COMPARATIVE ANALYSIS OF PHOSPHOPROTEOME OF MUSCLE INDUCED BY SALT CURING

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Abstract –The purpose of the study was to examine phosphorylated proteins in ovine muscle induced by salt curing. The topside muscles of eight crossbred sheep were used. Muscle samples were cured for 16 h with 0% salt and 3% salt, respectively. Muscle total proteins were extracted from salted meat with 0 % and 3 % salt, and analyzed by two dimensional electrophoresis coupled with Pro-Q Diamond and SYPRO Ruby staining. The differentially phosphorylated proteins were identified by Liquid chromatography-tandem mass spectrometry (LC-MS/MS) and the UniProt database. There were thirteen different phosphoprotein points (>1.5 fold change, p < 0.05) induced by salting, which were triosephosphate isomerase, glycogen phosphorylase, creatine kinase M-type, myoglobin, troponin T fast skeletal muscle type, actin, myosin light chain 1/3, tropomyosin beta chain, etc. Most of the different phosphoproteins were involved in glycometabolism and protein degradation. In a word, salt curing may influence meat quality through protein phosphorylation.

Key words–differential phosphorylated proteins, myofibrillar protein, salting

I. INTRODUCTION

Salting is widely used to preserve meat and to improve the quality of meat products, such as water holding capacity, tenderness, texture and so on. Although phosphate, collagen, sodium alginate and other ingredients are applied to improve meat quality, salt is still the most essential salting material in cured or cooked meat products [1].

Protein phosphorylation is one of the most ubiquitous post-translational modifications (PTMs). Reversible protein phosphorylation plays an important role in protein structure, function, signaling and regulation [2]. The majority of muscle proteins exists phosphorylation in vivo in mammalian cells. Protein phosphorylation may affect meat quality by three ways, including protein degradation, glycolysis and muscle contraction. Protein phosphorylation affects the degree of protein degradation, which influences meat tenderness [3-4]. Glycolytic enzymes can be phosphorylated by the upstream protein kinases. The phosphorylation of glycolytic enzymes affects their activity and stability, and then affects the glycolysis [5]. Protein phosphorylation affects muscle contraction, especially the phosphorylation of myosin regulatory light chain 2 (MYLC2) was reported to change protein structure [6-7].

Protein phosphorylation in relationship to muscle pH decline and meat tenderness was previously investigated in postmortem muscle [2,5]. Electrical stimulation (ES) alters protein phosphorylation [8]. All these studies reveal that protein phosphorylation plays an important role in meat quality. Many proteins are salt-responsive phosphoproteins in vivo in plants [9]. Salt influences the phosphorylation of SPAK and NKCC1.

Our previous study revealed that salt curing affected the global phosphorylation level of muscle proteins, however, the differential phosphorylated proteins present in salting meat remain unclear. The objective of the study is to investigate the differential phosphorylated proteins in response to salt curing.

II. MATERIALS AND METHODS

The topside muscles from eight male (not castrated), 8-month old sheep (small Tailed Han sheep ×large Tailed Han sheep) with an average carcass weight from 25 to 30 kg were used to the experiment. The mixed muscle were divided into two groups for salted treatments, with 0 % (control group) and 3 % green iodized salt for 16 h at 4 °C. The samples were snap frozen in liquid nitrogen and stored at -80℃ for further analysis.

Frozen samples were crushed in a mortar containing liquid nitrogen, and then extracted

