EFFECT OF FROZEN-THEN-CHILLED STORAGE ON FREE CALCIUM AND ACTIVITY OF CALPAIN-2 OF PORK *LONGISSIMUS* MUSCLE

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I. INTRODUCTION

The calpain system has been suggested to be the primary proteolytic enzymes to degrade myofibrillar proteins and tenderize meat. The activity of calpain-1 and calpain-2 is regulated by free Ca^{2+} and the inhibitor calpastatin. Autolysis of calpain-1 is important for early post-mortem proteolysis and meat tenderization [1]. Decrease of calpain-2 activity later post-mortem has been observed in pork [2] and beef [3], suggesting a role for calpain-2 in proteolysis during aging. Freezing is currently playing an essential role as preservation in meat technology. The freezing process prior to aging could be a method to increase proteolytic activity of calpains during aging and, thus, meat tenderness [4]. This paper aims to examine the effect of frozen-then-chilled storage on the level of free Ca^{2+} and the activity of calpain-2.

II. MATERIALS AND METHODS

Eight porcine *longissimus* muscles were obtained 6 h after slaughter. Each loin was divided into five pieces. One half of each piece was used as non-frozen control group which was aged at 2 ± 1 °C for 1, 2, 4, 6 and 9 days; the other half was frozen immediately at -20 ± 1 °C for 1 week and then thawed at 2 ± 1 °C overnight, followed by aging for the same period used in the non-frozen group. Calpains were measured using a casein zymography method [2]. Free Ca^{2+} was determined in sarcoplasm as described by Pomponio *et al.* [5]. Each analysis was performed in triplicate. The significant differences between means (significance was defined at $P < 0.05$) were evaluated by Tukey HSD test by the IBM SPSS Statistics 24 software.

III. RESULTS AND DISCUSSION

The free Ca^{2+} was measured since it is central to activation of calpain. Ca^{2+} in the range of 0.4-0.8 mM is required for half-maximal activity of calpain-2 [6]. In this study, we observed a significant increase of free Ca^{2+} concentration from 0.13 mM at day 1 to 0.40 mM at day 9, and a decrease of calpain-2 activity at day 9 to 87% of the initial activity in the non-frozen group (Fig.1) and a parallel increase in autolyzed calpain-2 (results not shown). Accordingly, 0.40 mM of free Ca^{2+} is only able to activate a small proportion of calpain-2 in fresh pork, whereas 0.5 mM of free Ca^{2+} in sarcoplasm is sufficient to decrease calpain-2 activity to only half of the initial activity in frozen-then-chilled pork. The freezing-thawing process resulted in a higher free Ca^{2+} level (0.42 mM) at day 1 (Fig.1), presumably due to rupture of the sarcoplasmic reticulum by ice crystals. However, no significant changes of calpain-2 activity was found at day 1 in the frozen-thawed group (Fig.1). During storage, we observed an additional smaller increase in the free Ca^{2+} concentration, and a substantial faster decrease in the calpain-2 activity in the frozen-thawed group (Fig.1). Apparently, a delay occurred between an increase in the free Ca^{2+} level and the activation of calpain-2. In agreement, a decrease in calpain-2 activity was also observed in beef following a freezing-thawing process [7].

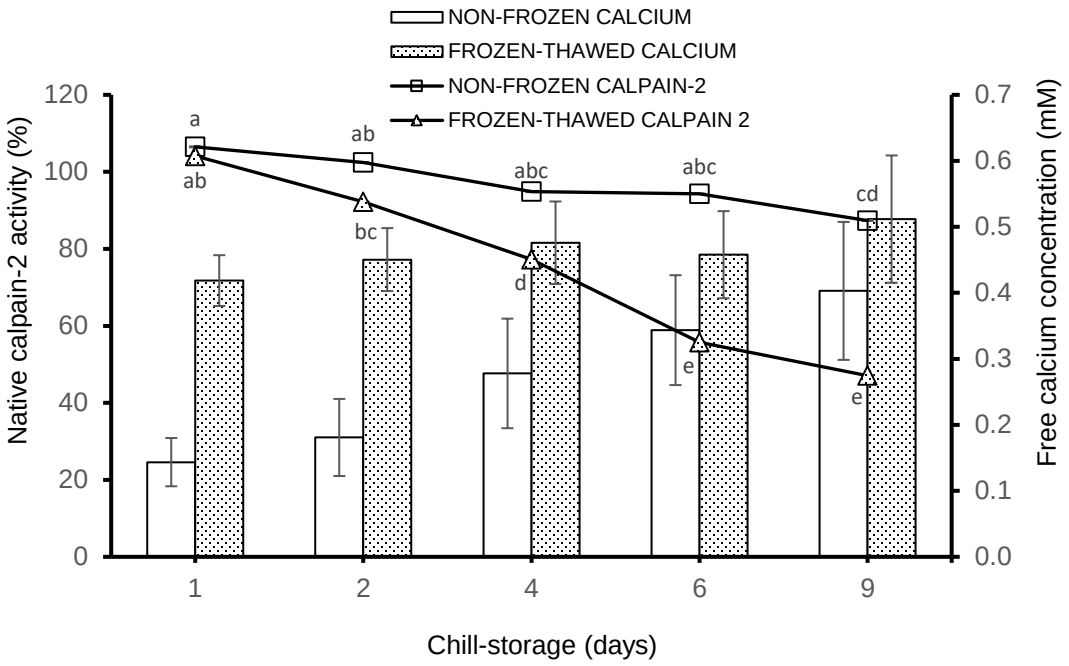

Fig. 1. Effect of chill storage following freezing-thawing on sarcoplasmic free Ca^{2+} concentration and the activity of native calpain-2. Calpain-2 activity at 12 h in the non-frozen samples were taken as 100%. Free Ca^{2+} values are given as means ± standard deviation. Superscripts with the same letter do not differ (P >0.05).

IV. CONCLUSION

Freezing-thawing of pork *longissimus* muscle resulted in a substantial release of free Ca^{2+}, and subsequent chilled storage activated about 50% of calpain-2. Frozen-then-chilled storage can be a method of increasing the overall activity of calpain-2, possibly leading to improved tenderness.

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