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EFFECT OF CHILLING RATE ON CALCIUM ION RELEASE THROUGH RYANODINE RECEPTOR 1 REGULATING POSTMORTEM TENDERNESS

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I. INTRODUCTION

Very fast chilling (VFC) offers potential benefits in saving chilling time, reducing weight loss, and improving meat quality [1]. Compared to conventional chilling (1.94 ℃/h), VFC (15.1 ℃/h) significantly increased free Ca^{2+} concentration in lamb at pre-rigor time and promoted meat tenderization [2]. The role of ryanodine receptor 1 (RyR1) as a calcium channel appears to participate in postmortem Ca^{2+} release under VFC. The objective of the present study was to explore the effect of chilling rate on Ca^{2+} release through RyR1 regulating postmortem tenderness.

II. MATERIALS AND METHODS

Longissimus thoracis et lumborum muscles (n=8) were obtained from carcasses (Landrace × Yorkshire × Duroc pig, 6 months old) at 1 h postmortem. Each muscle was cut out approximately 70 g marked as 1 h postmortem sample. Remaining muscle was sectioned into three portions (6 × 12 cm; diameter × length), bag-packaged, and randomly allocated to one of three treatments: low (LCR, 0-4℃ conventional air chilling, 4.26℃/h), medium (MCR, crushed ice chilling, 8.89℃/h), or high chilling rate (HCR, -1℃, 50% (v/v) propy-glycol solution immersion chilling, 13.33℃/h). Each muscle was divided into six blocks and immediately transferred to a 0-1℃ refrigerator when core temperature reached 1℃, randomly allocated to six time points: 2-5, 6, 12, 24, 72, 120, and 168 h postmortem. To determine shear force, samples were boiled at 71℃ for 35 min and cut parallel to muscle fibers into strips (1.0 cm^2 cross-section × 1.5 cm length) after being cooled, conducted with a tenderometer. Ca^{2+} concentration was measured using kits. In addition, total protein solution was run at SDS-PAGE (4% stacking/4% separation gel, 120V) and transferred to nitrocellulose membranes (380 mA, 100 min), incubated with RyR1 primary antibody (1:1000, 4℃, overnight) and secondary antibody (1:2500, room temperature, 1.5 h). Nitrocellulose membranes treated with enhanced chemiluminescence were scanned by Imaging System to analyze RyR1 expression.

III. RESULTS AND DISCUSSION

Shear force exhibited differential postmortem patterns across treatments, with LCR and MCR groups showing first increased and then decreased, whereas that in HCR samples gradually decreased ($P < 0.05$, Figure 1A). At 24 h postmortem, the samples treated with HCR were observed the lowest shear force with exhibiting intermediate values in MCR group and the highest shear force in LCR treatment samples. The Transmission electron microscope analysis revealed that I-bands and A-bands in MCR and HCR samples were not visible at 2-5 h postmortem (Figure 1B). HCR-treated samples were found more misalignment of the sarcomeres with fracture at Z lines as similar as super-contraction [3]. The pork under HCR (13.33℃/h) did not produce a peak from rigor mortis and pork tenderness was improved by sarcomere damage, likely caused by the excessive calcium release.

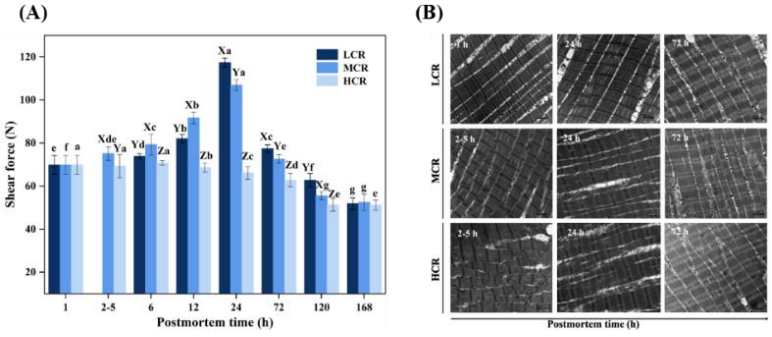


Figure 1. Effect of chilling rate on shear force (A) and sarcomere structure (B). Transmission electron microscope images were taken at 15000 ×. a-e: Different letters under the same chilling rate group indicate significant differences at different postmortem times ($P < 0.05$). X-Z: Different letters at the same postmortem time indicate significant differences between different chilling rate groups ($P < 0.05$).