total muscle proteins. The protein concentration was determined using the Bradford assay. 450 µg proteins were incubated with 24 cm 3-10 linear IPG strip (GE, Fairfield, USA) in the IEF tray, passive hydration 12 h. Then run on the PROTEAN IEF system. The isoelectric focusing running conditions were as follows: 6 h at 50 V, 1 h at 200 V, 1 h at 500 V, 2 h at 1000 V, 3 h at 3000 V, 4 h at 5000 V, 4 h at 10000 V, 10000 V for 50000 Vh, and 2 h at 500 V. After IEF separation, the strips were equilibrated in equilibrated buffer I for 15 min, followed by the equilibrated buffer II 15 min at room temperature. The mixed proteins were separated by two-dimensional electrophoresis with 12.5% separating gel. Firstly, running condition was 1 W/gel for 1h, and then 13 W/gel until the bromophenol blue reached the bottom of the gel. Firstly, the gels were stained by Pro-Q Diamond to detect the phosphoproteins according to the manufacturer's instruction with minor modification. Then the same gels were stained by SYPRO Ruby dye for total protein. Finally, the gels were stained with colloidal Coomassie Brilliant Blue R-250 to visualize the protein locations. The differential protein identification was performed by LC-MS/MS instrument.

The Image Master 7.0 software was used to identify and quantify the protein spots from 2-DE gel images. The phosphorylation levels of the individual spots were analyzed. The relative protein phosphorylation levels (P/T ratio) were evaluated by the ratio of the density of phosphoproteins (P) in the Pro-Q Diamond images to the density of total proteins (T) in SYPRO Ruby images[3].

All statistical analysis was carried out using SPSS Statistic 21.0. The difference was analyzed by independent-samples T test. Reported p values were evaluated at a 5% significance level.

III. RESULTS AND DISCUSSION

The main aim of the study was to identify the differential phosphoproteins of control group and salt group by 2-DE. There were 13 phosphoproteins (>1.5 fold change, $p < 0.05$) analyzed from the images of Pro-Q Diamond staining. The isoelectric points (pI) of phosphoprotein spots were 5-8 (Fig. 1). There were 4 significantly differential muscle proteins analyzed at SYPRO Ruby images (Fig. 2).

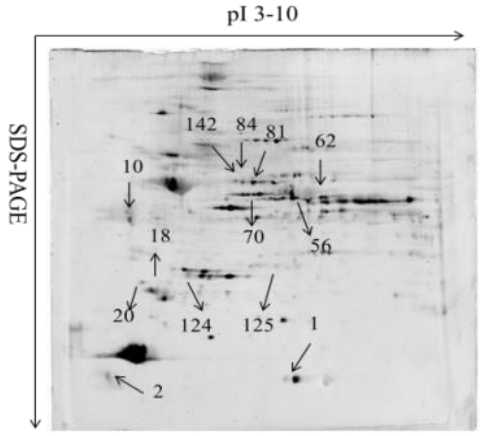

Fig. 1 Phosphorylated proteins of topside muscle under curing

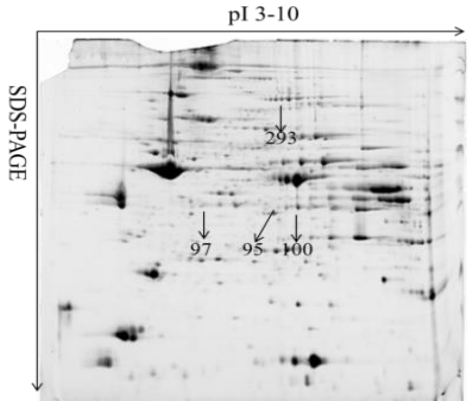

Fig. 2 Total proteins of topside muscle under curing

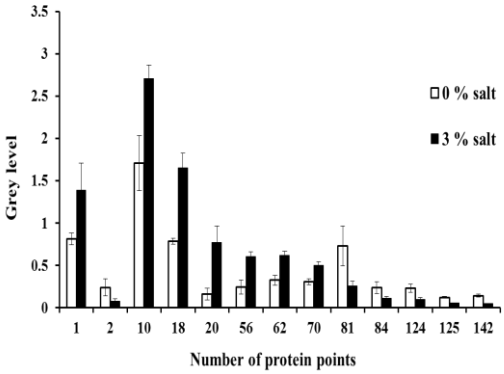

Fig. 3 The grey level of differential phosphoproteins of topside muscles under curing

