Targeted energy metabolomics analysis of postmortem pork in an *in vitro* model as influenced by protein S-nitrosylation

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Objectives: As a typical redox-dependent post-translational modification of the protein, protein S-nitrosylation is formed by cova- lently combining the sulfhydryl group of protein cysteine with nitric oxide (NO) (Stamler & Meissner, 2001). According to the pre- vious study, protein S-nitrosylation could affect meat quality by regulating postmortem energy metabolism (Liu et al., 2018). Thus, it is of great significance to explore how the protein S-nitrosylation influences energy metabolism for further regulating the quality of fresh meat. The aim of this work was to explore the targeted energy metabolite variation of early postmortem pork in an *in vitro* model as influenced by different levels of protein S-nitrosylation using UPLC-MS/MS based metabolomics multivariate statistical analysis.

Materials and Methods: 2.1 *In vitro* buffer system establishment referred as Matarneh et al. (2018). A total of 0.5 g pork samples (n = 6) were ground into powder in liquid nitrogen and then homogenized in the phosphate buffer. Then the samples were incubated at 37 °C for 1 h with the following three treatments: control (phosphate buffer), 1 mM NOR-3 (NO donor) and 0.1 M L-NAME (NOS inhibitor), respectively. Following this, the reaction buffers were added to the mixtures for building *in vitro* model and incu- bated at 25 °C for 24 h. 2.2 Protein S-nitrosylation level determination referred as Wang et al. (2019). 2.3 Sample extraction and UPLC-MS/MS determination referred as Yu et al. (2019). 2.4 Pathway analysis used MetaboAnalyst 5.0.

Results and Discussion: 3.1 Protein S-nitrosylation level NOR-3 group had significantly higher protein S-nitrosylation level com- pared to L-NAME group. The distributions of protein S-nitrosylation in the range of 30-100 kDa were more concentrated and the high abundance suggests that small molecule proteins are more susceptible to S-nitrosylation. 3.2 Identified metabolites The results showed that 38 energy metabolites were identified by UPLC-MS/MS with targeted metabolic profiling. These 38 metabolites were classified into five pathways including glycolysis, oxidative phosphorylation, pentose phosphate pathway, tricarboxylic acid cycle and purine metabolism. 3.3 Multivariate statistical analyses of metabolites From PCA results, L-NAME group was obviously sepa- rated with NOR-3 group. The metabolite profiling was significantly changed after protein S-nitrosylation treatment. Basing on the OPLS-DA of NOR-3 and L-NAME group, 16 metabolites (VIP > 1) were discriminated as significantly different metabolites. 3.4 Metabolic pathway Based on KEGG data library, four metabolic pathways were identified including all significantly differential metabolites screened by OPLS-DA model. Four metabolic pathways (glycolysis, TCA cycle, purine metabolism and PPP) were connected and summarized. Glycolytic metabolism (contained 10 metabolites) and TCA cycle (contained 12 metabolites) were fur- ther screened out as the major energy metabolism pathways influenced by protein S-nitrosylation according to the number of dif- ferential metabolites in the metabolic pathways.

Conclusion: Sixteen metabolites were discriminated as significantly different metabolites. Glycolysis and TCA cycle were significant metabolic pathways affected by different levels of protein S-nitrosylation to regulate the energy metabolism of postmortem pork.

Reference:

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Key words: Protein S-nitrosylation, Postmortem Energy Metabolism, In Vitro model, Meat Quality, UPLC-MS/MS