INFLUENCE OF DIFFERENT THAWING METHODS ON PHYSICOCHEMICAL CHANGE AND PROTEIN OXIDATION OF PORK

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***Abstract*—The objective of the present study was to elucidate the physicochemical change and protein oxidation of porcine longissimus muscle as influenced by the different thawing methods. Five kind of thawing methods were used including refrigerator thawing (RT, 4 ºC), ambient temperature thawing (AT, 20 ºC), water immersing thawing (WT, 14 ºC), lotic water thawing (LT, 9 ºC), and microwave thawing (MT). RT had the least quality losses and its physicochemical characteristic was more close to the fresh muscle than other thawing methods. MT had the most significant negative effect on the porcine quality compare to other thawing methods and it could significantly increase the thawing loss, cooking loss, cutting force, carbonyl content, and TBARS ($P < 0.05$), and decrease *a**-value, Ca- and K-ATPase activity ($P < 0.05$). The observation to muscle microstructure showed that MT also could obviously increase gapping between muscle fibers and tore muscle fiber bundles compare to other thawing methods. The decreases in Ca^{2+}-and K^{+}-ATPase activity ($P < 0.05$) content with concomitant increases in carbonyl content and TBARS value ($P < 0.05$) showed that all the thawing methods could cause the porcine protein and fat oxidation.**

***Index Terms*—Porcine longissimus muscle, thawing method, physicochemical properties, protein oxidation.**

I. INTRODUCTION

Although frozen storage is an important preservation method for meat and meat products, however, some deterioration in frozen food quality occurs during storage. The extent of quality losses in frozen meat is dependent upon many factors, including the rate of freezing and thawing, the specific storage temperature, and temperature fluctuations (Gómez-Guillén, Martínez-Alvarez, & Montero, 2003). Thawing is the final stage of chilling technology, aimed at restoring the best properties of meat, similar to those typical of fresh meat. The thawing process is affected by numerous factors like relative air humidity, effective thawing time, and thawing methods (Kondratowicz, Chwastowska, & Matusevicius, 2006). During the thawing process, meat rapidly warms up to the freezing point, and then remains there for a relatively long time. The damage was caused by the formation of large extracellular ice crystals during the thawing process. Inappropriate thawing of frozen meat may result in significant quality deterioration.

In meat, protein oxidation may lead to decrease eating quality of meat such as flavor deterioration, reduced tenderness and juiciness, and discoloration (Xiong, 2000). The freezing and thawing process may cause protein and lipid oxidation, which will affect texture of muscle. One of the consequences of protein oxidation is forming of protein aggregates through both non-covalent and covalent intermolecular bonds (Howell, Herman & Li-Chan, 2001). Our previous studies also showed that multiple freezing and thawing increased the carbonyl content and thiobarbituric acid-reactive substances (TBARS) value, caused the discoloration of meat, and destroyed structure and functionality of myofibrillar protein (Xia, Kong, Liu & Liu, 2009; Xia, Kong, & Xiong, 2010).

Some papers have been published on effects of freezing and thawing on sensory, chemical and physical quality of fish muscle (Ersoy, Aksan, & Ozeren. 2008) and Pork (Xia, et al., 2009), little has been reported about the influence of thawing methods on the physicochemical properties of pork. Our objective was to elucidate the physicochemical change and protein oxidation of porcine longissimus muscle as influenced by different thawing methods.

II. MATERIALS AND METHODS

A. Sample prepared and thawing methods

The longissimus muscles chops (500 g for every chop) were packaged in moisture-impermeable polyethylene bags and frozen and stored at -26 °C for 7 days. The samples were thawed by refrigerator thawing (RT, 4 ºC), ambient

temperature thawing (AT, 20 ºC), water immersing thawing (WT, 14 ºC), lotic water thawing (LT, 9 ºC), and microwave thawing (MT) until the center temperature of samples up to 4 ºC. Myofibril protein isolation (MPI) was prepared from porcine longissimus muscle according to the procedure of Xia et al. (2009). The final pellet (MPI) was stored in a tightly capped bottle, kept on ice, and utilized within 24 h.

