DYNAMICS OF METABOLITES AND FREE FATTY ACIDS IN BEEF DURING COLD STORAGE

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I. INTRODUCTION

Wagyu beef is characterized by excellent marbling from intramuscular fat and a sweet aroma produced when cooked [1]. In addition, the lipids in Wagyu beef contain high levels of oleic acid, a monounsaturated fatty acid that prevents lipotoxicity [2]. Wagyu beef, rich in oleic acid, is attracting attention as a healthier option for beef production. Metabolomics analysis can provide a comprehensive analysis of metabolite profiles using mass spectrometry. The development of this technique has greatly advanced our understanding of metabolites closely related to meat aroma, texture, marbling, and color [3]. This study uses omics analysis, including metabolomics, to examine changes in metabolites and lipids in beef during cold storage and propose an optimal aging period.

II. MATERIALS AND METHODS

1. Beef Samples and Wet Aging Conditions

For our study, we obtained commercially available loin (Longissimus thoracis) and Round (adductor muscle) from Japanese Black Wagyu, Holstein-Friesian, and crossbred Wagyu (F1; Japanese Black and Holstein-Friesian) cattle. These samples were then subjected to cold storage, with the loin and round muscles divided into 5 equal portions and vacuum-packed in oxygen barrier film (Varialon S, Asahi Kasei Corporation, Tokyo, Japan). The samples were frozen immediately after packaging on Day 0, and the remaining portions were stored in a refrigerator for 10, 20, 30, and 40 days. We divided the right side for omics analysis and the left for sensory evaluation and analysis of various nutritional components. Our findings from this meticulous process will contribute significantly to the understanding of beef quality control during cold storage.

2. Metabolomics Analysis

Metabolomics analysis for various amino acids and organic acids in beef was performed by GC/MS analysis using Shimadzu GCMS-QP2010 Ultra and a DB-5 capillary column. For sample processing, frozen beef samples (1 g) were ground, and homogenates were centrifuged with a solvent containing sinapic acid. The supernatant was vacuum-dried, derivatized, and subjected to GC/MS.
The peaks obtained were analyzed using GC/MS Metabolite Database v.2 [4].

For lipid analysis, Lipids were extracted from a 1 mg Bligh and Dyer variant sample using nitrogen gas drying and methanol redissolution, followed by LC-MS/MS analysis. The analysis was performed on a DIONEX UtiMate 3000 system with an L-column3 C18 column and Q Exactive Plus detector. HPLC utilized solvents with ammonium formate and ammonium hydroxide, maintaining a column temperature of 40°C, 10 µL injection volume, and 0.1 mL/min flow rate. MS settings included Full MS/dd-MS2 mode, scanning from 200 to 1800 m/z. Various internal standards were used for different lipid classes. TG molecular species were identified using Lipid Search software [5].

3. Free Fatty Acids analysis

Total lipids from 5 g of ground beef were extracted using methanol: chloroform (1:1) with heneicosanoic acid as an internal standard. FFAs were isolated, methyl esterified, and dehydrated with sodium sulfate. Samples were analyzed by GC-FID using a Shimadzu GC-2010 plus with an SP-2560 column [6].

III. RESULTS AND DISCUSSION

This study examined the effects of wet aging on several types of beef (Japanese Black Wagyu, Holstein-Friesian, and crossbred Wagyu) during cold storage. Loin and Round were stored in a refrigerator for up to 40 days. Sensory evaluation showed that wet aging enhanced umami, richness, and continuity of taste over time, with minimal changes in saltiness, sweetness, and acidity. GC/MS metabolomics analysis identified significant changes in metabolites such as amino acids, organic acids, and nucleotides. Principal component analysis classified metabolites into early, middle, and late stages of storage, and a heat map highlighted temporal changes in metabolite concentrations. Amino acids like leucine, tyrosine, and citric acid cycle metabolites increased, while creatinine and glucosamine 6-phosphate decreased. Lipid analysis revealed stable overall lipid composition but significant increases in free fatty acids during cold storage. These findings suggest that wet aging alters the taste of beef through changes in taste-related metabolites and lipid composition, enhancing flavor and quality.

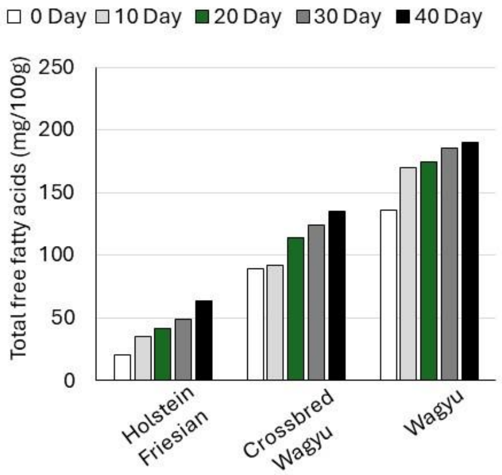

Figure 1. Increase in free fatty acids due to cold storage

IV. CONCLUSION

This study utilized metabolomics analysis to investigate the effects of wet aging on several beefs during cold storage. Initially, sensory evaluation confirmed that wet aging enhanced the umami and kokumi taste of hybrid Wagyu beef. GC/MS-based metabolomics analysis revealed significant changes in the levels of key taste-related metabolites, including glutamate, tryptophan, phenylalanine, acetyl-lysine, xylulose, citric acid, hypoxanthine, and creatinine. Additionally, lipid analysis indicated a significant increase in linoleic acids, which are precursors to aromatic compounds, due to wet aging. This study suggests that the changes in the taste of Wagyu beef resulting from wet aging are linked to alterations in taste-forming elements, including free amino acids, organic acids, and free fatty acids.

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