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MITOCHONDRIAL LIPID ALTERATIONS IN BEEF WITH DIFFERENT ULTIMATE pH: IMPLICATIONS FOR TENDERNESS DURING AGING

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I. INTRODUCTION

Beef tenderness is a crucial quality attribute for consumer acceptance and is influenced by several factors, including the ultimate pH (pHu). One of the main causes of variations in post-mortem pH decline is the reduction in muscle glycogen reserves, which results in beef with an intermediate pHu ($5.8 < pHu \leq 6.2$). This condition leads to reduced tenderness and necessitates a longer aging period to achieve the desired tenderness [1]. During aging, changes in mitochondrial lipid molecules affect mitochondrial apoptosis and the proteolytic activity of myofibrillar proteins, thereby modulating the tenderization process [2]. However, the impact of aging time on the mitochondrial lipidomic profile of the *longissimus lumborum* (LL) muscle in beef with intermediate pHu aged for 14 d remains poorly understood. Therefore, this study aimed to compare the mitochondrial lipid profile of LL muscle in beef with normal and intermediate ultimate pH aged for 14 d.

II. MATERIALS AND METHODS

Longissimus lumborum (LL) muscles from six grass-fed Nellore bulls (*Bos indicus*), aged 30–35 months, were obtained from a commercial slaughterhouse. Muscles were classified based on pHu into normal (5.40–5.79; n = 3) and intermediate ($5.8 < pHu \leq 6.2$; n = 3) groups. A 2.5 cm-thick LL steaks were sampled and aged under vacuum for 14 d at 4 °C. pH and Warner-Bratzler shear force (WBSF) measurements were performed. Muscle mitochondria were isolated by differential centrifugation and used for lipid extraction. Lipidomic analysis was conducted by ultra-Performance Liquid Chromatography–High-Resolution Mass Spectrometry (UPLC-HRMS). UPLC-HRMS raw spectra processing, including peak detection, peak alignment and peak annotation were performed using Metaboanalyst 6.0, which was also used for lipidome data analysis. Volcano plot analysis (fold change ≥ 2.0 and $P \leq 0.05$) and quantitative enrichment analysis (using relational database of metabolomic pathways [RaMP-DB]) were perfomed. pH and WBSF data were analyzed using a completely randomized design, in which a variance analysis (ANOVA) at a level of 5% (Tukey post-hoc comparison) was performed.

III. RESULTS AND DISCUSSION

Steaks with intermediate pHu exhibited higher final pH (5.96 ± 0.033 vs. 5.49 ± 0.033; $P < 0.001$) and WBSF (88.97 ± 1.89 N vs. 51.04 ± 1.89 N; $P = 0.013$) compared to those with normal pHu, respectively. The untargeted lipidome profiling initially detected 6,848 (including 5,434 positive ions and 1,414 negative ions) features in the isolated mitochondria samples. From those, 1,492 (1,302 in positive mode and 190 in negative mode) matched compounds based on HMDB were annotated and then considered for further statistical and bioinformatic analyses. Volcano plot analysis (Figure 1A) revealed 26 differentially abundant lipids between normal and intermediate pHu beef, in which 1,11-Undecanedicarboxylic acid, 3-Dehydrocarnitine, alpha-Tocopherol, CL(16:0/16:0/16:0/18:1(11Z)), PC(14:0/P-18:1(11Z)), Cer(d18:2(4E,14Z)/16:0), SM(d18:1/20:1(11Z)) were upregulated in intermediate beef pHu, while MG(0:0/18:3(6Z,9Z,12Z)/0:0), 9-Hexadecenoylcarnitine, DG(8:0/10:0/0:0), LysoPC(24:0/0:0), PC(15:0/22:5(4Z,7Z,10Z,13Z,16Z)), PE(P-18:0/20:4(5Z,8Z,11Z,14Z)) and PG(a-13:0/i-14:0) were upregulated in normal beef pHu. Quantitative enrichment analysis identified 18 significantly enriched pathways (Figure 1B). Specifically, an upregulation of lysophosphatydilcholine (LysoPC(24:0/0:0)) was observed in beef with normal pHu, which may suggest enhanced activity of phospholipase A2 (PLA2). This enzyme hydrolyzes phosphatidylcholine to generate LysoPC, which may lead to phospholipid membrane inversion and increased membrane permeability to calcium ions (Ca^{2+}). Elevated Ca^{2+} levels facilitate μ-calpain activation, a key enzyme in the degradation of myofibrillar proteins, thus improving beef tenderness [3]. Additionally, the enrichment of pathways associated with palmitoleic acid and PE(P-18:0/20:4) in beef with normal pH were involved in the response to oxidative stress and maintenance of mitochondrial function, which may promote the activation of apoptotic proteases. In contrast,