B. Thawing loss, cooking loss, cutting force and colour determination

The thawing loss and cooking loss of porcine chops were determined according to the methods (Xia et al., 2009). The cutting forces of the cooked chops were measured using a texture analyzer (Stable Micro System; TA: XT2i, England). The surface colour of porcine chop was measured by a Color Difference Meter (WSC-S, Shanghai Physics and Optics Instrument Co., Shanghai, China). The values were expressed as L^* (lightness), a^* (redness) and b^* (yellowness) units.

C. Thiobarbituric acid-reactive substances (TBARS), carbonyl content and ATPase activity determination

Lipid oxidation was evaluated by TBARS according to Sinnhuber and Yu (1977). Carbonyl groups were detected by reactivity with 2, 4-dinitrophenylhydrazine (DNPH) to form protein hydrazones (Oliver, Ahn, Moerman, Golstein, & Stadtman，1987). ATPase activities of MPI were determined according to Wells, Werber, and Yount (1979).

D. Gel electrophoresis and microstructure

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of pork was performed with a Mini-PROTEAN Tetra Cell (BIO-RAD, U.S.A). A 10% acrylamide resolving gel and a 3% acrylamide stacking gel were used. Microstructure of longissimus muscle sample was examined using a scanning electron microscope (SEM) (Xia et al, 2010).

E. Statistical analysis

The experiment was replicated twice with at least triplicate analyses. Analysis of variance (ANOVA) was done to determine the significance of the main effects. Significant differences ($P < 0.05$) among means were identified using Turkey procedures.

III. RESULTS AND DISCUSSION

The results of thawing methods on thawing loss, cooking loss and cutting force are presented in Fig. 1. Higher amounts of thawing loss and cooking loss were observed with different thawing methods compare to fresh meat ($P < 0.05$). The RT had the least thawing losses and cooking loss (2.09% and 19.72%), and MT had the highest thawing losses and cooking loss (6.64% and 20.78%). Water loss affected meat product weight, sensory properties, appearance, and water-holding capacity (Huff-Lonergan & Lonergan, 2005). The drip loss of muscle can lead to less acceptability, due to the loss of tasteful constituents, e.g. some amino acids or nucleotides. The higher cooking loss of meat samples may also be construed as a result of increased myosin denaturation as demonstrated, and possibly also weakening of the myofibril lattices due to protein degradation (Xia et al., 2010). The cutting forces of thawing chops increased 26.0%, 35.9%, 33.4%, 32.4%, and 42.0%, respectively, by RT, AT, WT, LT, and MT compare to fresh meat ($P < 0.05$). RT had the best tenderness and MT had the worst tenderness among the thawing methods. The decrease in cutting force suggested loss in integrity of muscle fibers, resulting in the weakening of muscle.

The L^*-, a^*-, and b^*-value changes of pork caused by different thawing methods are also shown in Fig. 1. All the thawing methods could significant decrease a^*-value, and increase L^*- and b^*-value ($P < 0.05$) except for RT, that showed RT had least negative effect on colour. Yu, Lee, and Jong (2005) found that the increase of lipid oxidation in cooked turkey muscle was correlated with the decrease in redness and the increase in yellowness.

The protein oxidation in pork was evaluated by measuring carbonyl content. All the thawing methods significantly increased the carbonyl content and TBRAS ($P < 0.05$), and the RT and LT had least carbonyl content and TBRAS (Fig. 2). Compare to control, RT, AT, WT, LT, and MT increased carbonyl content 2.30%, 4.37%, 3.41%, 2.10%, and 3.20%, respectively. AT and WT had higher TBRAS compare to control (0.18 mg/kg MPI), and increase 56.2% and 71.1%, because of their higher thawing temperature. Reznick, Witt, Matsumoto, and Packer (1992) showed that freezing and thawing cycle enhanced the carbonyl formation of proteins. Furthermore, the apparent relationship between protein carbonyls and TBARS indicated a strong likelihood that some dicarbonyl compounds derived from lipid oxidation,

notably malondialdehyde, formed complexes with proteins (Xiong, 2000).

