork quality and consumer evaluation of the meat at purchase. Pork colour can be objectively determined using the *L**, *a**, *b** values in CIELAB colour space [1]. Although meat colour may be monitored prior to retail, its variation during ageing and in the marketplace remains inevitable, therefore a better understanding of the underlying mechanisms and identification of biomarkers would be helpful for its improvement and control. Proteomics, a high-throughput technology for the analysis of proteins, has emerged as a powerful tool for investigating the molecular mechanisms underlying meat quality traits [2]. However, the large amount of data generated by meat research proteomics can be overwhelming. The integration of proteomics datasets through data mining and bioinformatics is a promising way to better understand complex biological systems and refine the list of candidate biomarkers. In fact, data reuse is a leading, active, and evolving field, recently applied in meat research to rediscover and reshape the publicly available proteomics data to decipher the unknowns of meat quality determination [3]. Foreseeing the above, this study aimed to integrate fresh pork colour proteomics studies and the identified proteins in a unique database with the aim of providing a more holistic understanding of the biological processes that underpin pork colour determination and variation.

II. MATERIALS AND METHODS

An integrative muscle biology approach was applied as previously described [3] to gather published proteomics studies on pork colour. A computerized literature search was performed using the keywords “color or colour”, “proteom*”, “protein”, “biomarker” in combination with “meat”, or “pork” or “pig” or “muscle”. Papers published up to June 2022 were identified. The inclusion/exclusion criteria were based on i) proteomics on *Longissimus* muscle; ii) only proteins that were significantly correlated (P<0.05) with *L**, *a**, *b** colour traits; and iii) exclusion of studies on DFD (Dark, Firm and Dry) or PSE (Pale Soft and Exudative) pork quality defects. The proteins were gathered in a database that was subsequently subjected to bioinformatics [4] for protein-protein interactions using STRING database (https://string-db.org/), and for Gene Ontology (GO) enrichment using Metascape® (https://metascape.org/). In this study, proteins were identified by their gene names retrieved from UniProt (https://www.uniprot.org/).

III. RESULTS AND DISCUSSION

Seven eligible studies allowed creating the first database of 46 putative protein biomarkers of pork colour (Figure 1A), from which 38 were correlated with lightness (*L**), 17 with redness (*a**) and 13 with yellowness (*b**). Four proteins: GPD1 (Glycerol-3-phosphate dehydrogenase [NAD(+)]), PKM (pyruvate kinase), TNNT2 (Troponin T) and MYH1 (Myosin-1) were commonly correlated with *L**, *a**, *b** colour traits (Figure 1A). Among them, TNNT2 and GPD1 were identified in more than one study amongst 10 other putative biomarkers (Figure 1B). The annotation of the 46 proteins allowed their clustering into 6 interconnected biological pathways (Figure 1B), which are consistent with those previously identified for beef colour [3]. In line with an earlier integromics study on beef colour [3], the dominating first two biological pathways of pork colour were catalytic and energy metabolism (half of which are glycolytic enzymes) and contractile and muscle structure, followed by protein folding and response to oxidative stress, binding and transport proteins, signalling, apoptosis and proteolysis. The GO analysis further confirmed “carbohydrate catabolic process” and “muscle system process” as the top enriched molecular

