Food Research International 89 (2016) 739–748

Contents lists available at ScienceDirect

Food Research International

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

The structural basis of cooking loss in beef: Variations with temperature and ageing

P.P. Purslow [a,*], S. Oiseth [b], J. Hughes [c], R.D. Warner [d]

[a] Facultad de Ciencias Veterinarias, Universidad Nacional del Centro de la Provincia de Buenos Aires, Argentina
[b] CSIRO Food and Nutrition, 671 Sneydes Road, Werribee, Victoria 3030, Australia
[c] CSIRO Food and Nutrition, 39 Kessels Road, Coopers Plains, Queensland 4108, Australia
[d] Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Royal Parade, Parkville, 3010, Victoria, Australia

ARTICLE INFO

Article history:
Received 8 June 2016
Received in revised form 5 September 2016
Accepted 7 September 2016
Available online 29 September 2016

Keywords:
Meat
Muscle
Cooking
Denaturation
Proteolysis
Actin
Myosin
Ageing

ABSTRACT

Meat loses fluid during cooking, resulting in textural changes and loss in cook yield. To understand the structural basis of cooking losses, this work used 10 bovine *semitendinosus* muscles and two ageing periods (1 vs 14 days) to examine micro- and macro-level dimensional changes in muscle during heating. Muscle blocks, muscle fibre fragments and myofibrils all showed similar maximum shrinkage in cross sectional area (20–24%) but maximum length shrinkage was less in myofibrils (15%) than muscle blocks and fibre fragments (25%). Dimensional changes were dominated by shrinkage in individual muscle fibres and myofibrils, indicating that connective tissue does not play a major role. Transverse shrinkage predominantly occurred over 50–65 °C whereas the longitudinal shrinkage predominantly occurred over 70–75 °C; we attribute these two separate shrinkage events to denaturation of myosin and actin respectively. Higher cook losses in samples aged for 14 days versus 1 day suggests that desmin, nebulin and titin denaturation are not major drivers of fluid expulsion as these proteins are degraded during ageing. We postulate that proteolysis during ageing produces protein fragments which are more easily lost from the structure during cooking, along with water.

1. Introduction

Meat loses volume and weight during the cooking process by expulsion of fluid. This change in fluid content brings about modifications in the textural qualities of meat which are in addition to the heat-induced changes in protein and fat. In addition, variations in cooked meat yield are of economic concern to meat processors.

Both temperature and time are important factors in the processes of heat and mass transfer, protein denaturation and, in some cases, protein solubilisation that occur on cooking. It is well established that the amount of weight lost from meat gradually increases with cooking temperature and that muscle (sarcomere) length, pH and salt content also affect the weight loss on cooking (Offer & Knight, 1988; Tornberg, 2005).

As the temperature of meat increases, the shrinkage in its volume is not equal in all directions. showed that There is a greater transverse shrinkage at temperatures between 40 and 60 °C than shrinkage along the muscle fibre direction, but in the temperature range from 60 to 80 °C this anisotropy is reversed, with longitudinal shrinkage being greater in this temperature range (Bouton, Harris, & Shorthose, 1976). In addition, the ratio of transverse to longitudinal shrinkage over the entire temperature range 40–80 °C is strongly influenced by sarcomere length, with cooking loss dominated by longitudinal shrinkage at long sarcomere lengths and dominated by transverse shrinkage at short sarcomere lengths. Differences in transverse versus longitudinal shrinkage of meat strips has also been documented by Tornberg (2005).

This temperature-dependent anisotropy (i.e. different dimensional changes in different directions) is not well explained by currently accepted models of cooking changes in meat, which suppose that it is the shrinkage of myofibrillar proteins (predominantly myosin) at temperatures of 40–60 °C followed by shrinkage of collagen at 60–70 °C, followed by denaturation of actin at 70–80 °C that largely determine cooking losses. Although careful DSC experiments correlate denaturation of major proteins to cooking effects (Martens, Stabursvik, & Martens, 1982), the denaturation of each of these components cannot adequately explain the differences in transverse versus longitudinal shrinkage at various temperatures. Thus, although there is considerable data measuring cooking losses in the literature, the mechanisms driving these, and variation in these, are still open to question.