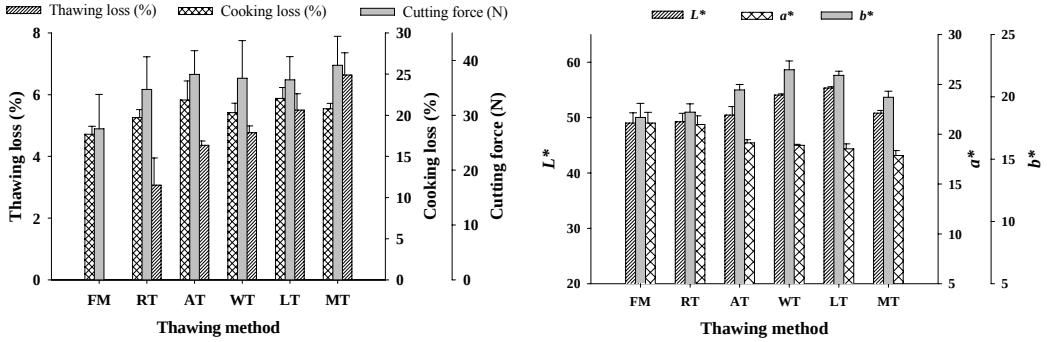

Fig. 1. Influence of thawing method on the thawing loss, cooking loss, cutting force and colour of pork. RT, refrigerator thawing (4 ºC); AT, ambient temperature thawing (20 ºC); WT, water immersing thawing (14 ºC); LT, lotic water thawing (9 ºC); MT; microwave thawing.

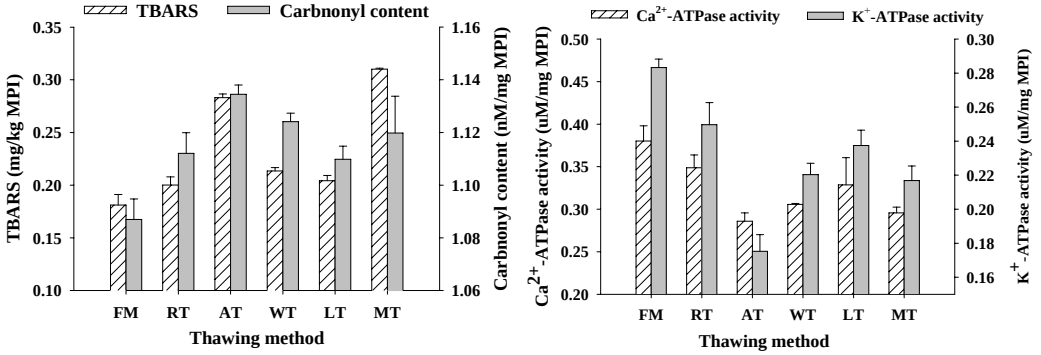

Fig. 2. Influence of thawing methods on TBARS, carbonyl content and ATPase activity of porcine myofibrillar protein.

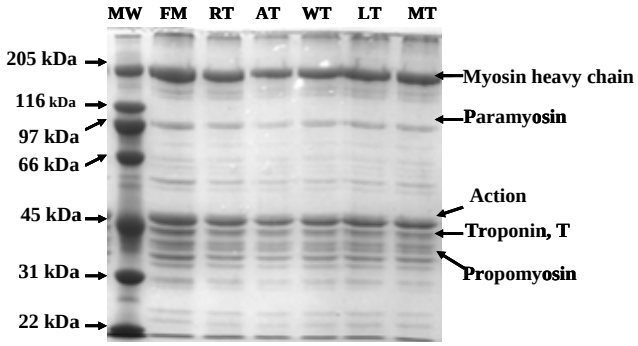

Fig. 3. Influence of thawing methods on the SDS-PAGE pattern of pork myofibrillar proteins.

