Metabolomic changes in porcine fast and slow type muscles during postmortem aging

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Abstract – We aimed to elucidate metabolomic changes in postmortem porcine longissimus lumborum (LL) and vastus intermedius (VI) muscles with different aging times, using capillary electrophoresis-time of flight mass spectrometry. A total of 188 water-soluble metabolites were detected in the postmortem muscles during aging. According to the principal component analysis, hydrophilic amino acids and β-alanine-related compounds were associated with the muscle type positively and negatively, respectively. Glycolytic and ATP degradation products contributed to aging time. At 168 h postmortem, LL was enriched in amino acids, dipeptides, and glycolytic products, whereas the VI was enriched in both sulfur-containing compounds. The contents of inosine 5'-monophosphate (IMP) accumulation in the LL were more than 4 times higher than in the LL after 24 h postmortem.

Key Words – Metabolomics, Pork, Postmortem aging

I. INTRODUCTION

Amino acids, peptides, nucleotide-related products, fatty acids, and sugars are sensory attributes of the quality of beef, pork, and chicken not only as flavor and taste components but also as the precursors of these components [1]. Most of those compounds are developed during postmortem muscle aging. In addition, the degradation of glycogen in postmortem muscle generates glucose and glucose-phosphate compounds, and the glycolytic metabolism consequently shows a decline in muscle pH due to lactate accumulation, which indirectly affects water holding capacity and meat tenderness through protein denaturation and protease inactivation. Sugars in meat are likely precursors of flavor components produced by the Maillard reaction, which is one of the most important pathways mostly occurring between amino acids and reducing monosaccharides for flavor formation in cooked foods. Lipids are developed to volatile components of meat flavor after cooking. Thus, most of the low-molecular weight (MW) compounds related to metabolism are the precursors of meat flavor components that develop during the meat aging and cooking processes. The aim of the present study was to comprehensively analyze the changes in the metabolite profiles of pork during postmortem aging in fast- and slow-type muscles. We used a capillary electrophoresis-time of flight mass spectrometry (CE-TOF MS) technique for the analysis of water-soluble charged metabolites such as amino acids, free fatty acids, and short peptides.

II. MATERIALS AND METHODS

From three sows of the Landrace × Large White × Duroc breed, aged 4.5 months (90–100 kg), LL and VI muscle samples from each animal were excised from the hanging carcasses at 0, 4, 24, 48, and 168 h after slaughter. The muscle samples were used for determination of the glycogen and glucose concentrations, pH measurement, determination of myosin heavy chain isoform (MyHC) contents, and CE-TOF MS analysis [2]. The samples were analyzed with CE-TOF MS by Human Metabolome Technologies (Tsuruoka, Japan). In brief, the water-soluble fraction from the frozen muscle tissue was extracted by homogenizing with Milli-Q water (Millipore) and chloroform (ratio = 2:5). After filtration thorough a Millipore 5-kDa cutoff filter, the upper aqueous filtrate was used for analysis with CE-TOF MS, using an Agilent CE Capillary Electrophoresis System including an Agilent 6210 Time of Flight mass spectrometer (Agilent Technologies, Waldbronn, Germany). The system was controlled by Agilent G2201AA ChemStation software version B.03.01 for CE (Agilent Technologies). Raw data obtained by CE-TOFMS were processed with the proprietary software MasterHands [3]. Statistical analysis of CE-TOF MS data including hierarchical clustering analysis (HCA) and principal component analysis (PCA) were performed as described previously [2,3]. Content of each metabolite was considered significantly different if $P < 0.05$ in the least significant difference analysis under the significant result in analysis of variance (ANOVA) ($P < 0.05$).

III. RESULTS AND DISCUSSION

The result of PCA revealed that PC1 completely separated the samples into LL and VI muscles with the proportion of 32.8% (Figure 1), indicating specific metabolomic profiles of LL (negative PCA score) and VI muscles (positive PCA score) through the entire postmortem aging period. VI muscle especially at 168 h was characterized by the presence of amino acids such as alanine, glycine, hydroxyproline, glutamine, and histidine as well as glutathione and pyridoxamine 5'-phosphate (PMP) with a positively high PC1 loading value, whereas LL muscle at 0 h was characterized by β-alanine and imidazole-dipeptides such as carnosine and anserine/homocarnosine with a negatively high loading value. In other words, changes or distribution of these metabolites were different between the muscles during the postmortem aging.
The PC2 was associated with the period of postmortem muscle aging at the proportion of 25.5%, since the early postmortem stage muscle samples were plotted in the positive area of PC2 whereas the late-stage samples were plotted in the negative area. Notably, compared to the LL samples, the VI muscle plots did not change after 24 h postmortem, according to the VI plots on the PC2 axis. This could reflect higher postmortem proteolysis in fast type muscle fibers [4]. The contents of metabolites related to glycolysis and the pentose-phosphate pathway were positively correlated with the late stage of aging. On the other hand, the early stage of aging was characterized by negatively high loading values of metabolites such as ATP and glycerol 3-phosphate, indicating that that glycolysis and the pentose-phosphate pathway were working by 24 h postmortem in the VI but also further thereafter in the LL muscle.

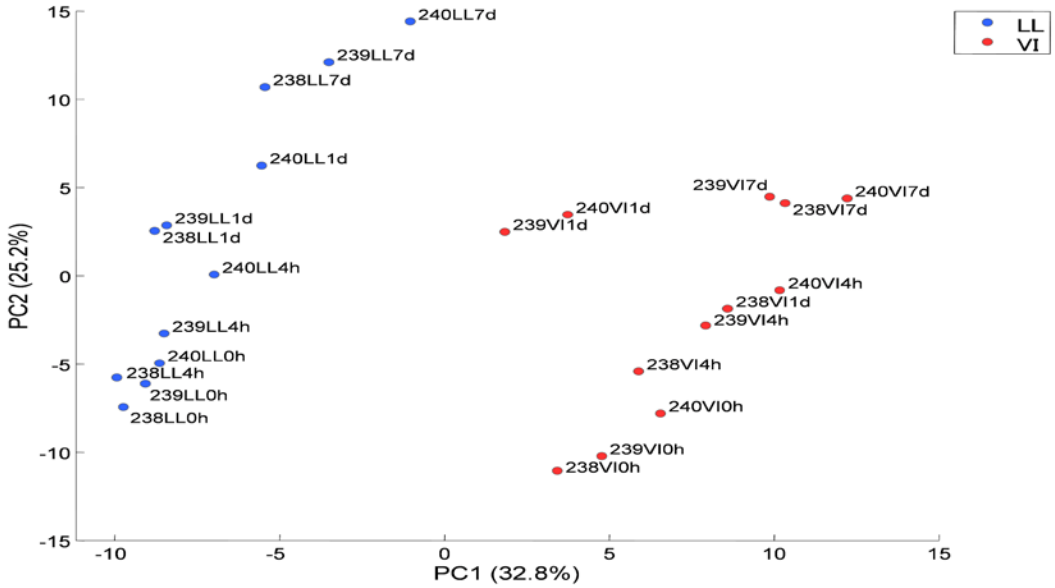

Figure 1. PCA plots of the porcine LL and VI muscle samples in the aging period (0h, 4h, 1d, 7d)

IV. CONCLUSION

Our CE-TOF MS-based metabolomic analysis of postmortem porcine muscle aging unveiled not only details of relevant metabolite changes in the fast and slow type muscles but also the intermuscular differences.

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