REAL-TIME MICROSTRUCTURAL ANALYSIS OF CONNECTIVE TISSUE FRAGMENTS DURING HEATING

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I. INTRODUCTION

Meat is cooked to make a palatable and microbiologically safe part of a meal. However, heating causes contraction of muscle tissue due to protein denaturation. This can result in moisture to be squeezed out making the meat denser and less juicy. Shrinkage of connective tissue is believed to be one of the mechanisms behind moisture loss and volume decrease upon cooking of meat [1]. Another viewpoint is that the myofibrillar proteins drive the overall shrinkage [2]. In this study, the change in structure of isolated connective tissue fragments with increasing temperature, up to 90 °C, was investigated by confocal laser scanning microscopy (CLSM) to obtain real-time understanding of microstructural events during cooking.

II. MATERIALS AND METHODS

Five eye round (*M. semitendinosus*) muscles (British breeds, average carcass weight 248 kg, average fat 16 mm, no permanent incisors) were used to extract endomysium. 20 g of muscle tissue was homogenised for 30 s in 180 mL cold Mannitol buffer (380 mmol, pH 5.6) using a Waring type blender. The connective tissue fraction was collected with an Abichem test sieve with 150 µm aperture, rinsed, resuspended and flurorescently labelled with Fast green.
A Leica TCS SP5 CLSM (Leica Microsystem, Germany) with a Linkam THMS600 temperature stage (Linkam Scientific Instruments Ltd, UK) was used to visualise the connective tissue fibres. Samples on sealed microscope cavity slides were heated at 7 °C/min to 90 °C. The extent of connective tissue shrinkage was calculated as percentage decrease of the area occupied by connective tissue fibres in each micrograph compared to their initial area.

III. RESULTS AND DISCUSSION

The connective tissue fibres in this study were composed of entangled, long, single, collagenous fibres. The large scale order of the connective tissue was destroyed through the removal of muscle tissue and subsequent homogenisation to obtain fragments trackable by microscopy. In Fig. 1, examples of an endomysial fibre entity along with a muscle fibre fragment are shown as the temperature increased from 25 to 90 °C. At 65 °C, the endomysial fibre fragments had shrunk less than 10 % while retaining their original shape. In comparison, epimysial fibres had fully undergone denaturation transformation at this temperature (data not shown). At higher temperatures, the endomysial structure quickly became more compact and changed its shape. The transformation was completed when reaching 80 °C with a total shrinkage of occupied area around 70 %. Interestingly, no signs of gelatinisation were observed, even in samples kept at 90 °C for 2 hours. In Fig. 1, the effect of heating on a muscle fibre fragment can also be seen. It displayed a small reduction in diameter from 60 °C but the major change, a decrease in length, occurred between 75 and 85 °C.

Two types of behavior of heated endomysium were observed; one with a greater degree and rate of shrinkage (around 70%) and another where the shrinkage was gradual and only 30-50% in total. These differences could possibly be explained by the endomysium consisting of various amount of thermally stabile cross-links [3] and, hence, would resist change upon heating to different temperatures. Further research is needed to classify these different types of connective tissue fibres.