Shrinkage of collagen fibres with longitudinal alignment at high sarcomere lengths and more transverse alignment at short sarcomere lengths is a commonly proposed mechanism for length-dependent variations in cooking loss. Although a model of cooking loss due to collagen

* 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, Tandil B7001BBO, Argentina.
E-mail address: ppurslow@vet.unicen.edu.ar (P.P. Purslow).

http://dx.doi.org/10.1016/j.foodres.2016.09.010
0963-9969/

orientation has been proposed (Lepetit, Grajales, & Favier, 2000), it is not a good fit with the observed cooking changes in meat. The transverse: longitudinal shrinkage ratio of both endomysial and perimysial collagen networks is expected to be independent of sarcomere length if the only action of heat is to shrink the collagen molecules along their length and not to reorientate them (Purslow, 1999).

The cooking loss from eleven grilled beef muscles ranged from 18.7% (biceps femoris) to 27.4% (*semitendinosus*).and no significant correlation between cooking loss and collagen content of the 11 muscles was found (Rhee, Wheeler, Shackelford, & Koohmaraie, 2004). This suggests that shrinkage of intramuscular collagen does not play a dominant role in driving variations of cooking loss. Similarly, cooking loss is similar between whole meat (connective tissue networks intact) and comminuted meat (connective tissue networks disrupted) (Tornberg, 2005).

In this study we focussed on the temperature-dependent anisotropy of heat-shrinkage in muscle to further understand the exact structural changes that force fluid out of meat as it is cooked. The observation that lateral and longitudinal shrinkage occurs over different temperature ranges suggest to us that several different mechanisms are involved.

One hypothesis that we test is that transverse shrinkage could be directly related to denaturation of transversely orientated cytoskeletal proteins (e.g. desmin), while longitudinal shrinkage could be driven by denaturation of longitudinally-orientated cytoskeletal proteins (e.g. titin, nebulin). Desmin extracted from pork longissimus muscle has a T_{max} of 46 °C in DSC studies using a heating rate of 10 °C/min (Dr Caroline P. Baron, personal communication). Titin from pork has a T_{max} of 78.4 °C and from beef 75.6 °C (Pospiech, Greaser, Mikolajczak, Chiang, & Krzywdzinska, 2002). Post mortem conditioning (ageing) of meat is known to preferentially degrade cytoskeletal proteins (Huff-Lonergan et al., 1996) and so a comparison of the heat-induced shrinkage in aged versus unaged meat is a potential means to examine the role of these structures.

Detailed comparison of the dimensional changes in whole meat, myofibrils and muscle fibre fragments (with endomysial connective tissue sheaths intact) and myofibrillar fragments from which myofibrillar proteins have been partially extracted is used to reveal the levels of structure within meat that determine cooking shrinkage. Immunolabelling of actin, myosin, titin and desmin is used to determine the residual structures within muscle fibres where myofibrillar proteins have been partially extracted. The aim was to use aged and unaged beef muscle to examine dimensional changes in muscle structure at various levels, as a consequence of heating to different temperatures.

2. Materials and methods

Investigations were carried out on meat blocks, muscle fibre fragments and myofibrils to investigate the relationship between cook loss, structural changes and cooking/heating temperature. Muscle blocks were cooked at various temperatures to investigate the dimensional changes in relation to temperature and weight loss. Muscle fibre fragments were heated in situ under a microscope to follow the structural changes of specific fragments with increasing temperature. Suspensions of single myofibrils were heated and compared to myofibrils prepared from cooked muscle blocks to investigate if freely floating, non-constrained myofibrils changed dimensions differently to myofibrils that were constrained in a muscle block while being cooked.

