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Pre-rigor temperature and the relationship between lamb tenderisation, free water production, bound water and dry matter

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ABSTRACT

The *M. longissimus* from lambs electrically stimulated at 15 min post-mortem were removed after grading, wrapped in polythene film and held at 4 (n = 6), 7 (n = 6), 15 (n = 6, n = 8) and 35 °C (n = 6), until rigor mortis then aged at 15 °C for 0, 4, 24 and 72 h post-rigor. Centrifuged free water increased exponentially, and bound water, dry matter and shear force decreased exponentially over time. Decreases in shear force and increases in free water were closely related ($r^2 = 0.52$) and were unaffected by pre-rigor temperatures.

1. Introduction

Water gradually appears on the cut surfaces of meat as it ages and tenderises; it appears in the bottom of trays where it is often mopped up by adsorbent pads; it accumulates in folds in packaging of vacuum packaged meat where it is called purge, drip or weep and is related to the water holding capacity (WHC) of the meat (e.g. Bertram, Andersen, & Karlsson, 2001; Otto et al., 2006). Not only is the appearance of water regarded as undesirable, but it is also often regarded as a loss of product. The sources of water are unclear and it has been suggested that there are several intramuscular compartments with varying properties affecting the production/release of water (Bertram, Purslow, & Andersen, 2002). One possible major source is the pre-rigor protein denaturation that is related to elevated temperature and low pH most widely studied in pork and modelled by Offer (1991). As the free water also increases over time, it has been suggested that shrinkage squeezes out water produced pre-rigor (Huff-Lonergan & Lonergan, 2005; Offer & Knight, 1988; Zhang, Lonergan, Gardner, & Huff-Lonergan, 2006), possibly facilitated by degradation of integrin (Lawson, 2004). The theoretical foundations of bulk water-holding are still lacking (Puolanne & Halonen, 2010).

While various theories may account for some water released around rigor, it doesn't account for how it is generated or released over longer periods post-rigor when the meat ages, a situation involving degradation of the cytoskeletal proteins. As water is released, the capacity to hold further water, (i.e. WHC) is reduced, thus WHC is lower in meat that has entered rigor and is even less in aged meat. Other studies also show that myosin denaturation is not the only cause of reduced WHC (Cheng & Sun, 2008).

Previous studies suggested that water appearing post-rigor is a component of cytoskeletal protein degradation during ageing for lamb (Devine, Wells, & Lowe, 2004; McGlone, Devine, & Wells, 2005) and beef (Rosenvold et al., 2009, 2008). Since cytoskeletal proteins such as titin comprise up to 10% of muscle protein (Labeit & Kolmerer, 1995), potentially there is considerable scope for water to come from degradation of the cytoskeletal protein tertiary structure during tenderisation, but it still needs to be established to what extent pre-rigor conditions can influence subsequent post-rigor tenderisation and water changes. Pre-rigor temperature conditions can influence myosin denaturation, especially in pork (e.g. Offer, 1991; Offer et al., 1989). For lamb semimembranosus, exercise stress pre-slaughter increased water loss and the rate of titin breakdown but had no effect on tenderness with no adverse effects of electrical stimulation (Bond, Can, & Warner, 2004).

Understanding the sources of water becomes important in light of a study by McGlone et al. (2005), where post-rigor changes in bound and free water were suggested to be responsible for the NIR spectral

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changes related to tenderness in lamb. The present study further examines the hypothesis that the water released from post-rigor muscle from cytoskeletal degradation during tenderisation is not significantly affected by pre-rigor temperatures 7–35 °C.

While the free water, bound water and tenderisation are all interrelated during post-rigor ageing of lamb at 15 °C (Devine et al., 2004), it is unclear how pre-rigor temperatures might affect these changes. In the present study, muscle was therefore exposed to a range of pre-rigor temperatures and the post-rigor free water, water binding, dry matter and tenderisation were followed during ageing at a constant temperature of 15 °C. The free water measurement techniques were based on a centrifugation method using small meat samples (Bertram et al., 2001; Kristensen & Purslow, 2001).

2. Materials and methods

2.1. Slaughter, electrical stimulation and pre- and post-rigor samples

Twelve-months old, Romney cross lambs ($n = 32$) consisting of five groups were obtained over 10 months. Each group was from a single mob. The lambs were head-to-back electrically stunned for 3 s at 400 V followed by a throat cut to sever carotid arteries and jugular veins. The lambs had electrical stimulation of 90 V peak, 180 mA peak (at 500 Ω load for calibration), 14.28 pulses s^{-1} for 30 s applied at 15 min post-mortem.

The *M. longissimus* (LL), between 5th rib (T5) and 6th lumbar vertebra (L6), were removed from each lamb side immediately after stimulation and were tightly wrapped in polyethylene cling film to prevent shortening (Devine, Wahlgren, & Tornberg, 1999). The wrapped LL was taken to the laboratory in polystyrene containers and placed either in a water bath at 4 °C ($n = 6$), 7 °C ($n = 6$), 15 °C ($n = 6$; $n = 8$) or 35 °C ($n = 6$).

