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The thermal shrinkage force in perimysium from different beef muscles is not affected by post-mortem ageing

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ABSTRACT

Differences in the thermal shrinkage and collagen solubility between bovine *Semitendinosus* (ST) and *Pectoralis profundus* (PP) muscles and their interactions with ageing were evaluated by studying collagen solubility, hydrothermal isometric tension and thermal denaturation properties of intramuscular connective tissue after 5–20 days post-mortem storage at 4 °C.

Collagen solubility was higher in ST than in PP muscle at 5–13 days, but the differences between the two muscles decreased at longer ageing times. A small decrease in the peak denaturation temperature of perimysium occurred with increasing ageing times in both muscles. Maximum force in isometrically-heated perimysium was broadly equivalent in both muscles. Although the amount and solubility of collagen varies between muscles and ageing decreases the stability of some of the collagen, thermal shrinkage forces in heated perimysium are not significantly diminished by ageing.

These findings support the idea of one collagen fraction easily degraded by ageing and heat, and another more resistant fraction that determines the physical properties of the tissue after ageing and cooking.

1. Introduction

Variations in the structure and composition of intramuscular connective tissues are known to exist between muscles and are related to difference in cooked meat toughness between muscles (Bailey & Light, 1989; Dransfield, 1977; Light, Champion, Voyle, & Bailey, 1985; Nishimura, 2010; Purslow, 2005). The perimysium has been shown to be the most variable component of intramuscular connective tissue (Purslow, 1999) and also to be the connective tissue component most involved in resisting the breakage of cooked meat (Light, Champion, Voyle, & Baley, 1985; Purslow, 1985). Torrescano, Sánchez-Escalante, Giménez, Roncalés, and Beltrán (2003) have shown differences in the collagen solubility between different muscles. In addition, Archile-Contreras, Mandell, and Purslow (2010) have demonstrated that changes due to nutritional treatments in the solubility of collagen from perimysium of different muscles differed from muscle to muscle.

Previous reports on the strength and extensibility (Lewis & Purslow, 1989; Lewis, Purslow, and Rice, 1991) and the hydrothermal isometric tension generated on heating isolated perimysial strips (Latorre, Lifschitz, & Purslow, 2016) have used samples isolated from the bovine *M. semitendinosus*. Although the perimysium from this muscle is relatively easy to isolate by dissection from both raw and cooked muscle samples, the connective tissue in bovine *M. Semitendinosus* also has an unusually high content of elastin. It is therefore quite possible that the perimysium from other bovine muscles may show different physical properties, or react to treatments affecting connective tissue properties in a different manner. Voutila, Mullen, Ruusunen, Troy, and Puolanne (2007) studied the stability of the connective tissue in the three porcine muscles by Differential Scanning Calorimetry (DSC) and concluded that the thermal properties differed between muscles.

Although it has long been recognized that there is some biochemical degradation of the intramuscular connective tissue (IMCT) in raw muscle during ageing, there has been some debate as to what this means for cooked meat toughness. Stanton and Light (1988) indicate that subtle modifications occur in intramuscular collagen during conditioning which can be correlated with catheptic action. Etherington (1987) demonstrated the comparable action of cathepsins to pepsin in attacking collagen at the non-helical terminal ends of molecule. Nishimura, Hattori, and Takahashi (1995) extracted raw perimysium by NaOH digestion from muscles aged for different periods and then

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measured the strength of the residuum. They infer that reductions in strength of the raw connective tissue during ageing must imply a lower contribution to cooked meat toughness. However, the studies of Bouton and Harris (1972) show no variation in the IMCT contribution to the shear force toughness of cooked beef with ageing. Lewis, Purslow and Rice (1991) clearly showed that the breaking strength of raw perimysium isolated from aged *M. semitendinosus* (ST) was lower than that of raw perimysium isolated from unaged ST – but that after cooking the meat to temperatures above 60 °C, the strength of both the aged and unaged samples of perimysium had fallen to similar values. These results explained why degradation of collagen could be seen biochemically (Stanton & Light, 1988) and mechanically (Nishimura et al., 1995) in raw meat, but have no effects on cooked meat toughness (Bouton & Harris, 1972). Purslow (2014) inferred from this that there may be two pools of collagen in the perimysium; one that is easily degraded by enzymes and/or heat, and another thermally and mechanically stable pool. Judge and Aberle (1982) and Mills, Smith, and Judge (1989) showed that the thermal denaturation temperature (as measured by T_{max} values from DSC) of intramuscular collagen decreases in the early post-mortem period (0–24 h).