2.1. Reagents

Primary antibodies against desmin (DE-U-10), myosin (MY-32) and actin (AC-40) were from AbCam (Cambridge, MA, USA), and anti-titin antibody 9 D10 was from Developmental Studies Hybridoma Bank (University of Iowa, USA). All antibodies are known to react with bovine proteins and are raised in mice. A FITC-conjugated secondary goat-anti-mouse antibody (ab6785) was purchased from AbCam (above). A broad-spectrum protease inhibitor cocktail (Complete - mini) was purchased from Roche Life Science, Australia. All other chemicals were of analytical grade and obtained from Sigma –Aldrich (Castle Hill, NSW, Australia).

2.2. Collection of muscles

Semitendinosus muscles from both sides of five beef carcasses (, average hot carcass weight 248 ± 5.2 kg, zero dentition) were collected from a processing plant at 24 h post-mortem. The pH of each muscle was measured at 24 h post-mortem using a TPS = WP-80 pH meter (TPS Pty Ltd., Springwood, Qld, Australia) with an Inode IJ44 polypropylene spear-type electrode (Ionode Pty Ltd., Tennyson, Qld, Australia) and temperature probe. All muscles were in the normal pH range (5.5–5.6) and this data is not presented. Muscles from one side of the carcass were studied on the same day of collection and the muscles from other side were vacuum packed and stored at 4 °C until 14 days post-mortem (aged samples).

2.3. Dimensional and weight changes in meat cooked as blocks

18 blocks (approximately 50 × 30 × 30 mm) were cut along their long axis exactly parallel to the muscle fibre direction from each muscle. The exact dimensions of each were determined by Vernier callipers and the raw weight recorded. Pins were placed in the dorsal aspect of each sample to provide a consistent distinction between the two axes perpendicular to the muscle fibre direction. The blocks (3 blocks/temperature) were individually placed in separate plastic bags and heated simultaneously in one of five stirred water baths set at 55, 60, 65, 70 or 75 °C for 60 min, or microwave heated in a Menumaster 3100i Microwave oven for 5 min at 30% of maximum power (i.e. 465 W). The time/power levels used were computed to achieve approximately 100 °C within the samples. Spot checks with rapid-response thermocouples, inserted into the centre of microwave blocks as soon as possible after heating, recorded average core temperatures of 104 °C. After heating, all samples were transferred to an ice bath and cooled for 15 min. The dimensions of the blocks after cooking were recorded and shrinkage in length as well as cross section area calculated. The blocks were then patted dry and their weight measured to determine the weight lost during cooking (cook loss).

2.4. Preparation of myofibrils, muscle fibre fragments extraction and immunolabelling

2.4.1. Raw myofibril preparation

1.5 g of each raw muscle was homogenized in 15 ml of cold mannitol buffer (380 mM mannitol, 5 mM potassium acetate, pH 5.6, (Winger & Pope, 1980) with added protease inhibitor) by an Ultra-turrax disperser (Ultra Turrax, T25 basic from IKA Labortechnik, Germany) with a 18 mm head at 16,000 rpm for 2 × 30 s homogenisation with 30 s rest between each homogenisation, to make myofibril suspensions. The sample tube was kept on ice during homogenization. Seven myofibril slurries per muscle were prepared for each muscle at each ageing time. One tube was saved as a control (raw, non-treated) and the remaining tubes were placed into their own floating collars for 1 h cooking in water baths (55, 60, 65, 70, or 75 °C), or subjected to microwave treatment as above.

2.4.2. Heated myofibril preparation

0.5 g from each of the cooked blocks described above was collected and combined (3 × 0.5 g) and, homogenized to extract myofibrils as described above.

2.4.3. Muscle fibre fragment preparation

2 × 1 g samples of each raw muscle were gently homogenized in 10 ml cold mannitol buffer, one with and one without protease