PROTEOMIC ANALYSIS USING ITRAQ REVEALS THE ALTERATIONS IN STRESS-INDUCED DYSFUNCTIONAL CHICKEN MUSCLE

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Abstract – The objective of this study was to investigate the changes in the protein profiles of pale, soft and exudative (PSE)-like muscles of broilers subjected to transportation under high temperature conditions, using isobaric tags for relative and absolute analysis quantitation (iTRAQ). *Pectoralis major* (PM) muscles were collected and classified as normal (T-NOR) or PSE-like (T-PSE). Results indicated that broilers in the T-PSE group exhibited higher activities of plasma stress indicators. The microstructure of T-PSE group showed a looser network and larger intercellular spaces in comparison to the other groups. Proteomic analysis, based on iTRAQ revealed 29 differentially expressed proteins in the T-NOR and T-PSE groups that were involved in protein turnover, signal transduction, stress and defense, calcium handling, cell structure and metabolism. In particular, proteins relating to the glycolysis pathway, calcium signaling, and molecular chaperones exhibited significant differences that may contribute to the inferior postmortem meat quality. Overall, the proteomic results provide a further understanding about the mechanism of meat quality changes in response to stress.

Key Words – Glycolysis, Calcium signaling, Molecular chaperone

I. INTRODUCTION

The selection of broilers for augmented growth rate and breast yield has been accompanied with muscle dysfunction and myopathy, among which pale, soft and exudative (PSE)-like meat is well known [1]. Previous studies have indicated that transportation of broilers during high ambient temperature can affect various physiological and metabolic functions which may result in PSE-like t meat [2, 3].

The development of proteomic approaches in meat science to understand the biological mechanism and to discover biomarkers of meat quality has been highlighted in recent years [4]. However, research on alterations in proteins in *PM* muscle of broilers subjected to stress and the mechanism triggering the development of PSE-like meat is limited. In addition, the reason for the variation in meat quality caused by intra-broiler variability remains unclear. Therefore, we applied iTRAQ-labeling coupled with LC-MS/MS to identify protein changes in *PM* muscle of broilers, based on categorized normal and PSE-like muscles collected after subjecting samples to acute transport under high ambient temperature.

II. MATERIALS AND METHODS

Broilers were subjected to transportation according to a previous study [3]. Muscle samples were categorized as normal ($46 < L^* < 53$, $5.7 < pH_{24h} < 6.1$, T-NOR) and PSE-like ($L^* \geq 53$, $pH_{24h} \leq 5.7$, T-PSE) groups. The experimental design and workflow are illustrated in Fig. 1. Data analysis was performed by Student's *t-test* or Duncan's multiple range tests using SAS 9.12 (SAS Institute Inc., Cary, NC, USA, 2003).

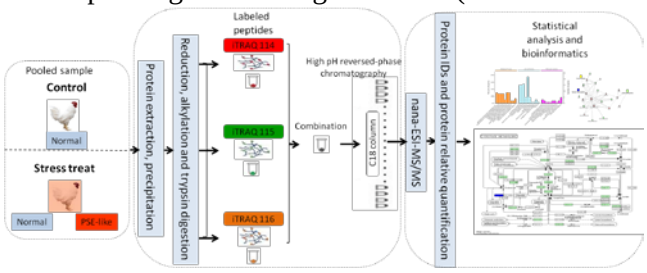

Figure 1. Experimental design and workflow for quantitative proteomic analysis using iTRAQ.

III. RESULTS AND DISCUSSION

Based on the results, 125 proteins were identified within the false discovery rate (FDR) of 5%, and 39 proteins were considered candidate proteins, which were differentially expressed between the T-NOR and T-PSE groups based on the fold change of >1.5 or <0.67 and $P < 0.05$. Among them, 19 were significantly

up-regulated and 20 down-regulated. The differentially expressed proteins were classified into seven functional categories: protein turnover, signal transduction, stress and defense, calcium handling, cell structure, energy and metabolism (Fig. 2). The KEGG pathway enrichment analysis indicated carbon metabolism, glycolysis/gluconeogenesis, and biosynthesis of amino acids processes were significantly low expressed, while the calcium-signaling pathway was highly over-represented. Differently expressed proteins involved in these processes include ENO3, MDH1, TPI1, ATP2A1 and TNNC1.

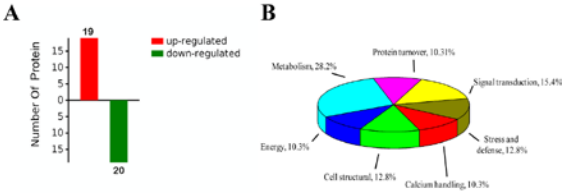

Figure 2. Differentially expressed proteins identified. (A) Up-regulated and down-regulated proteins. (B) Classification of the differentially expressed proteins according to their molecular functions.

Subsequently, we applied STRING for protein-protein interaction analysis (Fig. 3). The central network implied proteins related to signal transduction, energy metabolism, stress response, and the calcium signaling. These protein interactions regulated intracellular biological process, such as the ubiquitin-proteasome pathway, selective degradation of various forms of damaged proteins undeer oxidative stress, and the glycolysis pathway, which is critical in controlling metabolism and energy supply. The heat shock proteins family acts as molecular chaperones to maintain assembly and folding, translocation and interaction with damaged proteins under stress conditions

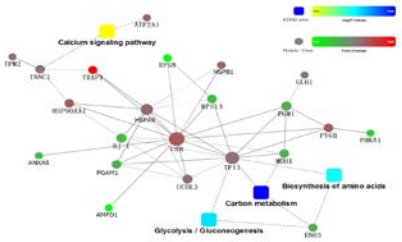

Figure 3. Interaction analysis of differentially expressed proteins. Circle nodes denote genes/proteins, and rectangle denotes biological process.

IV. CONCLUSION

Proteomics study identified differentially expressed proteins related to the glycolysis pathway, calcium signaling, and molecular chaperones play a crucial role in mediating meat quality changes in response to stress.

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