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DISTINGUISHING SPOILED AND UNSPOILED PORK USING MEAT PROTEIN DECOMPOSED BY BACTERIA

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I. INTRODUCTION

During meat storage, meat proteins are degraded into small amino acids and peptides by endogenous (calpain, caspase, cathepsin, and proteasome) and exogenous (microbial metabolism) enzymes [1, 2]. Peptides generated through the proteolysis of meat proteins have specific-properties [3]. Specific peptides are used as predictors of pork meat quality [4], and to discriminate species, meat cuts, and storage periods [5,6]. Therefore, there are differences in protein degradation between spoiled and un-spoiled meat owing to the activity of endogenous and exogenous enzymes. Protein degradation characteristics are potential markers for distinguishing between spoiled and un-spoiled pork using bacteria. Thus, this study aimed to distinguish between spoiled and un-spoiled pork by comparing the proteins decomposed by bacteria.

II. MATERIALS AND METHODS

In a commercial slaughterhouse, pork loins (M. *longissimus lumborum et thoracis*) were obtained from 15 carcasses at 24 h postmortem. Pork loins were divided into three groups by the storage time and microbial counts: Fresh (FR), internal region at day 1 and < 2.0 Log CFU/g; Aged (AG), internal region at day 14 and < 2.0 Log CFU/g; Spoiled (SP), external region at day 14 and > 7.0 Log CFU/g. Peptides were extracted from each sample and profiled using liquid chromatography with tandem mass spectrometry (LC-MS/MS) and Peaks Studio (Bioinformatics Solutions Inc., ON, Canada) [1, 7]. Decomposed proteins were quantified as sum of the intensities of the identified peptides. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were conducted to assess the differences among pork groups (FR vs. AG vs. SP). Decomposed proteins were subjected to partial least square discriminant analysis (PLS-DA) to distinguish the spoiled and unspoiled groups. Differences were considered statistically significant at $P < 0.05$.

III. RESULTS AND DISCUSSION

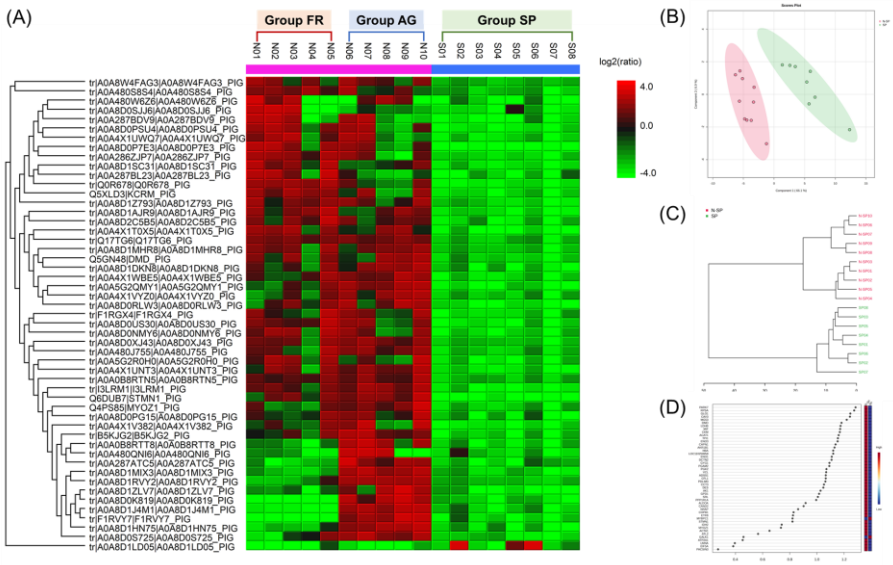


Figure 1. Comparison of decomposed proteins among the fresh (FR), aged (AG), and spoiled (SP) pork groups. (A) Heatmap showing the intensity of decomposed proteins, (B) Principle component analysis, (C) Hierarchical clustering analysis, (D) VIP scores of decomposed proteins showing distinguished spoiled (SP) and unspoiled (FR and AG) pork.

A total of 51 proteins were identified in the FR, AG, and SP groups (Figure 1A). Pork from FR and AG was clearly distinguished from pork SP based on these proteins according to the PCA and HCA results (Figure 1B and 1C). The

variable importance in projection (VIP) scores was represented using the PLS-DA model to distinguish among the three pork groups (FR, AG, and SP) (Figure 2D). The proteins having high scores were parkinsonism associated deglycase (PARK 7), small ribosomal subunit protein uS2 (RPSA), lactoylglutathione lyase (GLO1), caveolin-3 (CAV3), tripartite motif-containing protein 72 (MG53), dystrophin (DMD), L-lactate dehydrogenase B chain (LDHB), macrophage migration inhibitory factor (MIF), creatine kinase M-type (CKM), acetyl-CoA acetyltransferase (ACAT1), triosephosphate isomerase (TPI1), and beta-enolase (ENO3). Thirty decomposed proteins which showed significantly different intensities among the three pork groups (Figure 2). Among these proteins, GLO1, CAV3, DMD, LDHB, and ACAT1 showed significantly different intensities in the SP groups compared with the FR and AG groups (Fig. 2). These results indicate that bacteria utilized glucose, lactic acid, and glucose for energy metabolism [8].

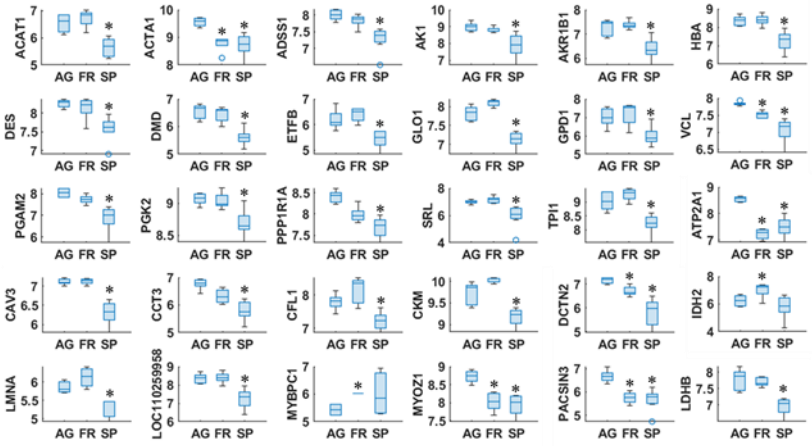


Figure 2. Comparison of the intensity of decomposed proteins among three different pork groups. AG, Aged; FR, Fresh; SP, Spoiled. Asterisk (*) indicates significantly higher or lower intensity in FR and SP than that in AG ($P < 0.05$).

IV. CONCLUSION

Protein decomposed by microbial metabolism, such as GLO1, CAV3, DMD, LDHB, and ACAT1, were clearly distinguished between spoiled and unspoiled pork, which had different bacterial counts: spoiled pork, > 7.0 Log CFU/g; and unspoiled (fresh and aged) pork, < 2.0 Log CFU/g. Thus, these proteins are potential markers for distinguishing between spoiled and unspoiled pork samples.

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