Impact of visual dark-cutting severity and aging on the metabolomic profile of beef *longissimus lumborum*

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I. INTRODUCTION

Meat color profoundly influences consumers' decisions when purchasing beef products, serving as an indicator of freshness and quality [1]. However, persistence of myoglobin in the purple deoxymyoglobin state in dark-cutting beef presents significant challenges for the global meat industry due to its muscle darkening effect. The incidence of dark-cutting beef in the U.S. stands at 1.7% [2], while Canada and Australia have reported incidences at 2-2.5% [3] and 2.8% [4]. Limited studies have evaluated dark-cutting beef based on the severity of muscle darkening and the subsequent impact on color and metabolite profile throughout aging. We hypothesize that increasing dark-cutting severity will impact the beef metabolome. In addition, aging time will differently affect metabolite profiles. Therefore, the objective was to assess how varying degrees of dark-cutting severity influence the metabolic profile of beef *longissimus lumborum* at three different aging periods.

II. MATERIALS AND METHODS

At the time of grading, beef carcasses (n = 8/treatment) were selected from a commercial beef packing plant based on the visual degree of dark-cutting. These carcasses originated from *bos taurus* steers of primarily Angus genetics. These animals were sourced from commercial U.S. feedlots fed with a high-concentrate grain diet during the finishing period. After fabrication, beef strip loins (boneless, *longissimus lumborum*) were collected to represent a normal cherry-red colored control, one-half dark, and full dark-cutting. Loins were vacuum packaged and stored at 4 ± 2°C for further analysis. One 2.54 cm steak from the anterior end of each loin was cut at 48-60 h postmortem for metabolomics profiling, pH measurement, and bloom color analysis. The remaining portion of each loin was halved and assigned to either 21 or 39 d of wet aging in vacuum bags in dark storage at 4 ± 2°C. Following aging, one 2.54 cm steak was cut from the anterior end of each half loin for metabolomics and bloom color analysis. For bloom color analysis, steaks were horizontally bisected to expose the interior surface of the steak with one side allowed to bloom for 1 h at 4 ± 2°C. After 1 h of bloom, the color was evaluated using a HunterLab MiniScan EZ spectrophotometer. The remaining portion of each steak was used for quantitative metabolomics profiling via untargeted gas-chromatography-mass-spectrometry. Bloom and muscle pH were analyzed using the Glimmix procedure of SAS. Metaboanalyst 6.0 was used to analyze metabolite data. Significance was determined at an alpha value of 0.05 for all analyses.

III. RESULTS AND DISCUSSION

Full dark-cutting steaks had a greater ($P < 0.05$) pH early postmortem than normal and half dark-cutter steaks (pH: 5.51<5.91<6.39). Moreover, full dark-cutting steaks were darker in color and had lower bloom values early postmortem than full dark-cutting steaks from d 21 and 39 of aging. A total of 122 metabolites were observed across all treatment groups and the principal component analysis revealed distinct clusters in metabolite profiles between treatments. A reduction in differentially abundant metabolites was observed with aging time. Pairwise comparisons of the differentially abundant metabolites showed that half dark-cutting steaks exhibited the least changes compared with normal-beef, with 30, 7, and 7 differentially abundant metabolites on aging d 0, 21, and 39, respectively, while full dark-cutting steaks showed 56, 32, and 29 metabolites. Correspondingly, full dark-cutting steaks had a change in L^* value of 2.15% from d 0 to 39, while half dark-cutting steaks had a 6.15% change in L^* value indicating the potential role of metabolites on dark color. Metabolites differentially less abundant in full and half dark-cutting steaks on d 0 of aging were enriched for carbohydrate metabolism, while over-abundant metabolites were enriched in compensatory amino acid and nucleotide metabolism. By d 21 of aging, the differentially abundant metabolites in half steaks were all downregulated and involved in carbohydrate metabolism. Additionally, 50% of the differential metabolites observed on d 21 and 39 of aging in full dark-cutting stakes were enriched in amino acid metabolism, specifically alanine, aspartate, and glutamate metabolism, suggesting enhanced aging induced proteolysis in full dark compared with half dark steaks.

IV. CONCLUSION

In this study we show that varying degrees of dark-cutting severity in beef prompt distinct aging-related metabolite profiles compared to normal beef. Subtle changes in metabolites are noted at lower levels of muscle darkening and intensify at extreme levels. The observed differences in metabolite profiles throughout the aging periods relate to changes from carbohydrate metabolism towards compensatory energy pathways such as amino acid and nucleic acid metabolism. Moreover, the over-abundance of amino acid metabolites, particularly associated with alanine, aspartate, and glutamine metabolism in full dark-cutting beef at 21 and 39 d of aging, signifies enhanced muscle turn-over, emphasizing the impact of aging on muscle color.

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