Latorre et al. (2016) highlight the theory of Lepetit (2008) that intramuscular collagen can contribute to cooked meat toughness either by the high strength of the perimysial network directly contributing to the Warner-Bratzler peak shear force, or by providing a thermal shrinkage force that drives out water from the myofibrillar proteins, so increasing their content in cooked meat and thereby providing a higher shear force. Whereas the measurements of Lewis, Purslow and Rice (1991) specifically exclude an effect of ageing on the first of these mechanisms, the possible effect of ageing on the second remains unclear. Hence, this study uses hydrothermal isometric tests as well as DSC measurements and collagen solubility measures to resolve the question of whether there are differences in the thermal shrinkage forces produced by perimysium from different muscles, and whether these react differently to post-mortem ageing.

2. Materials and methods

Three *Aberdeen Angus* steers were fed on pasture and slaughtered at their commercial weight (~400 kg) following standard handling procedures. All animals were slaughtered on the same day. The *M. semitendinosus* (ST) and *M. pectoralis profundus* (PP) muscles were removed from the right-hand part of chilled carcasses at 5 days post-mortem. The pH of all muscles samples was in the range of 5.5–5.7. Each muscle was divided in four equal pieces and each one was packed in polythene bag. One sample of each muscle was frozen immediately at − 20 °C (5 days aged) the other three pieces were stored at 4 °C and then frozen after a total maturation time of 7, 13 or 20 days. The samples of both muscles with either 5, 7, 13 or 20 days post-mortem ageing were then subjected to the following chemical, physical and thermal analyses.

2.1. Collagen solubility

Muscle slices from each animal were scissor-cut into small pieces and a 10 g sub-sample was used for thermal treatment according to the Latorre et al. (2016) procedure. Supernatant fluids and solid residues were separated by centrifugation (5.000 rpm, 10 min 25 °C) and both were dried in an oven at 60 °C. Each fraction was then hydrolyzed in 5 ml HCl (6 N) at 110 °C for 16 h. After hydrolysis, samples were neutralized and the hydroxyproline concentration was determined by the spectrophotometric determination of hydroxyproline by the colorimetric method of Bergman and Loxley (1963). The % of soluble collagen was calculated as 100 × the hydroxyproline content of the soluble phase divided by the total hydroxyproline in both the soluble phase and the solid residue.

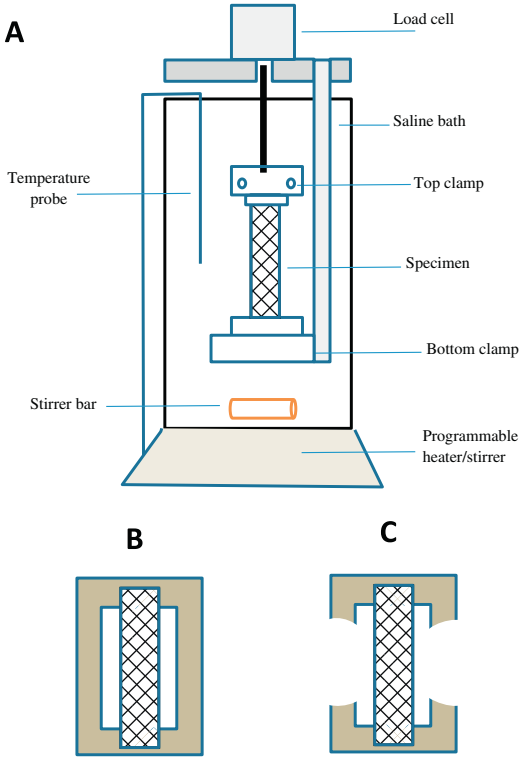

Fig. 1. Schematic diagram showing (a) the hydrothermal isometric tension apparatus, (b) the attachment of a perimysial strip onto an aluminum foil frame, and (c) the cutting of the frame after mounting it into the grips of the apparatus. The aluminum frame allows placement of the specimen in the apparatus without undue stretching.

2.2. IMCT - perimysium extraction

Small strips of perimysial connective tissue were dissected from subsamples of each muscle and each post mortem time point as described by Latorre et al. (2016).

