Effect of intermediate ultimate pH beef over ageing time on *Longissimus lumborum* muscle proteome from grass-fed Nellore

Monique M. Krauskopf[1], Daniel S. Antonelo[2], Chimenes D. L. Araújo[1], Eduardo F. Delgado[3], Carmen J. Contreras-Castillo[1*]

[1] Department of Food Science and Technology, Luiz de Queiroz College of Agriculture, University of Sao Paulo, Piracicaba, SP, 13418-900, Brazil

[2] Lipid Marker Omics Sciences, Pirassununga, SP, 13638-407, Brazil

[3] Department of Animal Science, Luiz de Queiroz College of Agriculture, University of Sao Paulo, Piracicaba, SP, 13418-90, Brazil

*Corresponding author email: ccastill@usp.br

I. INTRODUCTION

The ultimate pH (pHu) is a crucial factor in beef quality, influenced by various factors such as animal diet, exercise, and pre-slaughter stress. These factors can lead to muscle glycogen depletion and result in abnormal pHu beef (> 5.80) [1]. In Brazil, the prevalence of beef classified as intermediate (5.8 to 6.19) or high pHu (≥ 6.2) accounts for about 40% of production, causing significant economic losses [2]. Hence, this study aims to evaluate the impact of intermediate pHu beef over ageing time on the *Longissimus lumborum* (LL) muscle proteome from grass-fed Nellore (*Bos indicus*) bulls, a topic of great importance in the field of animal science and meat quality.

II. MATERIALS AND METHODS

Three LL muscles from grass-fed Nellore bulls (30 - 35 months old) classified as intermediate pHu (pHu 5.8 to 6.19) were obtained from a commercial slaughterhouse. The muscles were divided into 2.5 cm thick steaks and assigned to vacuum-aging treatments: 1-d (72 h post-mortem) and 14-d vacuum ageing at 4°C. Samples from each ageing time were stored at −80 °C for proteomic analysis. Protein extraction was performed as described by Wiśniewski *et al.* [3]. LC-MS/MS analysis was performed on a Xevo G2-QTof mass spectrometer (Waters Corporation) coupled directly to the chromatographic system. Differentially abundant proteins (DAPs) over ageing time were defined through volcano plot analysis (fold change ≥ 1.2; $P \leq 0.05$). Enriched Gene Ontology terms and pathways were investigated using the open-source tool Metascape® ($P \leq 0.05$, minimum overlap of 3, and enrichment factor > 1.5).

III. RESULTS AND DISCUSSION

PCA discriminated beef at 1-d and 14-d ageing (Fig. 1A). Volcano plot analysis (Figure 1B) revealed 26 DAPs between ageing times comparison, of which 12 were overabundant at 1-d ageing, and 14 were overabundant at 14-d ageing. Enrichment analysis revealed ten enriched terms (Figure 1C), most of which were related to energy metabolism and muscle structure. Succinate-CoA ligase subunit alpha (SUCLG1), NADH dehydrogenase ubiquinone flavoprotein 2 (NDUFV2), ubiquinone biosynthesis monooxygenase COQ6 (COQ6) and NADH dehydrogenase ubiquinone one subunit C2 (NDUFC2) were abundant at 1-d ageing indicating increased oxidative metabolism, as also observed by Zhai et al. [4]. These results suggest increased oxidative stress in meat with intermediate pH, resulting in reduced proteolysis and degradation of structural proteins. The overabundance of candidate biomarkers for tenderness, such as aldehyde dehydrogenase (ALDH2), myozenin-1 (MYOZ1), malate dehydrogenase, mitochondrial (MDH2), troponin T (TNNT1) and under abundance of heat shock protein HSPA5 observed in intermediate pHu beef at 14-d ageing compared to 1-d ageing are indicative of a delay in tenderisation, which partially explain the toughness of intermediate pHu beef, as reported by [1, 5].

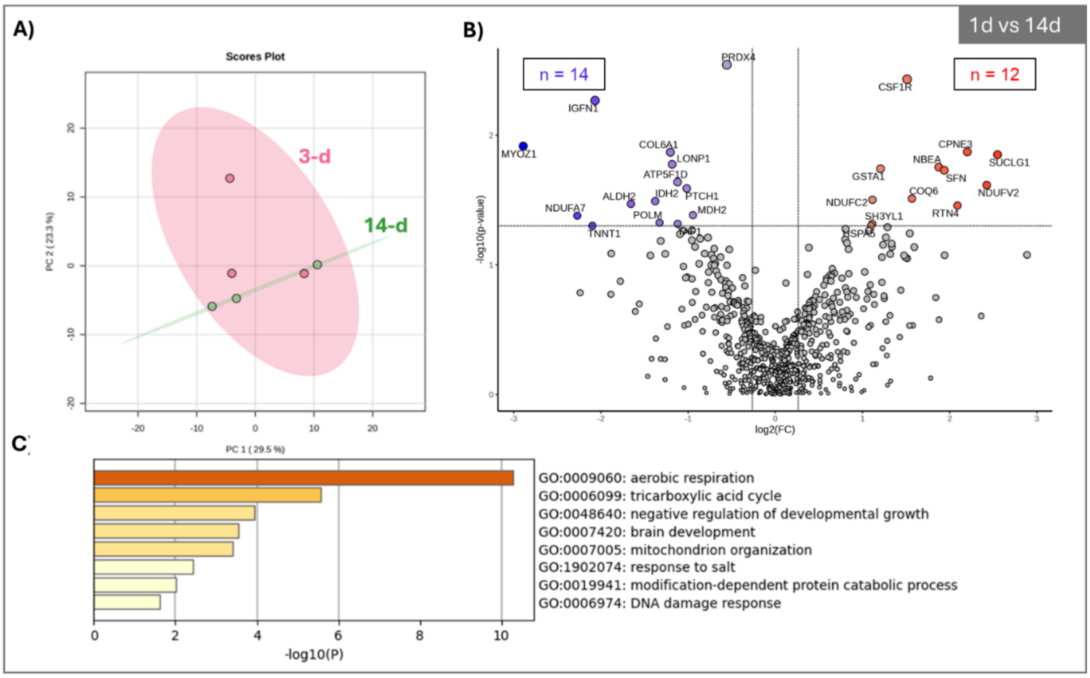

Figure 1. A) Principal Component Analysis (PCA); B) Volcano plot showing DAPs between 3 and 14 days of ageing; C) Bar chart of significantly enriched GO cluster terms according to *P*-values ($P \leq 0.05$).

IV. CONCLUSION

The main proteomic changes of intermediate pHu beef over aging time are related to energy metabolism and muscle structure, revealing that some proteins are essential for the main meat quality attributes, such as beef colour and tenderness.

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