EFFECTS OF CONTROLLED FREEZING POINT STORAGE ON TENDERIZATION OF LONGISSIMUS THORACIS ET LUMBORUM IN LAMB

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Abstract – The objective of this study was to investigate the effects of controlled freezing point storage on tenderization of lamb muscle in comparison with refrigeration storage. The *longissimus thoracis et lumborum* (LTL) muscles from eight crossbred sheep were collected immediately after bleeding and assigned to the following treatments: refrigeration storage (RE) for 5 days, controlled freezing point storage (CF) at the freezing point temperature (-1 to -2 °C) for 5 days, controlled freezing point storage for 3 days and then refrigeration storage for 5 days (CF3), and controlled freezing point storage for 5 days and then refrigeration storage for 5 days (CF5). The pH, shear force, sarcomere length and relative activities of the Calpains were measured during the 5 days storage for samples in RE and CF treatments and during the 5 days refrigeration storage for samples in CF3 and CF5 treatments. The pH and sarcomere length were significantly different between treatments but no difference was observed on shear force results. The tenderization process was slower in samples treated by controlled freezing point storage compared with refrigeration storage. Controlled freezing point storage could be used as an option to extend the shelf life of fresh meat.

Key Words – Ageing, Meat quality, Meat storage

I. INTRODUCTION

The transformation from muscle to meat is due to the tenderization process during post mortem. Temperature is one of the key factors influencing tenderization [1]. Controlled freezing point storage was innovated based on the difference of freezing point between water and cellular fluid. The metabolism activity is at the lowest level under controlled freezing point storage [2]. Controlled freezing point storage has been applied for storage of fruit and vegetable. However, the effects of controlled freezing point storage on muscle tenderization are still unknown. The objective of this study was to investigate the effects of controlled freezing point storage on lamb muscle tenderization in comparison with refrigeration storage to explore a possible solution for extension of fresh meat shelf life.

II. MATERIALS AND METHODS

Sampling and treatments

Eight crossbred sheep with the same age and feeding management were slaughtered on the same day at a commercial slaughter house according to their standard slaughter procedures. The *longissimus thoracis et lumborum* (LTL) muscles from both sides of the carcass were collected immediately after bleeding.

The LTL muscles from each animal were divided into four cuts, quickly chilled at -18 °C and randomly assigned into the following treatments: (1) refrigeration storage (RE): the samples were quickly chilled to 4 °C and stored for 5 days, (2) controlled freezing point storage (CF): the samples were quickly chilled to 0 °C and stored at freezing point temperature (-1 to -2 °C) for 5 days, (3) controlled freezing point storage for 3 days and then refrigeration storage for 5 days (CF3), (4) controlled freezing point storage for 5 days and then refrigeration storage for 5 days (CF5). The samples were collected at 1, 12, 24, 72 and 120 h during storage for RE and CF treatments and during refrigeration storage for CF3 and CF5 treatments.

pH

The pH of muscles was measured with a pH meter (Testo 205, Germany) manually at 1, 12, 24, 72 and 120 h during corresponding storage.

Warner Bratzler shear force

The Warner Bratzler shear force (WBSF) was measured as D'alessandro *et al.* [3] using samples collected at 24 h. Muscle samples were cooked in an 80 °C water bath until a core temperature of 70 °C. The samples were cooled at 4 °C for 6 h and then cut into 1 × 1 × 2 cm in size with the long axis paralleled to the direction of muscle fibers. The WBSF values were measured using a TA-XT2i texture analyzer (Stable Micro System Ltd., Godalming, UK) equipped with a WBSF device.

Sarcomere length

Muscle ultra-structure was examined by transmission electron microscopy as described by Mestre Prates *et al.* [4]. The sarcomere length was measured using Image-Pro Plus software (version 6.0) and the average value of 30 measurements was recorded.

Relative activities of Calpains

The relative activities of Calpain-1 and Calpain-2 were determined using casein zymography according to Veiseth *et al.* [5]. Samples at 6, 48, 96 h storage were also collected besides the time points mentioned above.