2.3. Hydrothermal isometric tension (HIT)

Isolated perimysial strips from each muscle sample were placed in an apparatus designed to measure force at a fixed length (Fig. 1), which was modified from the apparatus described by Purslow, Wess, and Hukins (1998). Three perimysial strips from each animal and each muscle (ST and PP) at each of four ageing times (5, 7, 13 and 20 days) were analyzed (total n for ST = 36; total n for PP = 36). The temperature in the bathing solution was increased at a linear rate of 3 °C per minute by an EchoTherm™ programmable digital hot plate (Torry Pines Scientific, California, USA) with constant stirring until a target temperature of 85 °C was reached. The temperature was then held constant at 85 °C for a further 30 min. The temperature at which force began to rapidly develop in the strip (T_{onset}) was quantified by back-extrapolating the linear portion of the rise in the load-temperature graph to zero load. Peak force at the 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 was calculated as a percentage of the maximum force (% relaxation). Lack of relaxation from the peak load is taken as an indication of a high concentration and heat-stability of covalent cross-linking present (Allain, Le Lous, Bazin, Bailey, & Delaunay, 1978).

2.4. Differential scanning calorimetry (DSC)

Thermal denaturation was studied using a Rheometics Scientific SP differential scanning calorimeter fitted with an Intracooler-I1 unit.

Table 1
Mean values for the total collagen, percentage of soluble collagen, maximum isometric tension load, percentage of relaxation from the maximum load and onset temperature of isometric load for perimysium from *M. pectoralis profundus* (PP) and *M. semitendinosus* (ST) at 5 days post-mortem ageing.

	PP	ST
Total collagen (mg/g w.w.)	10.30 ± 2.42 (1.40)	8.08 ± 2.02 (1.17)
% Soluble collagen (g soluble collagen/100 g total collagen)	6.8 ± 1.3 (0.75)	8.0 ± 1.7 (0.97)
Max load (N)	8.2 ± 1.6 (0.9)	7.3 ± 0.8 (0.5)
% Relaxation (Load t_{end}/Load t_0)	9.64 ± 0.60 (0.35)*	3.04 ± 0.39 (0.22)**
T_{onset} (°C)	66.4 ± 1.9 (1.1)	63.2 ± 2.5 (1.5)

Values given are means ± standard deviation and numbers in parentheses represent the standard error of the mean. Asterisks (*; **) indicate differences between muscles in each row, by *t*-test.
* $p < 0.05$.
** $p < 0.01$.

Samples (10–15 mg of isolated perimysial strips) were heated from 25° to 90 °C at 10 °C/min in a small sample pans (part n° L7168), using argon as a sweeping gas and high purity sapphire as a reference, similar to the conditions used by Stabursvik and Martens (1980). DSC was performed in triplicate and the temperature reproducibility of reported thermograms was ± 0.5 °C. Enthalpies of denaturation (peak areas) and mean denaturation temperatures (T_{max}) were calculated.

2.5. Statistical analysis

Data are expressed as means and standard errors (SE) of three animals for each of the two muscles. Data were statistically analyzed by two-way or one-way analysis of variance as appropriate. Post-hoc multiple comparisons were made using Tukey's critical range test. Statistically significant differences were considered at $p < 0.05$.

3. Results

3.1. Initial differences between muscles at 5 day post-mortem

Table 1 shows a comparison between the ST and PP muscles of total collagen content, % soluble collagen, maximum load in the HIT test on perimysium, % relaxation from maximum load, and the temperature at which isometric tension began to be generated in the HIT test (T_{onset}). The collagen content of the PP muscles was higher than that of the ST muscles, and a higher proportion of collagen in the ST muscles can be solubilized on heating than in the PP muscles, although none of these trends were significant. The maximum load generated in heating isometrically-held perimysial strips is not significantly different between the two muscles, but there is a significantly greater relaxation of this maximum load in perimysium from PP than ST, which may indicate a higher proportion of mature cross-links in the ST muscle, or may be due to the presence of elastin in the perimysium of this muscle. The T_{onset} values tend to be a little higher in perimysium from the PP muscle, but this tendency is not significant.