Thermocron iButton temperature loggers (Dallas Semiconductor Corp., Dallas, TX, USA) accurate to ±1 °C were inserted into the muscles to monitor the desired temperatures. The pH of the muscles was measured at hourly intervals using a pH meter with a combination puncture electrode (Mettler Toledo, GmbH Process, Switzerland) until rigor mortis was reached (defined as the time point at which two consecutive hourly pH measurements did not change). Full rigor mortis occurs after all muscle fibres have entered rigor sequentially (Jeacocke, 1984) culminating in maximum peak isometric tension before its decay at commencement of ageing (Devine et al., 1999). The time to rigor mortis also varied widely with the different pre-rigor temperatures affecting rate of glycolysis (Jeacocke, 1977). The ultimate pH (pH_u) was measured and recorded 24 h after rigor mortis.

Following rigor mortis, each of the wrapped LL muscles was cut into four sequential caudal to rostral equal lengths (approximately 50 mm), placed into a polyethylene bag and held in a 15 °C room to age for 0, 4, 24 and 72 h respectively and samples were taken for shear force and free water measurements. The four samples from each muscle were treated as similar, as the wrapping, by producing an even pressure along all LL muscle removed to a large extent any sarcomere length variation that normally occurs along the LL on the carcass (Devine et al., 1999, 2002). This method meant that each sample was compared with the comparable sample on other LLs. The LL portions were frozen and stored at −20 °C until cooked (20 batches) for shear force measurements.

2.2. Centrifugation measurements

A sliver of meat was removed from the exposed surface at 0, 4, 24 and 72 h so the sample of meat removed for centrifugation was not affected by potential spurious surface water movements or drying. Then at each of these ageing times, a sample (approximately 2 g) from each LL section was excised and accurately weighed. Clearly this sample could only approximately represent the bulk of that meat sample. Each 2 g sample was placed, with fibres vertically-aligned, into a plastic centrifugation tube containing 5 mm diameter polycarbonate beads on top of a stainless steel mesh (0.5 mm) inserted in order to keep the fluid released during centrifugation separate from the meat sample. Each sample was centrifuged for 15 min at 1800 G (Hettich Zentrifugen Tuttlingen, Germany). The duration of centrifugation was based on the extensive data from Kristensen and Purslow (2001) for pork. The water lost, termed "free water" or centrifuged free water, was the percentage difference in weight before and after centrifugation.

2.3. Bound water and dry matter measurements

After centrifugation, each remaining sample was dried in a 105 °C oven for 24 h to determine the residual water content in the sample. The water dried off in the oven was termed "bound water" and the dried sample remaining was termed "dry matter". The sum of the free water, bound water and dry matter was 99–100% indicating that all components had been accounted for.

2.4. Shear force measurements

Meat samples for shear force measurement (approximately 50 mm long, 75 g portions of LL muscle) were obtained at each ageing time as the free water samples. The portions were from each experiment (maximum 8, minimum 6), cooked from the frozen state in a stirred 85 °C water bath to an internal temperature of 75 °C (measured by a thermocouple), removed when that occurred, and then immediately placed in 0 °C ice–water slurry. Because of the small numbers of animals involved, the treatments were not randomly allocated: each of the five treatments was a separate cooking batch. Once cooled, each LL was cut along the muscle fibres into 10 sub-samples, each with a 10 mm × 10 mm cross section. Each sub-sample was sheared across the muscle fibres with a MIRINZ tenderometer (Graafhuis, Honikel, Devine, & Chrystall, 1991) and the peak shear force was recorded.

2.5. Statistical methods

There were four pre-rigor temperatures and a constant ageing temperature with samples removed at 1, 4, 24 and 72 h for shear force, free water and bound water measurement. There were two experiments at 15 °C that were combined. Each temperature regime was a separate experiment.

The data were analysed using the REML directive of GenStat (2012) (15th edition). The random effects were 'experiment' and 'animal within experiment', with % drip, ageing time, and (linear) temperature as fixed effects. Treatment effects were assessed using the Wald test. Shear force was log transformed (base e) for analysis, and all conclusions are drawn from the analysis on that scale. The curves were produced using the solver programme in Excel and the figures drawn using *STATISTICA* statistics software (StatSoft Inc Tulsa, USA, Hoare Research Software, New Zealand).

3. Results

There are four aspects of the study. The first explores the relationships of the free water increase and bound water, dry matter and shear force decrease over time; the second explores whether there is a pre-rigor temperature effect on these relationships; the third explores the relationship between free water and shear force, and the fourth explores the changes in dry matter. The mean pH value for these studies was 5.64 (range pH 5.5–5.8) with four elevated pH values. As measurements were made every hour, the time of full rigor mortis (zero time in this study) only had an accuracy of one hour — this may slightly affect any initial values immediately after zero ageing from which point subsequent changes are initially rapid. Any effect of pre-rigor temperatures on sarcomere length and, hence, toughness, were moderated/forestalled by the wrapping of the samples.