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**ACCEPTED MANUSCRIPT** 

Effects of frozen-then-chilled storage on proteolytic enzyme activity and water-holding capacity of pork loin

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Abstract

This study aimed to determine the effect of frozen-then-chilled storage on free Ca<sup>2+</sup>, proteolytic

enzyme activity of calpains and the proteasome, water-holding capacity and shear force of porcine

longissimus thoracis et lumborum muscle. Pork loins were subjected to either chilled storage at  $2 \pm$ 

1 °C for 1, 2, 4, 6 and 9 days, or frozen-then chilled storage (-20  $\pm$  1 °C for 1 week followed by

thawing overnight). Free Ca<sup>2+</sup> increased with chilled storage in the non-frozen group. Frozen-then-

chilled storage increased free Ca2+ concentration, followed by a faster decrease of calpain-1 activity

and activation of around 50% of calpain-2. Proteasome activity was reduced by around 40% following

freezing-thawing. Purge loss increased and water-holding capacity of myofibrils decreased in the

frozen-thawed group, suggesting considerable denaturation of myofibrillar proteins. Shear force was

not affected by freezing-thawing, and we speculate that the tenderizing effect of calpain activation was

counteracted by loss of proteasome activity and substantial exudate loss.

Keywords: Freezing, Calpains, Proteasome, Purge loss, Shear force

1. Introduction

Freezing is currently playing an essential role in extending the shelf-life of meat and meat products by

preventing microbial spoilage and retarding oxidative deterioration. Often no major changes are

observed in sensory properties of frozen-thawed beef (Holman, Coombs, van de Ven, & Hopkins, 2017;

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