

Effect of pre-rigor temperature on Ca²⁺ concentration and translocation of calpain-1 from sarcoplasm to myofibrils in pork longissimus muscle

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Objectives: The calpain system is generally recognized to play an important role in meat tenderization during storage, and temperature is one of the factors that influences its activation and thereby postmortem proteolysis [1]. It has been reported that during early storage, calpain-1 gradually binds to myofibrils in meat [2-4] induced by Ca²⁺ [4, 5], and myofibril-bound calpain-1 has been suggested to be the reason of degradation of myofibrillar proteins [4]. However, the link between temperature and translocation of calpain-1 from the sarcoplasm to the myofibril is currently not known. The aim of this study was to investigate the effect of pre-rigor temperature on Ca²⁺ concentration and translocation of calpain-1.

Materials and Methods: Pork *longissimus* muscle was obtained 1 h postmortem, then pre-rigor incubated at 14, 22, 30 and 38 °C until 6 h postmortem, followed by another 2 h incubation at 14 °C and thereafter stored at 2 ± 1 °C for 4 days. Free Ca²⁺ level in the sarcoplasm was measured according to the method described by Zhang & Ertbjerg (2018) [6]. Western blot and quantification of calpain-1 in the sarcoplasmic and myofibrillar fractions were done according to the Lyu & Ertbjerg (2022) [4].

Results and Discussion: The amount of myofibril-bound calpain-1 on day 4 became greater with the increased pre-rigor incubation temperature, while the amount of sarcoplasmic calpain-1 decreased. In parallel, the free Ca²⁺ concentration in the sarcoplasm increased with the improved incubation temperature. The results suggest that calpain-1 gradually translocated from the sarcoplasm to the myofibrils with a greater level of free Ca²⁺ due to elevated pre-rigor temperature. It has been also reported that *in vitro*, added purified calpain-2 became increasingly bound to isolated myofibrils with increased concentrations of Ca²⁺ [4]. Additionally, an increased amount of calpain-1 was found to bind to myofibrils in beef [2], lamb [3] and pork [4] during early storage, and it is well known that the Ca²⁺ concentration in the sarcoplasm increases during storage of meat [6, 7]. Thus, there is supporting evidence that calpain-1 translocates from the sarcoplasm to the myofibrils induced by an increase of free Ca²⁺ concentration during storage.

Conclusions: During postmortem storage, calpain-1 translocates from the sarcoplasm to the myofibrils with increased concentration

of sarcoplasmic Ca²⁺ due to higher pre-rigor temperature.

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Key words: Pre-rigor temperature, Ca²⁺, Calpain translocation, Myofibril-bound calpain, Pork