3.2. Time post-mortem affects collagen characteristics in each muscle differently

As expected, there are variations between animals for the collagen content, % soluble collagen and HIT characteristics of the connective tissue from both muscles at day 5 post-mortem, as evidenced by the standard errors in Table 1. However, a noticeable feature of the results obtained was that the pattern of changes in each parameter with increasing days post-mortem was remarkably consistent between the animals studied. To demonstrate this, the inter-animal differences have been removed in this section by dividing the value for each measurement by the initial value (at day 5 post-mortem) for that animal and muscle. The relative change from this initial value is then plotted.

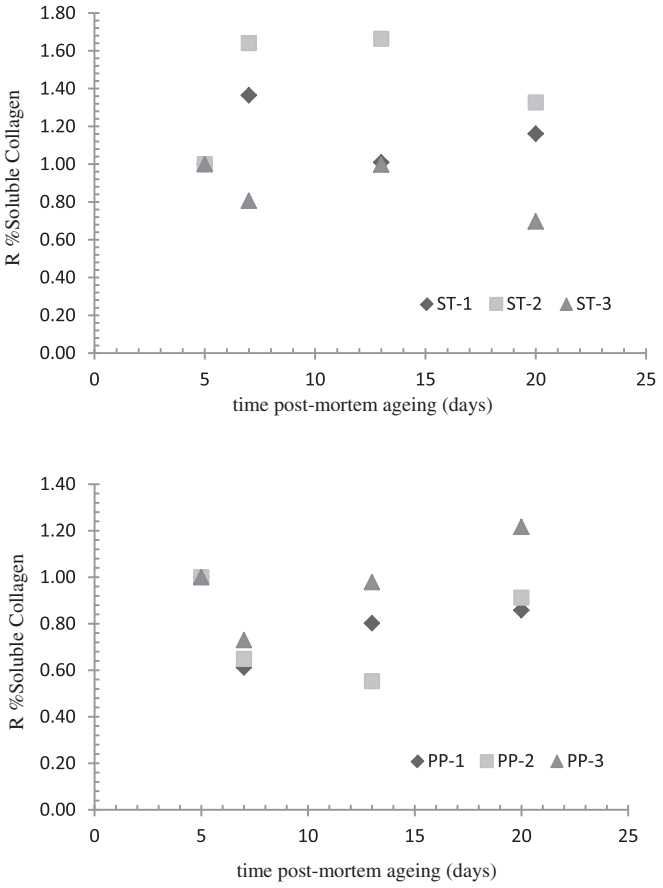

Fig. 2. Relative change in the percentage of soluble collagen in perimysium versus days of post mortem ageing. Top: perimysium from *M. semitendinosus* (ST). Bottom: perimysium from *M. pectoralis profundus* (PP). The R % soluble collagen (y-axis) values are calculated as the percentage of soluble collagen expressed as a ratio to the value on day 5. Points are shown for each animal.

3.2.1. Percentage soluble collagen

Fig. 2 clearly shows that the pattern of change in the % soluble collagen with increasing days post-mortem is different between the ST and PP muscles. For each PP muscle, the relative percentage of soluble collagen shows a decrease between 5 and 7 days post-mortem, followed by an increase to higher values at day 20, whereas the % soluble collagen for the ST muscles shows a biphasic relationship with days post-mortem in all animals, increasing at 7–13 days and falling again at 20 days post-mortem.

3.2.2. Hydrothermal isometric tension

Fig. 3 shows that there are also differences between muscles for the relative changes in HIT characteristics with time post-mortem.

The maximum isometric load (Fig. 3a) in the perimysium from ST muscles changed by only a little with days post-mortem, but is lower at 13 days than 7 days. The small error bars reveal that the maximum load values were very consistent between animals for perimysium from this muscle. In contrast, there is a tendency for perimysium strips from the PP muscle to show a biphasic relationship between relative maximum load and days post-mortem, although the greater variability in these samples precludes any significance being reached. Isometric contraction loads in the PP samples increase between 7 and 13 days post-mortem, in direct contrast with the loads in the ST samples.