The Ca^{2+} concentration of sarcoplasm and sarcoplasmic reticulum was determined to characterize the degree of Ca^{2+} release. Both samples in MCR and HCR group occurred a sharply increase of sarcoplasm Ca^{2+} concentration and decrease of sarcoplasmic reticulum Ca^{2+} concentration at 2-5 h postmortem, which was earlier than those in the LCR group ($P < 0.05$, Figure 2A & B). But the effect of MCR and HCR had a difference on Ca^{2+} release extent ($P < 0.05$). The Ca^{2+} concentration resulted in an increase by 636% to 386 μM in the HCR group, while that in the MCR chilling rate group by 424% to 275 μM ($P < 0.05$). The expression of RyR1 was also measured to explain how chilling rates affect the Ca^{2+} release. A higher protein expression of RyR1 was observed in the MCR and HCR groups from 2 to 24 h postmortem ($P < 0.05$, Figure 2C & D), especially in the HCR group. The expression of calcium channel RyR1 significantly increased reflecting on the increase of Ca^{2+} release under HCR treatment [4].

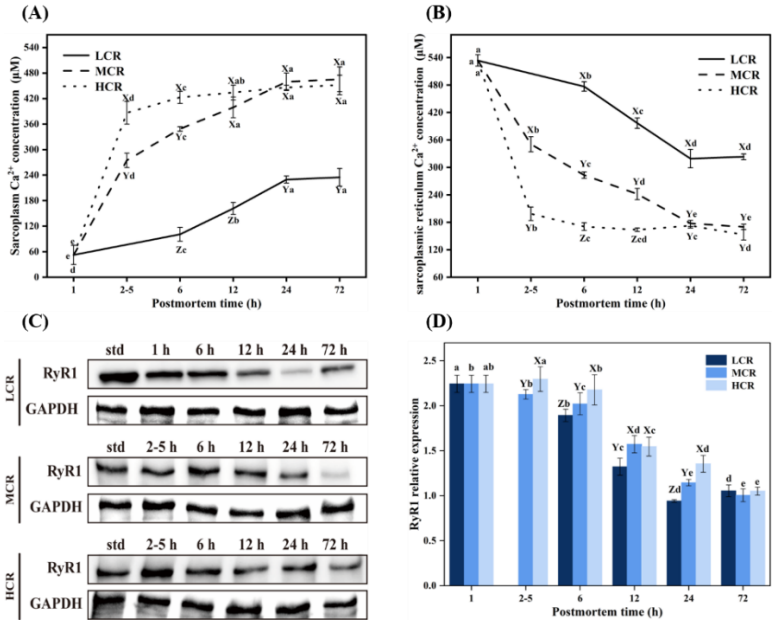


Figure 2. Effect of chilling rate on of Ca^{2+} concentration in the sarcoplasm (A) and sarcoplasmic reticulum (B), and RyR1 expression (C & D). a-e: Different letters under the same chilling rate group indicate significant differences at different postmortem times ($P < 0.05$). X-Z: Different letters at the same postmortem time indicate significant differences between different chilling rate groups ($P < 0.05$).

IV. CONCLUSION

The use of HCR (13.33℃/h) in comparison to LCR (4.26℃/h) may increase the level of RyR1 enhancing Ca^{2+} leakage from the sarcoplasmic reticulum to the sarcoplasm at early postmortem. This excessive increase of sarcoplasm Ca^{2+} concentration, causing the super-contraction bands in muscle, which stretched surrounding sarcomere and damaged microstructure in fibers, thus improving pork tenderness.

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