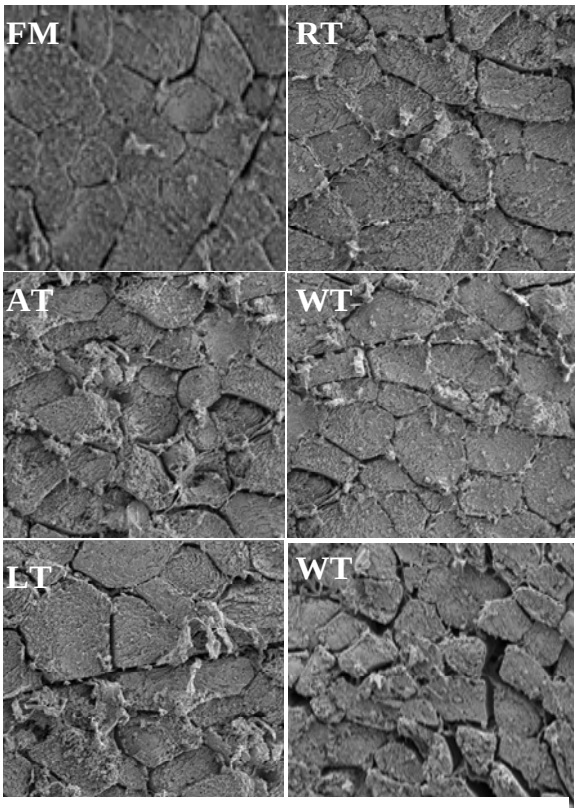

Fig. 4. Influence of thawing methods on the microstructure of pork (Magnification 500).

The activities of both Ca^{2+}-ATPase and K^{+}-ATPase of MPI extracted from porcine muscles subjected to different thawing methods are depicted in Fig. 2. The Ca^{2+}-ATPase and K^{+}-ATPase activities of fresh meat was 0.380 and 0.287 μM/mg. After the thawing process, RT had the highest Ca^{2+}-ATPase and K^{+}-ATPase activities compare to other thawing method, and AT had the lowest ATPase activities. ATPase has been used as an indicator of myosin integrity, and the loss in ATPase was possibly associated with the proteolysis of myosin molecule (Benjakul, Seymour, Morrissey, & An, 1997).

The decrease in ATPase activity was possibly due to the conformational changes of the myosin globular head as well as the aggregation of this portion. Rearrangement of proteins, by means of protein-protein interaction induced by the freezing and thawing process, might contribute to the loss in ATPase activity.

SDS-PAGE patterns of porcine muscle subjected to different thawing methods are shown in Fig. 3, and it exhibited no detectable loss or increase of myosin heavy chain, actin, and other bands. The result showed because of relative short thawing time, different thawing methods couldn't cause obviously protein aggregates and protein fragments. That also showed that protein oxidation is not so severity in all thawing methods in our present study.

The microstructure of the transverse section of muscle samples of different thawing method is shown in Fig. 4. The results showed that RT had the least negative role on the microstructure of muscle, and its microstructure is similar to the fresh meat. MT had the greatest destructive effect on the microstructure, and it could obviously increase gapping between muscle fibers and tore muscle fiber bundles compare to other thawing methods.

IV. CONCLUSION

Microwave thawing had the most significant negative effect on the porcine quality compare to other thawing methods and it could significantly increase the thawing loss, cooking loss, cutting force, carbnonyl centent, and TBARS, and decrease *a**-value, Ca- and K-ATPase activity. Refrigerator thawing has less detrimental effect on the pork quality compared to other thawing methods. The findings suggested that it is important to adapt proper thawing method of frozen pork in order to get a maximum protection of meat quality.

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