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Metabolomic changes and the relevant pathways in *longissimus*muscle of Japanese Black cattle during postmortem aging (#231)

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Introduction

Skeletal muscle metabolites contribute to meat quality as flavor components. Some of those compounds can be developed to 'meaty' or 'roasted' aroma by Maillard reaction in the cooking process of meat. Water-soluble free amino acids, peptides, nucleotide-related products, fatty acids, and sugars are a major part of those compounds in beef. Despite the importance of these compounds in meat, metabolomic changes by which these compounds are generated in beef have been poorly explored. A comprehensive understanding of changes in postmortem muscle metabolites could provide novel information on exploration of key compounds associated with meat quality improvement and monitoring.

Japanese Black (JB) cattle are well known for their genetically superior intramuscular fat depot, which makes beef tender, and gives JB beef the high marbling score and preferable flavor. However, contribution of water-soluble compounds to JB beef remains poorly understood. In the present study, we focused on generation of the water-soluble compounds that are potentially associated with flavor and food-functionality in JB beef. To explore these compounds and clarify mechanism of the compound generation during postmortem aging, we analyzed metabolomic changes in lean portion of JB beef using capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS). The resultant CE-TOFMS data were followed by bioinformatic analyses to interpret relevant molecular pathways in postmortem JB muscle aging.

Methods

Lean portions of Longissimus thoracis (LT) muscle in 3 JB steers aged 28 months were collected at 0, 1, and 14 days after slaughter. Avoiding contamination of intramuscular fat, several small pieces of lean-muscle were picked up in the core part of each LT muscle at 30 min after slaughter, thereafter at 1 d and 14 d during the storage at 2°C. The muscle pH was measured, and normal range of pH decline of the beef was confirmed. CE-TOFMS was carried out using an Agilent CE Capillary Electrophoresis System (Agilent Technologies) in Human Metabolome Technologies (https://humanmetabolome.com/). After the raw data were processed, the compounds were annotated in the Human Metabolome Database (ver. 4.0, http://www.hmdb.ca/) and the Kyoto Encyclopedia of Genes and Genomes database (KEGG; http://www.genome.jp/kegg/). Data were also applied to statistical and pathway enrichment analyses using MetaboAnalyst (https://www.metaboanalyst.ca/MetaboAnalyst/faces/home.xhtml) under the autoscaling normalization. Using the annotated compounds, multivariate analyses were conducted after one-factorial ANOVA with postmortem time as the main effect. The extracted compounds showing significant differences were used for hierarchical clustering analysis (HCA) and principal component analysis (PCA).

Results

Among the detected compounds, a total of 171 compounds (117 cations and 54 anions) were annotated across all the beef samples. Of the 70 compounds, the contents of 31 increased over time, while the contents of 14 decreased ($P < 0.05$); the rest showed no difference between 0 and 14 d. The lactic acid content increased significantly within the first 24 hrs ($P = 0.02$), indicating the progress of glycolysis in the LT muscle in the early postmortem period. ATP degradation, temporary inosine 5'-monophosphate (IMP) accumulation at 1 d, and the subsequent accumulation of hypoxanthine and inosine at 14 d progressed in a coordinated manner during the aging.

Using 84 compounds screened by significance of changes, we obtained heatmap result of HCA, which indicated that the changing compounds in the metabolome profile was separated into several categories according to the pattern of changes over time (Fig. 1). The samples were grouped by the pattern of postmortem changes in water-soluble compounds detected in the lean portion of JB beef. Similar result was observed in PCA, which resulted in the plot segregation patterns grouped into 0, 1, and 14 d beef samples and a clear association of PC1 with postmortem aging. The loading scores of cysteine-glutathione disulfide (Cys-GTdS), thiamine, nicotinamide, gluconic acid, Cys, gluconic acid, and hypoxanthine were highly positive for PC1.

In the pathway analysis using the annotated compounds, 8 pathways were significantly extracted as the representative pathways in postmortem aging of the beef (Holm's $P < 0.05$). These pathways included Purine metabolism, Pyrimidine metabolism, Arginine and proline metabolism, Glycerophospholipid metabolism, Glutathione metabolism, Nicotinate and nicotinamide metabolism. These metabolic pathways are expected to be prominent in the lean portion of JB beef during postmortem aging. We used only three steers in the present study, however, the overall tendency of metabolomic changes observed in this study would not contradict that observed in biochemical studies of meat aging using larger numbers of animals.

Conclusion

In the present study, we extracted the key compounds and the relevant

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pathways in postmortem aging of JB beef, which were mainly associated with metabolisms of purine, pyrimidine, glycerophospholipid, glutathione, and nicotinamide. The results contribute to further understanding of the beef quality of JB and other breeds.

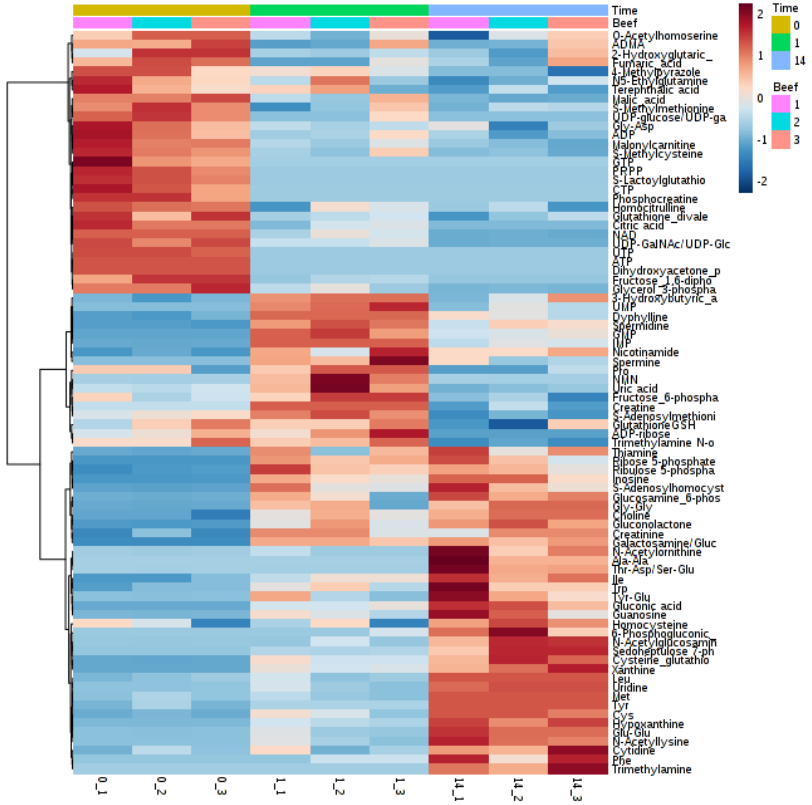

Fig. 1. Heatmap result of hierarchical clustering analysis.
The row displays the metabolite and the column represents the sample. Metabolites with relatively low contents are displayed in blue, while metabolites with relatively high contents are displayed in red. The brightness of each color corresponds to the magnitude of the difference when compared with the average value.

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