Meat Science 135 (2018) 109–114

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

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

María E. Latorre[a,c,*], Diego E. Velázquez[a,b], Peter P. Purslow[c]

[a] Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Argentina
[b] Instituto de Física de Materiales Tandil (IFIMAT), Facultad de Ciencias Exactas, Universidad Nacional del Centro de la Provincia de Buenos Aires (UNCPBA), Tandil, Argentina
[c] Departamento de Tecnología y Calidad de los Alimentos, Facultad de Ciencias Veterinarias, UNCPBA, Tandil, Argentina

ARTICLE INFO

Keywords:
Meat
Ageing
Intramuscular connective tissue
Collagen
Thermo-physical properties

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

* Corresponding author at: Departamento de Tecnología y Calidad de los Alimentos, Facultad de Ciencias Veterinarias, Universidad Nacional del Centro de la Provincia de Buenos Aires. 7000 Tandil, Buenos Aires, Argentina.
E-mail address: elatorre@vet.unicen.edu.ar (M.E. Latorre).

http://dx.doi.org/10.1016/j.meatsci.2017.09.003
Received 19 January 2017; Received in revised form 24 August 2017; Accepted 13 September 2017
Available online 20 September 2017
0309-1740/

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.

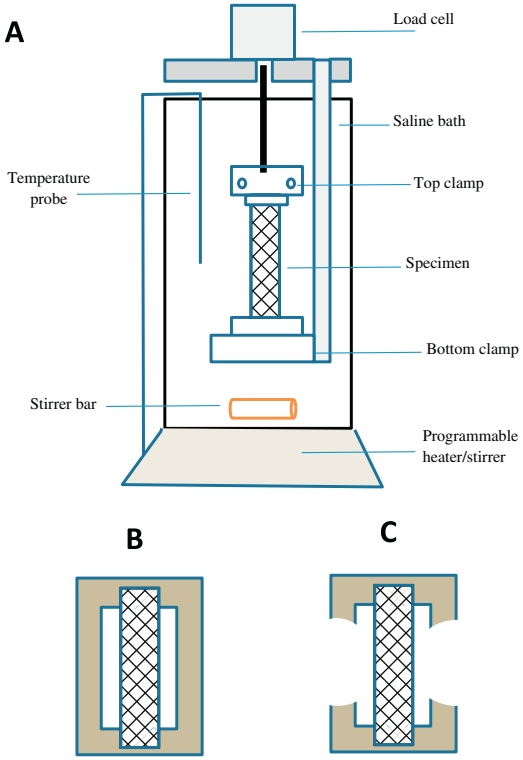

Fig. 1. Schematic diagram showing (a) the hydrothermal isometric tension apparatus, (b) the attachment of a perimysial strip onto an aluminum foil frame, and (c) the cutting of the frame after mounting it into the grips of the apparatus. The aluminum frame allows placement of the specimen in the apparatus 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.