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METABOLOME DEVELOPMENT OF RAW AND COOKED GLUTEUS MEDIUS MUSCLE AT EXTENDED AGING TIMES

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I. INTRODUCTION

Beef flavor is a matrix of confounding factors and intricate interactions between the chemical composition of tissues, post-mortem enzymatic activity, muscle location and type, and Maillard reaction products created while cooking. Aside from tenderness, flavor is also a critical component to a consumer's overall eating experience and ultimately their repurchasing decisions [1]. Aging beyond 14 days significantly impacts flavor development as it allows for oxidation to occur and for calpains and cathepsins to breakdown structural proteins and release flavor precursors [2] that will further develop during the Maillard reaction with the application of heat. Metabolomic analysis can identify sugars, amino acids, ketones, aldehydes, nucleotides, and other metabolic compounds produced within biochemical pathways within muscle tissue that change throughout aging. Further analysis allows these flavor precursors to be tied to sensory attributes such as umami, sweet, and savory found in aged meat [3]. When considering the locomotive gluteus medius muscle, its mitochondrial density, muscle fiber type, and energy metabolism is specific to that muscle, which will make an impact on its overall beef flavor. Profiling muscles individually should allow for development of a targeted aging strategy to better market cuts and increase overall consumer eating experience, which will be critical for the beef industry. Therefore, the objective of this study was to define the metabolic profile of the gluteus medius muscle, in both a raw and cooked state, over eight aging periods ranging from 14 to 70 days, with the goal of correlating the metabolic profile to beef flavor attributes when considering aging time.

II. MATERIALS AND METHODS

Eighty UDSA Average Choice (Modest 500-599) carcasses were randomly assigned an aging group. Paired gluteus medius muscles, still intact with the subprimals (IMPS #184, NAMP 2014) from USDA Average Choice carcasses were aged in a dark, 2.2°C cooler for their assigned aging times of 14, 21, 28, 35, 42, 49, 56, 63, or 70 d postmortem. The gluteus medius was isolated and was further sliced into 2.54-cm-thick steaks. Steaks were stored at -20°C in vacuum-packaged bags until needed. Each steak was trimmed of connective tissue and fat, snap frozen in liquid nitrogen, powdered in a blender, and stored at -80°C in labeled bags until needed. For the collection of cooked samples, steaks were thawed in refrigeration for up to 24 hours and cooked on a flat-top grill set to 190.5°C until 35°C, then flipped and cooked until a final internal temperature of 71°C when they were then removed, trimmed of external fat and connective tissue and sectioned. Samples for High Performance Liquid Chromatography-quadruple Time-of-Flight (HPLC-qTOF) analysis were extracted using chloroform and methanol [4]. The metabolite fraction was isolated and dried under nitrogen and stored in -80°C until ready to be run in the HPLC. Each of the stored samples were reconstituted using 1mL of a 95% water, %5 acetonitrile, 0.1% formic acid solution, two 450µL fractions of each reconstituted sample was placed in labeled vials with screw caps with septa while 40µL went into a quality control vial. Acquired data were integrated, log10-transformed, and then filtered for those present in at least 75% of samples in at least one treatment, with a coefficient of variation within 25%, normality with Shapiro-Wilk cutoff of 0.1, and analysis of variance ($P < 0.05$). Filtered data were then summarized using a heatmap to visualize abundance and principal component analysis to determine the relative relationship among treatments.

III. RESULTS AND DISCUSSION

For the gluteus medias raw samples, 142 metabolites and small molecules were detected, and for the cooked samples 75 metabolites and small molecules were detected after being sorted for their relevance. The relative abundance, regardless of whether the samples were cooked or raw, increased over the aging times. The 28-, 35-, and 42-day aging periods of the raw samples showed a slow growth in abundance of metabolites, and then from 49 days of age to 70 days there was a steady abundance of most metabolites. The cooked samples reacted similarly,

but there was only moderate abundance in only a fourth of the metabolites at 14-, 28-, and 35-day aging periods, and a more gradual increase in abundance occurred from the 42, 49, 56, 63, and 70 days of aging. The raw PCA data segregated the 14 days of age into its own quadrant, the 28, 35, and 42 into another, and finally any treatment group of 49 days of age or more into the upper left quadrant. The cooked data responded slightly differently, as 14 days of age was still alone but in the upper left quadrant, 28, 35, 42, and 49, were found clustered in the lower left quadrant, and finally the 56, 63, and 70 days groups clustered in the lower right quadrant.