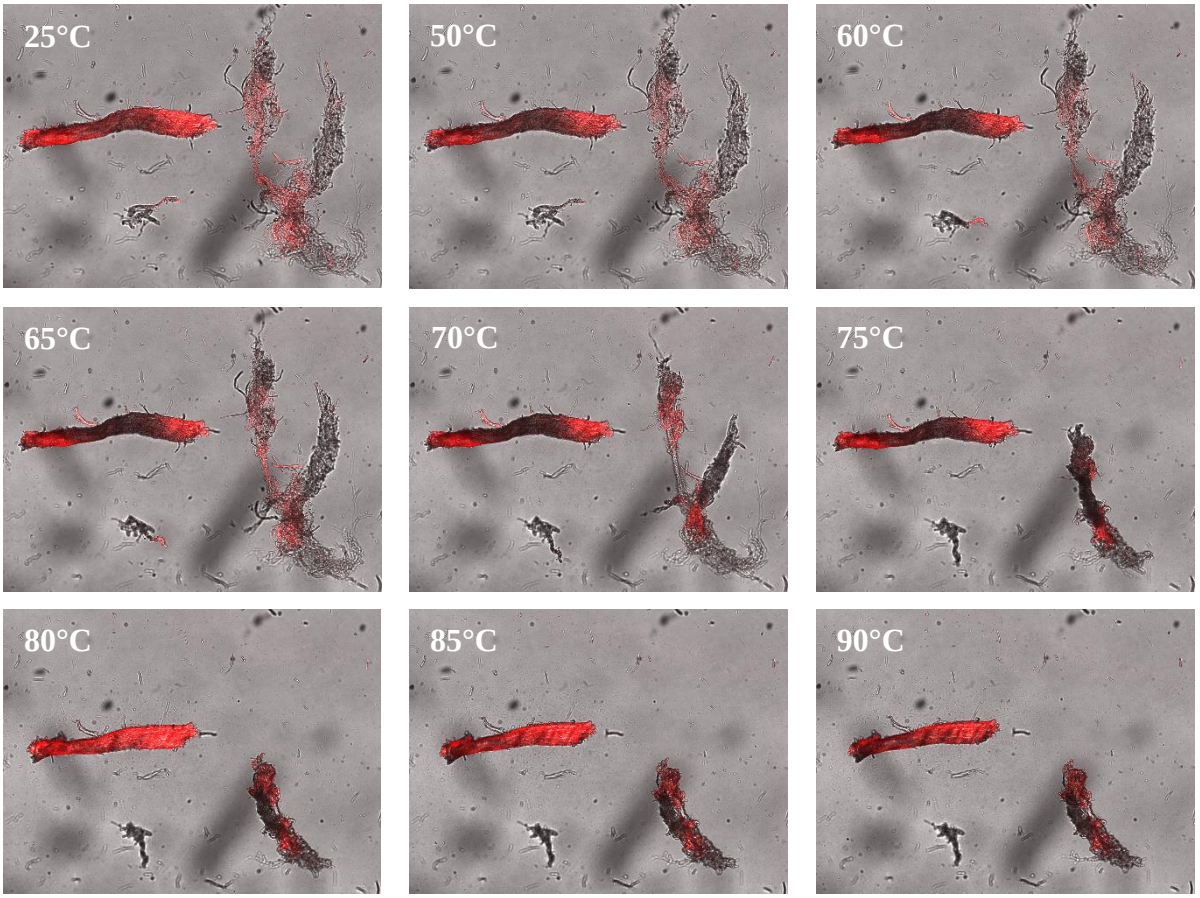

Figure 1. CLSM images of muscle fragments captured during heating from 25 to 90 °C. The feature to the left shows a muscle fibre fragment and the one to the right an endomysial fibre assembly. Image size: 580 µm x 775 µm

IV. CONCLUSION

Differences in the degree of structural change upon heating and denaturation temperature between various types of connective tissue and muscle tissue were observed. It should be noted that these experiments were carried out with freely floating connective tissue fibres, hence, the behavior when restricted in a muscle structure might be different. Even though one type of endomysial fibres shrunk to 70% of their original size, the contraction force of the denatured proteins in a muscle setting would not be strong enough to compress the meat to the same degree.

This study confirmed that when beef is cooked above 70 °C, both the individual connective tissue and muscle fibres shrink considerably and consequently, fluid (cook loss) could be squeezed out through this action. Hence, it is recommended to keep the meat at or slightly below 70 °C to retain its juiciness when cooking.

ACKNOWLEDGEMENTS

The authors thanks the Australian Meat Processor Corporation for co-funding of this research.

REFERENCES

1. Lepetit, J., Grajales, A., & Favier, R. (2000). Modelling the effect of sarcomere length on collagen thermal shortening in cooked meat: Consequence on meat toughness. Meat Science, 54(3), 239-250.
2. Hughes, J. M., Oiseth, S. K., Purslow, P. P., & Warner, R. D. (2014). A structural approach to understanding the interactions between colour, water-holding capacity and tenderness. Meat Science, 98(3), 520-532.
3. Tornberg, E. (2005). Effects of heat on meat proteins - Implications on structure and quality of meat products. Meat Science, 70(3), 493-508.