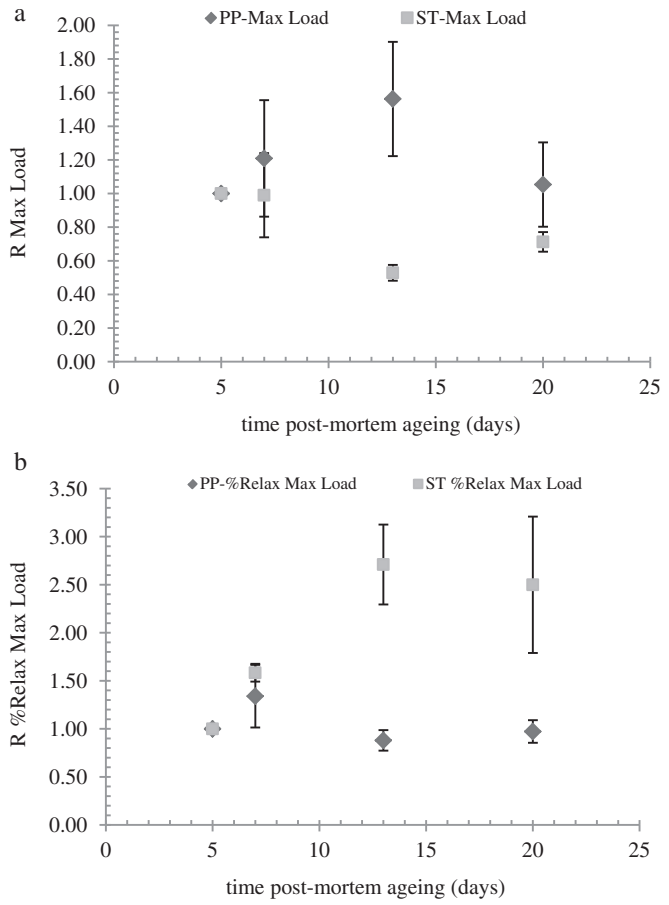

Fig. 3. Relative change in hydrothermal isometric tension (HIT) parameters versus days post mortem. A (top); relative maximum load. B (bottom); relative percentage of relaxation from maximum load after holding at 80 °C for 30 min. The relative change in maximum load (R max load) is calculated as the maximum load divided by the value on day 5. The relative change in Percentage relaxation from the maximum load (R % relax max load) is calculated as the percentage of relaxation from the maximum load divided by the value on day 5. Values shown are the means across the three animals studied, ± one standard error.

The relative % relaxation of peak load (Fig. 3b) shows no change with time post-mortem for the PP perimysium, whereas there is a clear increase with time post-mortem in the relaxation of the ST perimysium in all animals studied.

3.2.3. Comparison of values of T_{onset} from HIT and T_{max} from DSC

Fig. 4 demonstrates that there is a small but insignificant trend for T_{onset} to decrease slightly with time post mortem for the perimysium from both muscles. The absolute values of T_{onset} are generally in the same range as the temperatures at the maximum rate of thermal denaturation (T_{max}) measured by DSC (Table 2). It is also clear from Table 2 that there are significant differences in ΔH and T_{max} between the perimysium of ST and PP muscles. Perimysium from the PP muscle takes more energy to denature and the thermal denaturation transition has a T_{max} that is higher than for ST-perimysium. However, the effects of extended ageing are non-significant in the perimysium from both muscles.

4. Discussion

The main purposes of this study were to investigate (a) differences in the thermally-induced contractile forces in the perimysium from two bovine muscles, and (b) the effects of ageing on these, in order to assess whether enzymatic degradation in the perimysium during ageing could

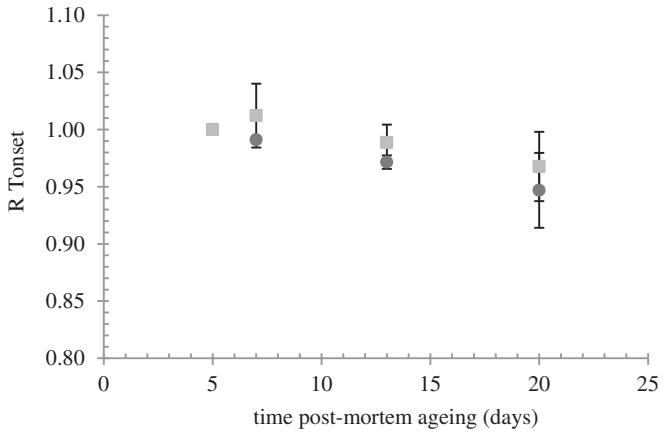

Fig. 4. Relative change in the onset temperature for isometric load in the perimysium versus days post mortem. The relative change in the onset temperature (R Tonset) is calculated as the onset temperature divided by the value on day 5. Values shown are the means across the three animals studied, ± one standard error. Circles; perimysium from *M. pectoralis profundus* (PP). Diamonds; perimysium from *M. semitendinosus* (PP).