Statistical analysis

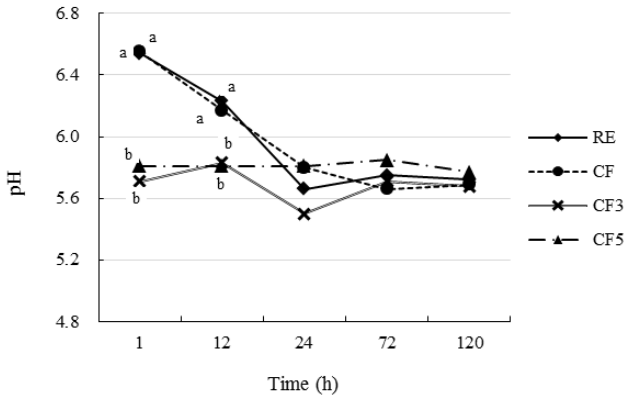
Statistical analysis was carried out with the Statistical Analysis System (SAS), version 9.2 (SAS Institute, Cary, NC, USA). The MIXED procedure was used with treatment as fixed factor. The LSMEANS statement was used for calculating least-squares means (LSM) and the PDIFF option was used for calculating *P*-values for differences between treatments.

III. RESULTS AND DISCUSSION

pH

The pH value was significantly different between treatments after 1 and 12 h storage (Fig. 1, $P < 0.05$). The pH values of samples in RE and CF treatments were significantly higher than those under CF3 and CF5 storage at 1 and 12 h.

Figure 1. pH of lamb muscle under different storage conditions and times (SE = 0.10). Different letters indicate significant difference between treatments at the same time ($P < 0.05$).



The glycogen is metabolized through glycolysis in muscle tissue after slaughtering of animal [6]. The pH of muscle decreased because of the accumulation of lactic acid in muscle. Therefore, pH value is an indicator of muscle tenderization. The lower pH values of samples in CF3 and CF5 treatments than those in RE and CF treatment could be attributed to the ageing process during 3 or 5 days storage under controlled freezing point condition. However, the ageing process was slower than at refrigeration condition and there was no difference on pH value between groups after 24 h storage.

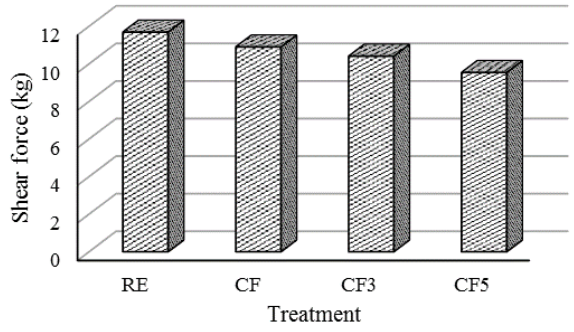
Shear force

There was no significant difference on shear force between samples in different treatments (Fig. 2). The tenderness of meat, as indicated by shear force value, was not influenced by the controlled freezing point treatments in this study, i.e. it is possible to use this kind of treatment for extension of shelf life without negative effects on meat tenderness.

Sarcomere length

The sarcomere lengths of samples were significantly different between treatments after 12,

Figure 2. Shear force of lamb muscle under different storage conditions and times (SE = 1.33)



24 and 72 h storage (P < 0.05, Table 1). The sarcomere length of samples in RE and CF3 treatments were higher than that of samples in CF and CF5 treatments after 12 h storage. The sarcomere length was higher for samples in CF3 than the other three treatments after 24 h storage and it was also higher for samples in CF5 treatment than those in RE and CF treatments. The samples in CF and CF5 treatments showed higher sarcomere length after 72 h storage than those in RE and CF3 treatments.

