TENDERIZING EFFECTS OF TWO EXOGENEOUS ENZYMES ON BOVINE SEMITENDINOSUS MUSCLE

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I. INTRODUCTION

Tenderness is a crucial factor for consumer acceptance of meat, and there is huge variation in quality between the different muscles of a carcass. Although ageing improves tenderness, the majority of muscles cannot reach “steak-quality” by traditional ageing. Polkinghorn et al. stated that less than 10% of a bovine carcass are prime grilling cuts [1]. Therefore, the majority of cuts will need some kind of processing to obtain high eating quality. Several studies have documented that exogenous proteases can improve meat tenderness [2,3]. The most widely used enzymes for meat tenderization are the plant enzymes papain, bromelain and ficin, which all are classified as GRAS (Generally Regarded As Safe) by food authorities. Kiwifruit contains actinidin, which is a cysteine enzyme in the same family as the previously mentioned. However, actinidin is not GRAS certified despite kiwifruit is consumed worldwide. Recently it was reported that aspartic enzymes obtained from the GRAS classified fungus *Rhizomucor miehei* is capable to tenderize meat [4], although they usually are applied in cheese manufacturing. The purpose of this study was to compare the tenderizing effects of actinidin with Tail21, which is an aspartic enzyme obtained from *Rhizomucor miehei*.

II. MATERIALS AND METHODS

Ten *Semitendinosus* muscles (ST) from young bulls (approximately 17 months old) were purchased from a commercial slaughterhouse. Seven days post mortem each muscle was cut into 4 pieces. Three pieces were injected to 110% weight with solution A, B or C, while the fourth was used as control. Solution A, B and C were all composed of 2% NaCl and 3% sodium triphosphate. In addition, solution B and C had 0.5% of the enzymatic products OT-1005X and Tail21, respectively. OT-1005X is a food grade powder made from kiwi fruits, which contains the cysteine protease actinidin. Tail21 is an acid fungal food grade protease intended for meat tenderization produced by submerged fermentation with a selected strain of the fungus *Rhizomucor miehei*. Both enzymes were obtained as kind gifts from the suppliers. After brine injection the meat samples were stored in sealed plastic bags for 8 days at 4ºC. Then a 3 cm slice, from each meat sample, was cooked at 70ºC before Warner-Bratzler (WB) shear force measurements (Instron) were conducted. The rest material (uncooked) were frozen until further analysis. Degradation of myosin were measured by Western blotting of SDS-PAGE gels. Protein bands were quantified with the Image Quant software (GE Healthcare). Statistical analysis was performed with the software Minitab (Version 18.1).

III. RESULTS AND DISCUSSION

The average WB shear force value was significantly ($p<0.001$) smaller for samples injected with solution B (23.65 N/cm^2) compared to solution A (35.12 N/cm^2) and C (32.41 N/cm^2), while no difference was found between A and C. This result shows that actinidin has a tenderizing effect when injected into bovine muscles, and is in agreement with the findings of Christensen et al. [3] who marinated porcine *biceps femoris*. Usually the maximum peak value is used when reporting WB values. However, by inspecting the complete shear force curves it is possible to get information about myofibrillar and connective tissue composition in the sample. As reported by Møller [5] peaks appearing late in the shear force curve comes from connective tissue, while myofibrillar proteins give peak in the middle. Figure 1 shows typical shear force curves for samples injected with the three solutions A, B and C, which clearly indicate that actinidin degrade connective tissue.

Myosin is one of the key proteins in the myofibril but is not substantially degraded by the endogenous proteases. Therefor enzymes capable to degrade myosin could improve tenderness. Although the samples injected with the C solution had similar WB shear force as the control samples, an interesting result was found when myosin degradation was compared before and after cooking. Figure 2 shows that Bc and Cc, which is

the cooked samples injected with solution B and C respectively, have clear myosin degradation spots. In contrast, Br and Cr, which is the raw samples injected with solution B and C respectively, have no degradation spots. This means the enzymes degrade myosin during the cooking process ahead of the WB shear force measurement, but not during cold storage. Based on the intensity of the degradation spots it seems like the *Rhizomucor miehei* enzyme degrades myosin more effectively than actinidin. Maybe a slower cooking process, which could have given longer action time for the enzymes, had changed the final shear force result. However, that has to be explored in future studies.

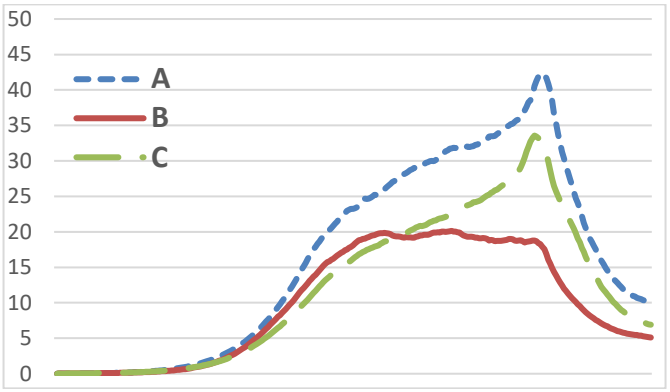

Figure 1. Mean WB shear force curves obtained from sub-samples of the same muscle and animal, but injected with the three solutions A, B and C.

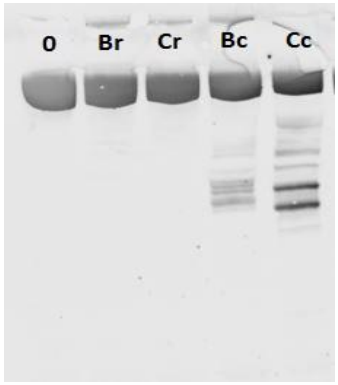

Figure 2. Westen immunoblot showing degradation products of myosin heavy chain. 0: uncooked (raw) control sample, Br: raw sample injected with solution B 8 days p.m., Cr: raw sample injected with solution C 8 days p.m., Bc: cooked sample injected with solution B 8 days p.m.,Cc: cooked sample injected with solution C 8 days p.m.

IV. CONCLUSION

This study has shown that actinidin tenderize the *semitendinosus* muscle, while the Tail21 enzyme obtained from *Rhizomucor miehei* may have potential to improve bovine tenderness.

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