Table 2
Comparison of mean values (± standard deviation) of DSC data with HIT data at 5 versus 20 days of ageing post mortem for the perimysium from *pectoralis profundus* (PP) and *semitendinosus* (ST) muscles. Values of denaturation enthalpy (ΔH) and T_{max} in the DSC experiments were measured at a heating rate of 10 K/min.

		Days (4 °C)	*Pectoralis Profundus*	*Semitendinosus*
DSC	ΔH (J/g IMCT-peri w.b.)	5	4.7 ± 0.2	2.8 ± 0.4
		20	8.5 ± 0.8	2.1 ± 0.8
	T_{max} (°C)	5	67.0 ± 1.1	64.3 ± 0.6
		20	66.0 ± 1.5	65.0 ± 0.2
HIT	Max load (N)	5	8.2 ± 1.6	7.3 ± 0.8
		20	10.2 ± 1.1	5.3 ± 1.9
	T_{onset} (°C)	5	66.4 ± 1.9	63.2 ± 2.5
		20	59.2 ± 2.8	61.6 ± 0.3

contribute to differences in cooked meat toughness by altering the degree and extent of forces having the potential to affect water loss. The results presented clearly show that there are differences in the intramuscular collagen characteristics between ST and PP muscles, but that the effects of post-mortem ageing may only be different to some extent in the connective tissue of the two muscles. Cartaginese and Purslow (2014) demonstrated by hydrothermal isometric tension (HIT) that shrinkage forces in perimyium heated above 65 °C are considerable, and they speculated that these forces may contribute to fluid loss from meat on cooking and affect the texture of the myofibrillar component. Latorre et al. (2016) also found similar HIT forces generated in the perimysium from bovine *M. semitendinosus*, and came to similar conclusions. The maximum load values reported from the HIT measurements in the current work are consistent with the values previously reported.

Collagen content and % heat-soluble collagen were determined on a wet weight basis so as to compare current results with the values reported by Torrescano et al. (2003), who also analyzed collagen content per gram of wet muscle tissue. Torrescano et al. (2003) analyzed muscles from four Swiss Brown bulls of approximately 300 kg empty carcass weight, and reported collagen contents of the PP and ST muscles corresponding to 6.7 and 4.5 mg collagen/g wet tissue, respectively. The three Angus animals used here were of comparable empty carcass weights, but mean values for total collagen contents of the PP and ST muscles were found to be 10.3 and 8.1 mg collagen/g wet tissue, respectively. The current results are slightly higher than those of Tossecano et al. (2003), but show the same relation between muscles. However, in terms of collagen solubility, Torrescano et al. (2003) used

extreme conditions (2 h of heating at 90 °C in a 0.23 M solution of NaCl) to solubilize collagen, whereas the conditions used in our present study matched those of the HIT test. This explains why Torrescano et al. (2003) report % soluble collagen values of 17% and 23% for PP and ST muscles, respectively, whereas the mean values in the current study were 6.8% and 8.0% for PP and ST, respectively, at 5 days post-mortem. It can nevertheless be noted that a higher proportion of collagen can be solubilized from ST than PP muscles in both the current results and those of Tossecano et al. (2003).

It has long been considered that different muscles differ in their cooked meat tenderness due in part to differences in their connective tissue characteristics (Dransfield, 1977) despite the fact that correlations of collagen content with shear force values in cooked meat are poor (Torrescano et al., 2003). In this study, we consider the possibility that the different collagen characteristics in different muscles may indirectly contribute to variations in cooked meat tenderness by shrinking on heating with more or less force, potentially causing different amounts of cooking loss. The results of show that the collagen content and collagen solubility is similar in PP versus ST muscles and the peak force generated on heating isometrically-constrained perimysial strips from the two muscles is not significantly different at 5 days of ageing. A lack of differences between muscles in collagen shrinkage forces that may drive cooking loss is consistent with previous literature. Rhee, Wheeler, Shackelford, and Koohmaraie (2004) analyzed the cooking loss of eleven bovine muscles (not including *M. pectoralis profundis*) that differed in collagen content, but could not find a strong correlation between cooking loss and collagen content. Purslow, Oiseth, Hughes, and Warner (2016) demonstrate a negligible contribution of collagen denaturation to the shrinkage of bovine *M. semitendinosus* on cooking to temperatures above 60 °C.