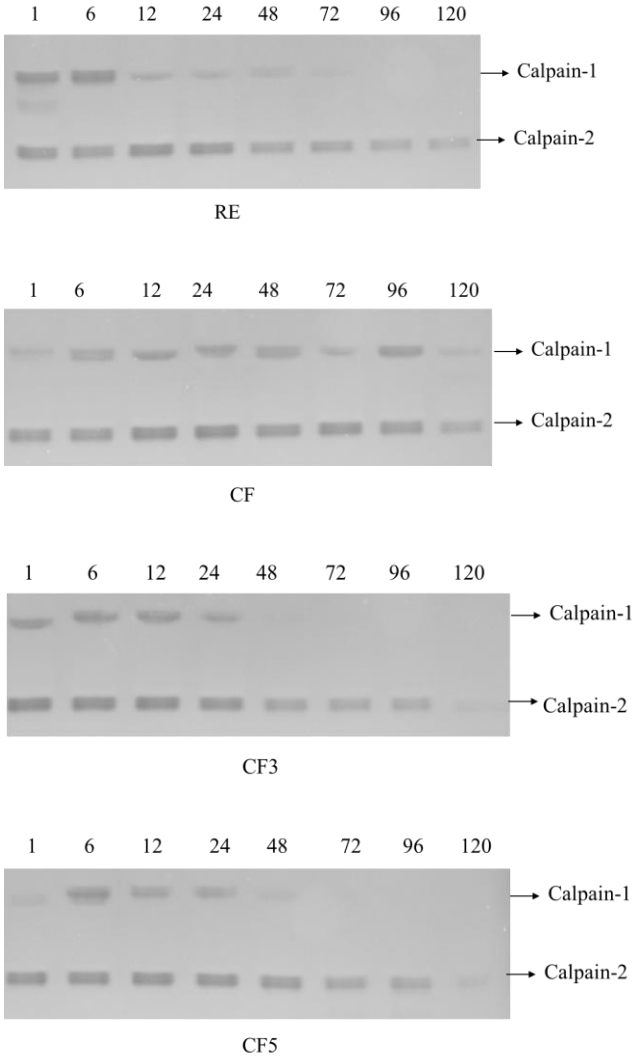
The sarcomere length changes during tenderization because of the rigor mortis of muscle tissue and it can be used to detect the stage of tenderization. The results indicated similar pattern between RE and CF3 treatments, meanwhile, CF and CF5 showed the same trend on the changes of sarcomere length. The rigor mortis process varied among treatments based on the controlled freezing point storage.

Table 1 Sarcomere length of lamb muscle under different storage conditions and times

Treatment	Storage time (h)		
	12	24	72
RE	1.70[a]	1.26[c]	1.14[b]
CF	1.02[b]	1.15[c]	1.33[a]
CF3	1.71[a]	1.95[a]	1.19[b]
CF5	1.15[b]	1.50[b]	1.35[a]
SE	0.05	0.06	0.03
P-value	< 0.05	< 0.05	< 0.05

Relative activities of Calpains

Figure 3. Casein zymography profiles of the relative activities of Calpains in lamb muscle under different storage conditions and times (h)



The relative activities of Calpain-1 and Calpain-2 were presented in Fig. 3. The relative activity of Calpain-1 for samples in RE treatment increased from 1 h to 6 h storage and then decreased until 3 days of storage. The relative activity of Calpain-1 for samples in CF3 and CF5 treatments showed similar pattern with that in RE treatment except higher Calpain-1 relative activity at 12 and 24 h storage. The Calpain-1 relative activity of samples in CF treatment existed until the end of observation. The relative activity of Caplain-2 didn't show significant difference between treatments. Whereas samples in RE and CF treatments still had Caplain-2 activity at 5 days

storage compared to CF3 and CF5 treatments which can not detect the Calpain-2 activity.

Meat is tenderized during post mortem due to the degradation of myofibrillar proteins by Calpains [7]. Rhee *et al.* [8] found that the increase of temperature during post mortem can activate the Calpains. The peak value of Calpain-1 activity is within 48 h post mortem under refrigeration [9]. The results of this study suggest that the expression of Calpain proteolytic ability is similar or slower for samples treated by controlled freezing point storage. The ageing process of lamb muscle is regulated by controlled freezing point storage under the experimental condition of this study.

IV. CONCLUSION

The tenderization process was slower for muscle samples in controlled freezing point storage than in refrigeration under the experimental condition of this study. Controlled freezing point storage could be used as an option to extend the shelf life of fresh meat.

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