increased levels of cardiolipin (CL(16:0/16:0/16:0/18:1(11Z)), ceramides (Cer(d18:2(4E,14Z)/16:0)) and vitamin E compounds such as alpha-tocopherol and 13'-Carboxy-alpha-tocopherol were observed in beef with intermediate pHu. CL is particularly susceptible to oxidation at pH near 6, which may impair mitochondrial function and delay the activation of apoptosis-related proteases, potentially reducing the effectiveness of the tenderization process [4]. Moreover, the accumulation of Cer also destabilizes membranes and interferes with cell death pathways [5]. The upregulation of these antioxidants may suppress lipid oxidation and ferroptosis, contributing to the enrichment of pathways related to fat-soluble vitamin metabolism and the selenium micronutrient network. These effects may reduce proteolysis and help explain the greater toughness observed in intermediate pHu beef.

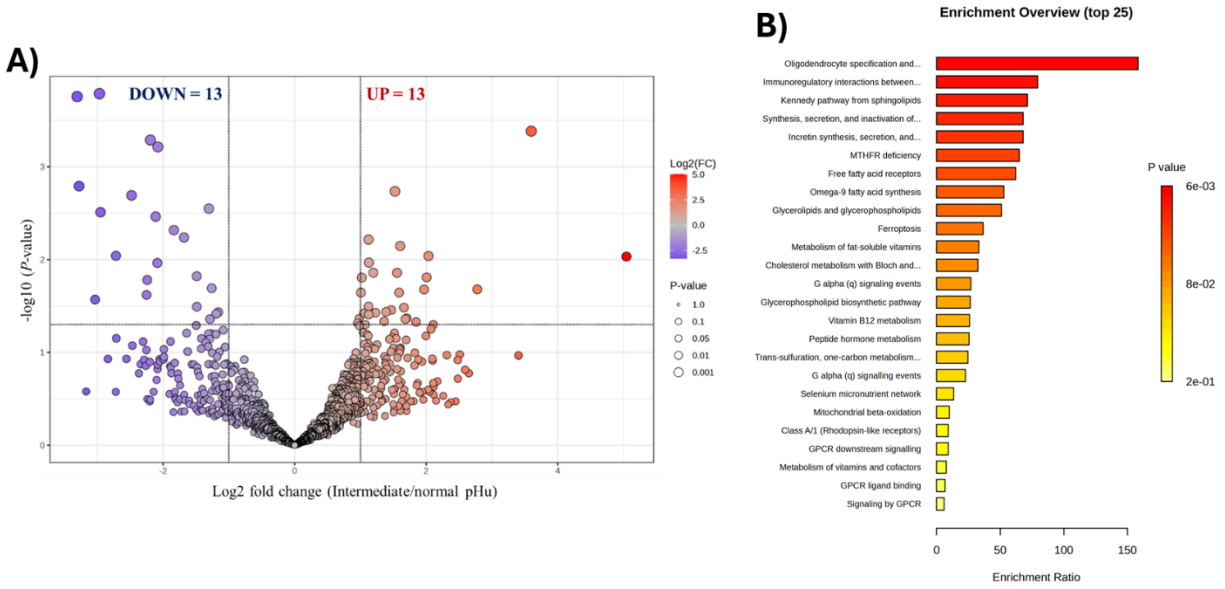


Figure 1. Volcano plot (A) showing 26 differentially abundant lipids between normal and intermediate pHu beef and the top 25 enriched pathways (B) identified in the quantitative enrichment analysis by using the differentially abundant compounds.

IV. CONCLUSION

Beef with intermediate pHu exhibited greater toughness after 14 days of aging, whch was associated with mitochondrial dysfunction. The upregulation of cardiolipin and ceramides suggests impaired apoptotic activity, , while increased LysoPC production via phospholipase activity at normal pHu may promote protease activation. These findings indicate that mitochondrial lipid remodeling plays a critical role in regulating beef tenderization during post-mortem aging.

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