Turning to the interaction of the ageing process with thermally-induced contraction forces in the perimysial collagen, the current findings of changing collagen solubility in the second week of ageing in ST muscles can be related to the findings of Nishimura et al. (1995), who showed structural changes in the IMCT after 14 days of post mortem ageing. They also agree with the biochemical observations of Stanton and Light (1988) who showed considerable degradation in intramuscular collagens during ageing. However, PP muscle showed a different pattern of solubility changes with time post-mortem that ST muscles.

The most significant difference between the thermal behavior of the perimysium of ST and PP muscles was in the relaxation of thermally-induced forces (% Relaxation) over a 30 min period at 85 °C. At 5 days post-mortem, the relaxation in the PP-perimysium was approximately three times greater than in the ST-perimysium (Table 1). However, with increasing time post mortem, the % relaxation in the ST-perimysium increased significantly, whereas it remained relatively constant in the PP-perimysium (Fig. 3b). This implies that the level of heat stable, mature cross-links in the perimysium from the two muscles may differ, and that their stability may be affected by ageing. Table 2 shows that the apparent enthalpy of denaturation of collagen in the PP-perimysium is higher that the ST-perimysium at both 5 and 20 days post-mortem.

Judge and Aberle (1982) observed a decrease in the thermal denaturation temperature of intramuscular collagen between 45 min, 24 h and 7 days post-mortem ageing, as measured by T_{max} from DSC scans performed at 10 °C/min. Mills et al. (1989) found an exponential-type fall of T_{max} over the 0–24 h post-mortem period. The DSC measurements in the present study did not find significant differences between T_{max} after 5 or 20 days ageing, but the T_{onset} measured in the HIT test shows a small downward trend between 5, 7, 13 and 20 days ageing for perimysium from both muscles, which supports the idea that there are still some changes in the thermal stability of the intramuscular collagen going on with ageing between 5 and 20 days. It is clear that proteoglycans, as well as some collagen, are degraded in post-mortem ageing (Nishimura, 2015), and the T_{max} of intramuscular collagen is known to be affected by its interaction with proteoglycans (Blackwell & Gelman, 1975). It is therefore uncertain whether it is proteolysis in the proteoglycans or the collagen molecules themselves, or both, that is responsible for changes in T_{max} with ageing.

In the DSC measurements of the present study there are large differences in the apparent denaturation enthalpy, ΔH, and it is a limitation of this study that we cannot explain these. In reality, ΔH is a quantity from equilibrium thermodynamics so should either be measured under equilibrium conditions or estimated by theoretical model fitting using data measured at various scanning rates. This is rarely performed in food science studies, and consequently we quote figures measured at 10 °C/min, in common with previous studies on IMCT (Judge & Aberle, 1982; Mills et al., 1989). However, a more rigorous analysis of ΔH estimates for intramuscular collagen should be the subject of future work.

While there are some differences between the % soluble collagen and the DSC and HIT properties of the ST-perimysium and the PP-perimysium, there is no statistically significant evidence to suggest that post-mortem ageing significantly decreases the contractile force generated by collagen denaturation in the perimysium during the cooking of meat. Thus, the hypotheses that ageing reduces the forces of perimysial contraction on cooking and hence may reduce high-temperature development of toughness on cooking to temperatures above 60 °C are not supported by the current results.

5. Conclusion

While there are small differences between the collagen content and in the thermal solubilization of the perimysial collagen between of ST and PP muscles, there is no strong evidence that the ageing of the meat leads to a change in the contractile forces produced in the perimysium on cooking, or that these forces are markedly different in the perimysium from the different muscles. The % of soluble collagen does not appear to be related to the thermal contraction of the tissue. There are differences in the relaxation of heat-induced contractile forces with time. This further strengthens the idea of two pools of collagen in the perimysium of these muscles; one easily degraded by post-mortem proteolysis and also easily extracted by cooking, and another fraction which is more resistive to thermal degradation and provides most of the post-cooking characteristics of intramuscular collagen (Purslow, 2014). One interpretation of this is that future studies should not concentrate on the easily-removed soluble collagen to explain differences in meat texture, but rather focus on the properties of the resistant residue of collagen left intact by the cooking process.

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