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NMR-BASED METABOLOMICS ANALYSIS OF BEEF EXUDATES FROM DIFFERENT BEEF MUSCLES DURING AGING

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I. INTRODUCTION

Exudate, which is naturally released from muscle tissue during postmortem aging, has traditionally been considered a waste by-product. However, recent studies have reported that this fluid can be a biologically informative source rich in water-soluble metabolites and proteins that reflect the biochemical status of the muscle [1]. In fact, the components of exudate originate from ongoing metabolic pathways, enzymatic activities, and redox reactions within the muscle tissue, making it a promising target for indirectly tracking intrinsic mechanisms underlying meat quality changes [2]. Postmortem aging is a critical process for improving meat tenderness and flavor, but its effects vary substantially depending on muscle type [3]. These differences are attributed not only to muscle-specific biochemical compositions such as myoglobin content, antioxidant enzyme activity, and mitochondrial function but also to variations in the activity of proteolytic enzyme systems such as calpains and caspases [3,4]. Consequently, the quality characteristics observed after aging differ across muscle types, particularly in terms of discoloration and oxidative stability during retail display [4,5]. Based on this background, the present study aimed to analyze exudates released during aging as an alternative analytical resource, by comparing the metabolite composition of exudates derived from different bovine muscles. In particular, nuclear magnetic resonance (NMR)-based metabolomics was employed to track changes in metabolite profiles during aging and to assess whether exudate-derived information could reveal muscle-specific aging responses. This study is expected to offer insight into the potential of exudate as a non-invasive indicator for evaluating aging-related meat quality characteristics.

II. MATERIALS AND METHODS

Pairs of five muscles including *longissimus lumborum* (LL), *gluteus medius* (GM), *biceps femoris* (BF), *semitendinosus* (ST) and *infraspinatus* (IF) were obtained at 1d postmortem from sixteen beef carcasses (USDA Choice). Muscles were divided into four equal sections, vacuum packaged, and randomly assigned to one of four aging times (2, 21, 42, and 63 days). Upon completion of each assigned aging time, the exudate from muscle section was collected and utilized for further analysis. A 50 µL aliquot of each exudate sample was transferred to a corresponding NMR tube and diluted with an additional 550 µL of deuterated water (D2O). All samples were the analyzed using a Bruker Avance III™ HD 600 MHz NMR instrument. A modified standard 1H pulse sequence was used for data collection with 64 scans repetition for each sample. Data pre-processing was performed in NMRProcFlow v1.4. All exudate spectra were baseline corrected, phase corrected, alignment adjusted, and calibrated according to the internal standard used, TSP. Finally, the spectra were divided into “bins” according to the Adaptive, Intelligent Binning method [6]. This process attempts to collect resonances (peaks) together across all samples such that the same resonance is as equally represented in every spectrum as possible. The resulting feature tables were then imported into MetaboAnalyst 6.0 for further processing.

III. RESULTS AND DISCUSSION

A total of 645 bins were detected from NMR spectra. Based on the bins, partial least square-discriminant analysis (PLS-DA) plot was clearly divided with four groups by aging time (Fig. 1A). In the PLS-DA plot for muscle effect, *M. infraspinatus* and *M. longissimus lumborum* were classified into different groups (Fig. 1B). The top 30 features from aging period and muscle type effect was selected based on the variable importance in projection (VIP) score of more than 1.7. The top 30 features showed notable abundance from 2 to 63 days of aging (Fig. 2A). Metabolites with high VIP scores for muscle type varied in abundance across muscles (Fig. 2B). Notably, several compounds exhibited opposite trends between LL and IF, with some highly abundant in LL but present at low levels in IF. While the precise identification of these features is still in progress, their differential abundance patterns suggest biological relevance related to aging period and muscle type. These exudate-derived features may serve as useful biomarkers for distinguishing aging periods and muscle types.

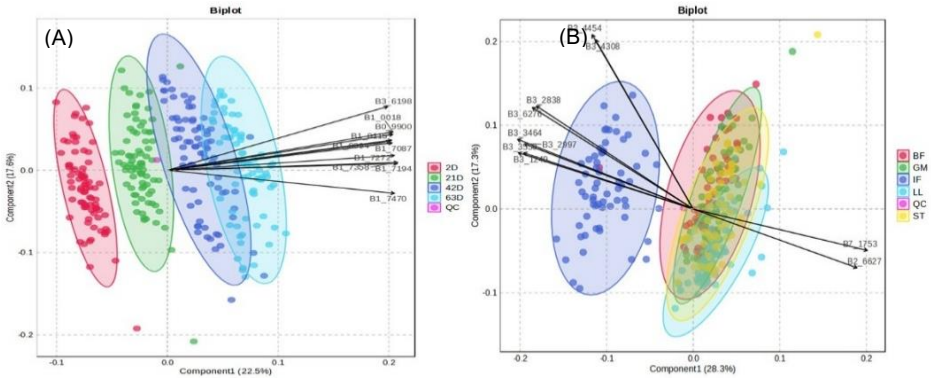


Fig. 1. PLS-DA plot of NMR-based metabolomics profile for exudate. (A) and (B) images presented by aging period and muscle type effect on metabolites, respectively.

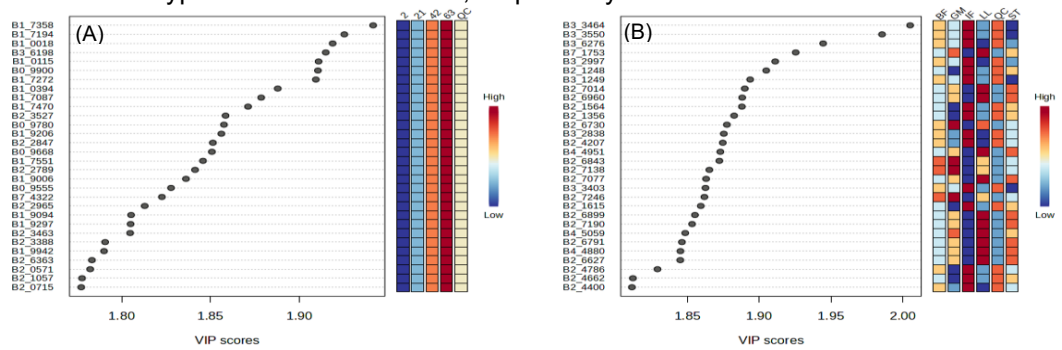


Fig. 2. VIP score plot of NMR-based metabolomics profile for exudate. (A) and (B) images presented by aging period and muscle type effect on metabolites, respectively.

IV. CONCLUSION

The NMR-based metabolome spectra detected in the exudate exhibited clear clustering patterns according to aging period and muscle type. These results highlight the potential of exudate as a practical analytical medium for monitoring metabolic changes associated with meat quality. Further identification of the candidate bins is currently underway to determine their chemical identities, which will contribute to the selection of key metabolites consistently present in both exudate and muscle tissues. Ultimately, these biomarkers may be applied for the targeted evaluation of specific meat quality traits.

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