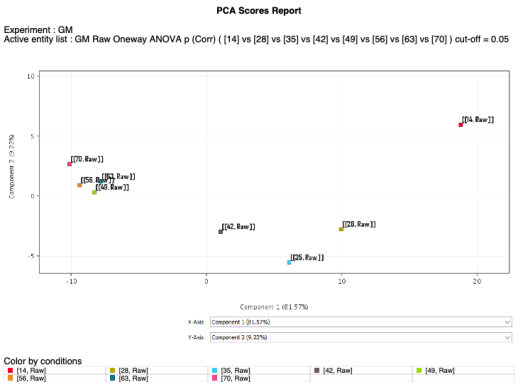


Figure 1. GM Raw PCA.

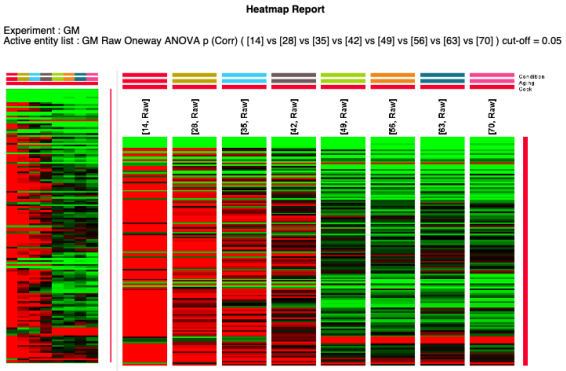


Figure 2. GM Raw Heatmap.

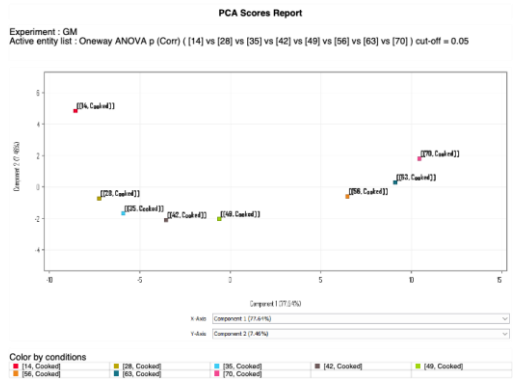


Figure 3. GM Cooked PCA.

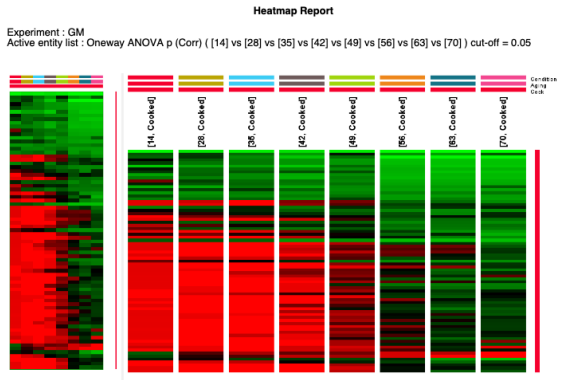


Figure 4. GM Cooked Heatmap.

IV. CONCLUSION

As the aging period was extended, significant differences in metabolite abundance occurred. As seen on the heatmaps, the metabolic profile becomes drastically different when comparing 14 days of age to 70. There was a significant increase in abundance of metabolites and small molecules in both the raw and cooked gluteus medius samples, and these compounds play a large role in the overall flavor of the muscle. This study suggests that the metabolic profiling of the gluteus medias is crucial to understanding the role aging plays in beef flavor development, and this further suggests that the use of metabolomics could be a revolutionary tool the beef industry in developing appropriate aging protocols for individual cut in order to ensure the highest consumer satisfaction.

ACKNOWLEDGEMENTS

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