Hydrothermal isometric tension properties of perimysial connective tissue in bovine *semitendinosus* muscle

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Abstract: Heat-induced shrinkage of collagen is generally believed to be a driving force of meat shrinkage on cooking. Previous studies of shrinkage forces in collagenous tissues have not included direct measurements on the major intramuscular connective tissue structure, i.e. the perimysium, despite its presumed role in changes in tenderness during the cooking of meat. Hydrothermal isometric tension tests were performed on perimysial tissue samples isolated from raw meat. Under a regime of heating at 3°C/min from room temperature to 87°C, individual perimysium strands from 9-day post-mortem bovine *semitendinosus* muscle developed on average a tension of 16.8g (S.E. 4.35g, n=21). Subsequent holding the perimysial samples at 85°C for a further 30 minutes caused only a 12.5% reduction in force, indicating that the perimysium in this muscle is very heat-stable. The results support the hypothesis that forces generated in the perimysiual network within meat during cooking could contribute significantly to cooking losses above 65^{0}C.

Key words: perimysium, collagen, meat tenderness, cooking, shrinkage forces

I. Introduction

As meat is cooked to progressively higher temperatures, both the toughness of the cooked product and the amount of cooking exudate (fluid loss) increases (Davey and Gilbert, 1974; Tornberg 2005). Correlations between thermal denaturation of proteins in meat and changes in tenderness with cooking temperature (Martens et al, 1982) suggest that increased meat toughness at cooking temperatures above 65°C may be associated with denaturation of collagen within the intramuscular connective tissue (IMCT). The largest component of IMCT is the perimysium (McCormick, 1994, Purslow 2005, 2014) and the amount of perimysium varies between muscles. Lepetit (2008) suggested a role for IMCT in the development of toughness in cooked meat due to the rubber-like elasticity of heat denatured collagen networks stabilised by covalent crosslinks and the pressure on muscle fibre contents that is presumed to occur due to the heat-induced shrinkage of these networks.

Perimysial IMCT can contribute to variations in meat toughness with cooking temperature in three ways. Firstly, the tensile strength of the perimysial strands can change with temperature Lewis and Purslow (1989) showed that the strength of perimysium isolated from cooked beef *semitendinosus* increases from room temperature to 50°C

and decreases at temperatures above this. Secondly, the number of perimysial strands per unit cross sectional area in a piece of meat may increase as the muscle fibres and fascicles shrink laterally. Thirdly, the forces produced in the perimysial network surrounding the fascicles on cooking may squeeze fluid out of muscle fibres, making them tougher.

Considerable forces are produced in collagenous tissues if heated while restrained from changing length, as measured by hydrothemal isometric tension (HIT) studies (Allain et al., 1978, Horgan et al., 1990, Kopp & Valin, 1980-81). However, the HIT studies reported to date are either on collagenous tissues such as skin tendon or epimysium from muscle. There does not appear to be data in the literature for the tension that can be generated in perimysium. HIT tests were therefore conducted to assess the forces that can be generated by the most important IMCT component in relation to meat cooking.

II. Materials and Methods

Semitendinosus muscles were obtained from two commercially-available Angus heifers (approximately 30-34months old) and excised from the carcasses 48 hours post mortem. Muscles from both animals were cut transverse to the muscle fiber direction, into 1-inch thick slices, vacuum packed immediately after cutting, and aged in a refrigerator at 4°C for a further 7 days prior to freezing at -20°C. All samples remained frozen until use. Small strips of perimysial connective tissue were dissected from thawed raw muscle slices using a surgical scalpel. Each strip was placed in the apparatus described by Purslow et al (1998) to measure force at a fixed length and suspended in phosphate buffered saline (PBS). Using an EchoTherm^{TM} Programmable Digital hot plate/stirrer, the specimen was heated in the PBS saline bath at a linear rate of 3º per minute with constant stirring until a target temperature of 85ºC was reached. Due to thermal inertia, there was an overshoot of 2ºC in the system, so that the maximum temperature reahed was actually 87ºC in al specimens. The temperature was then held constant at 85 ºC for a further 30 mins. Peak force at maximum temperature was recorded. At the end of the 30 min holding period, the residual force in the specimen was measured. The drop in load from peak force to this value was expressed as a % relaxation from the peak load.

III. Results and Discussion

Figure 1 shows a graph of load and temperature versus time for a typical specimen.

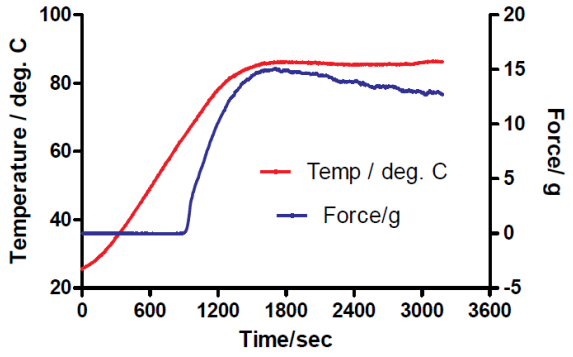

Fig.1. Force (right axis) and temperature (left axis) versus time during a typical test.

No appreciable load is generated until the temperature passes 65°C, and thereafter the relationship between load and temperature during the heating ramp is linear, with gradient of 0.67 g/°C. After the maximum temperature is reached, the small drop of 2°C

to the holding temperature of 85°C was accompanied by a small drop in load, but during the subsequent holding period there was only a small relaxation of load. The average behavior of all 21 samples tested is summarized in table 1.

Table 1. Mean values and standard errors for maximum force and % relaxation (n=21)

	Maximum force / g	% relaxation from peak force to end of test
Mean	16.85	12.48
Standard error	0.95	0.71

These results show that perimysium from 7 day-aged bovine *semitendinosus* muscle generates considerable shrinkage forces only when heated above 65°C. Unlike the majority of results reported for skin (Allain et al., 1978), tendon (Horgan et al., 1990) and epimysium, (Kopp & Valin, 1980-81), the relaxation in load in the perimysium at high temperatures is very small. When held at 85°C for 30 mins there was only a relaxation in isometric force by 12.5% of te maximum value. Shrinkage forces within the perimysium in a piece of meat being held at high temperatures during long cooking procedures could therefore continue to be significant and contribute to the forces driving cooking loss.

Kopp & Valin (1980-81) reported that almost half of collagen in the epimysium from the longissimus dorsi muscle of 16 month-old bulls could be solubilised on heating to 96°C. They also showed that this amount could be increased slightly by proteolysis of the tissue. In our samples of perimysium tsken 9 days post-mortem form bovine *semitendinosus* muscles, collagen solubilisation on heating to 85°C with a holding period of 30 mins was measured on 5 samples and was found to be on average only 3.62% of the total. This small degree of solubilization is commensurate with the small relaxation in shrinkage force. It uggests that the perimysium from this muscle is very highly resistant to solubilisation on cooking.

IV. Conclusions and future work

The results reported here are, to our knowledge, the first studies of thermal shrinkage forces that have actually been measured on perimysium directly, rather than other connective tissues or whole pieces of meat. The results support the hypothesis that shrinkage forces in intramuscular connective tissue at cooking temperatures above 65°C are considerable, and could contribute to fluid loss from meat and hence increase meat toughness at high temperature. However, Tornberg (2005) shows that cooking losses in comminuted meat (where the perimysial network is broken down) are not significantly smaller than in whole muscle, so that further research is necessary to clarify whether the shinkage forces of perimysium reported here actually contribute to cooking losses and increasing toughness at high temperature.

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