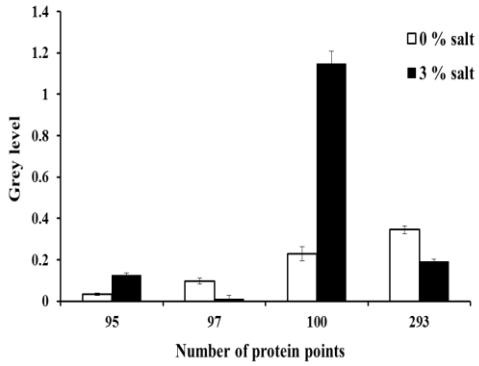

Fig. 3 The grey level of differential muscle proteins

The grey levels of differential phosphoproteins were shown in Fig. 3, while the grey levels of differential muscle proteins were shown in Fig. 4. The differential phosphoproteins at Pro-Q Diamond images included Myoglobin (1), Myosin light chain 1/3-skeletal muscle isoform (MLC; 2, 20, 81), Tropomyosin beta chain (10), Actin-alpha skeletal muscle (18, 62, 84), Troponin T (TnT; 56, 70), Apolipoprotein A-I (124), Triosephosphate isomerase (TPI, 125), F-actin-capping protein subunit alpha-2 (142). The differential muscle proteins at SYPRO Ruby images included Creatine kinase M-type (CK, 95), Troponin T (95, 100), Glycogen phosphorylase (GP, 293).

The relative protein phosphorylation levels were evaluated by the ratio of the density of phosphoproteins (P) in the Pro-Q Diamond images to the density of total proteins (T) in SYPRO Ruby images (P/T ratio). Sarcoplasmic proteins'relative phosphorylation level of salt group is higher than the control group included GP and Myoglobin. The relative phosphorylation level of sarcoplasmic proteins in salt group is lower than the control group included TPI and CK.

GP is a glycolytic enzyme which limits the glycolysis rate. Agius [4] found that phosphorylase kinase can phosphorylate GP on serine 14, which transform the inactive form (GPb) to the the active form (GPa). The protein phosphorylation level of GP of salt group was higher than the control group, and then accelerate glycolysis process, indirectly influence meat tenderness.

Triosephosphate isomerase (TPI) is an important is omerase involved in glycolysis. TPI catalyzes the reversible inter conversion of dihydroxyacetone phosphate (DHAP) and glucose aldehyde-3-phosphate (G3P), and G3P can proceed further down the glycolytic pathway. [10-11] Phosphorylated TPI was a direct substrate of cyclin-dependent protein kinase 2 (Cdk2), Lee et al. [12] found that phosphorylation of TPI decreases its enzyme activity, which is not conducive to the conversion of G3P to DHAP, and then decreases the rate of glycolysis. In our study, salt decreased the phosphorylation level of TPI, increased the enzyme activity,and accelerate glycolysis process, indirectly influence meat tenderness.

CK is an important enzyme related to the regeneration of ATP and energy metabolism [13]. Phosphorylation of CK increased its activity, whereas dephosphorylation decreased it [14].

Myoglobin was also the differential phosphoprotein between the two groups, while the meat color was different, so we speculated that phosphorylation of myoglobin might affect meat color.

The differential phosphorylated myofibrillar proteins identified between the two group included TnT (56, 70), Actin (18, 62, 84), Tropomyosin beta chain and MLC (2, 20, 81).

In our study, salt increased the phosphorylation level of TnT. The phosphorylation of TnT induced by protein kinase C make it more easier to be degraded by calpain [4]. The TnT of salt group is more easier to be degraded, then promoted the meat tenderization.

Tropomyosin is an important protein for the regulation of muscle contraction and the most important phosphorylated protein in the muscle filament. Lehman [15] found that tropomyosin is phosphorylated at serine 283, then enhanced the binding of the head and tail of tropomyosin. Myosin and actin accounted for the most of the myofibrillar proteins. Myosin light chain 1 / 3 is myosin regulatory light chain, which is related to muscle contraction.

IV. CONCLUSION

In summary, salt curing influences phosphorylation and dephosphorylation of individual muscle proteins, which may be a new mechanism to alter meat quality, especially the

tenderness of meat, which is related to protein degradation and muscle contraction.

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