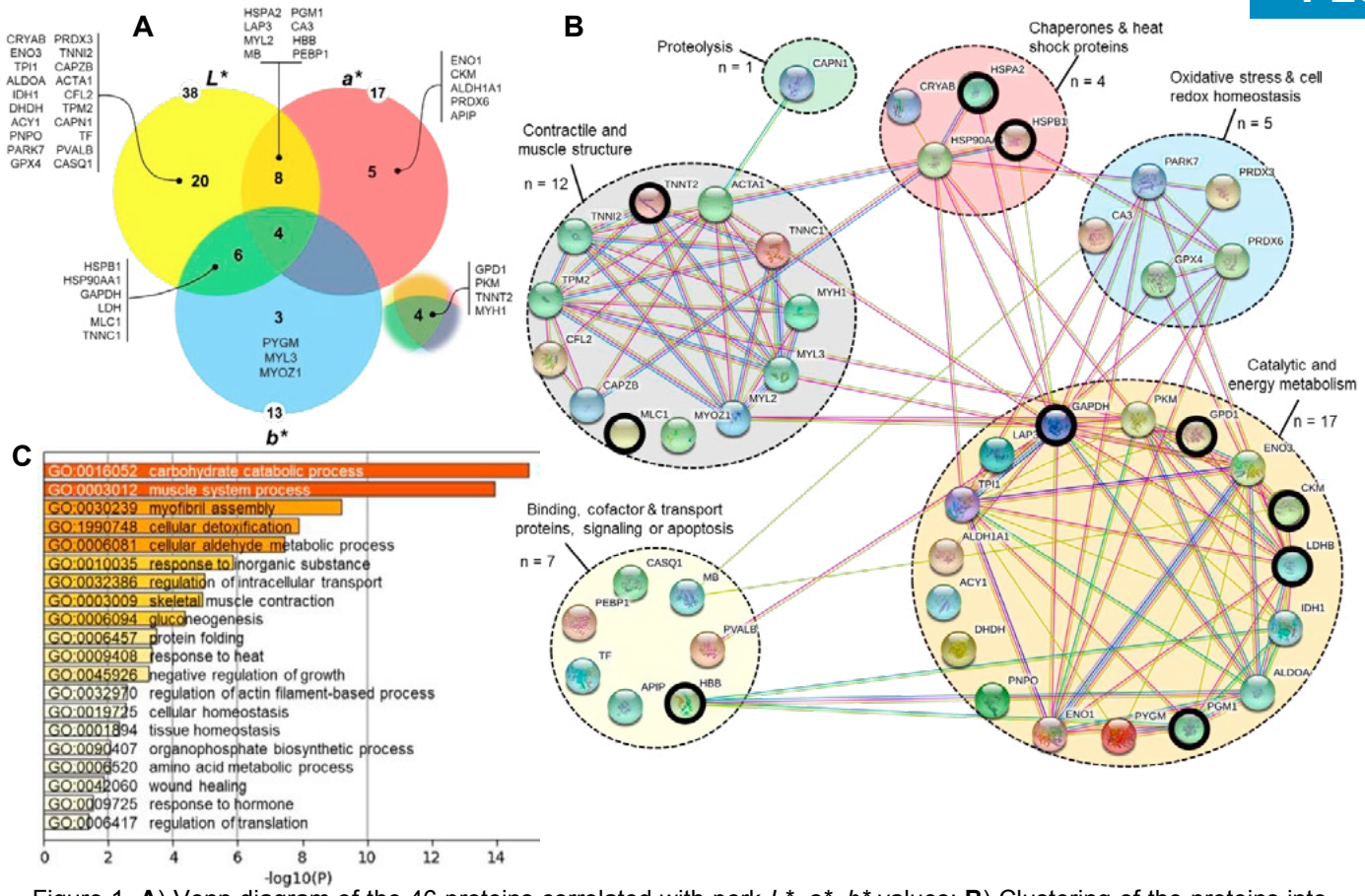

Figure 1. **A**) Venn diagram of the 46 proteins correlated with pork *L**, *a**, *b** values; **B**) Clustering of the proteins into six main biological pathways and String protein-protein interaction network; **C**) GO enrichment analysis using Metascape®. Proteins (n=10) identified >2 times from the 7 proteomics studies are indicated by black circles.

signatures underlying pork colour determination (Figure 1C). The pivotal role of muscle structure and related proteins can be ascribed to the achromatic sources of variations of meat lightness, known also to be related to variations in the microstructure and amount of light scattering [5]. Referring to the recent AMSA guidelines for measuring meat colour, it clearly stated that meat colour revolves around myoglobin [1]. Given this immense focus on myoglobin biochemistry in relation to meat colour (including pork), it is noteworthy that the myoglobin gene (MB), although included in our database, does not play a prominent role in this integromics analysis. Indeed, only one study flags MB as a biomarker of fresh pork colour. A possible inference from this is that the various factors affecting lightness of pork via the effect of post-mortem metabolism, post-mortem temperature and denaturation of muscle proteins (mostly enzymes from the energy metabolism) has a major role in pork colour variation. The higher number of proteins correlated with *L** than *a** and *b** (Figure 1A) supports such statement and asks for further investigations.

IV. CONCLUSION

Integromics studies are useful approaches allowing to gather and compare omics studies focused on meat quality performed by various platforms. This study showcased the myriad interconnected pathways driving fresh pork colour determination and proposes a list of 10 biomarkers for further evaluation.

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