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Meat Science 77 (2007) 2–6
MEAT SCIENCE
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Stratification of toughness in beef roasts

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Received 23 February 2007; received in revised form 3 April 2007; accepted 6 April 2007

Abstract

Most meat scientists adopt a reductionist approach to the study of meat toughness, taking a few intramuscular cores from one or more muscles to simplify the enormous complexity of toughness in all the retail cuts derived from a whole carcass. This is a valid approach to a complex problem, but we should also start to consider how consumers respond to bulk meat such as steaks and roasts. Probing whole roasts reveals a complex internal structure, detectable by both connective tissue fluorescence and resistance to penetration. The dorsal aponeurosis of the *Longissimus thoracis* is a major connective tissue stratum in beef rib roasts and its properties are correlated with those of adjacent intramuscular connective tissues. When the aponeurosis is cooked, its reflectance first increases with protein denaturation, then decreases with gelatinisation. Heat-induced contraction is concurrent with the increase in reflectance. Gelatinisation is reduced if the aponeurosis is mechanically restrained to resist contraction. Thus, mechanical restraint interacts with heat penetration in explaining stratification of toughness in bulk meat.

Keywords: Fluorescence; Penetrometry; Connective tissue; Meat toughness; Beef

1. Introduction

Meat scientists routinely analyse meat toughness using intramuscular core samples for methods such as the Warner–Bratzler shear test (Voisey, 1976). This reveals variation in toughness between and within major muscles of the carcass (Belew, Brooks, McKenna, & Savell, 2003; Gruber et al., 2006; Janz, Aalhus, Dugan, & Price, 2006). But when meat is consumed as a whole steak or roast, the stratification of connective tissues is superimposed on the toughness of individual muscles. The agreeable sensation of an internally tender core of meat may be spoiled by tough seams of connective tissue on the surfaces of, or between individual muscles. Overall tenderness in bulk meat is complicated by the amount of connective tissue gelatinisation, which depends on cooking method and depth in the meat. Building on our knowledge of intramuscular connective tissue toughness (Bailey & Sims, 1977; Dutson, Hostetler, & Carpenter, 1976; Loyd & Hiner, 1959; Smith & Judge, 1991), in this presentation we will review published experiments and other data. The objective is to look at the problems involved in understanding toughness in bulk meat containing different muscles and types of connective tissue, as when a consumer evaluates a whole steak or roast, or when intact sides of beef are probed in attempts to predict meat toughness (Wulf, Emnett, Leheska, & Moeller, 2002).

Lanius is the Latin word for a butcher (Lewis, 1891). We may use the adjective, *laniary*, to denote structures in cuts of meat prepared by a butcher, hence laniary strata for the layers of connective tissues in bulk meat. The thickness of the perimysium (connective tissue around bundles of muscle fibres) and the diameters of its collagen fibres both affect shear force (Brooks & Savell, 2004; Janz, Aalhus, & Price, 2006; Light, Champion, Voyle, & Bailey, 1985). But here we are more concerned with the epimysium (connective tissue on the muscle surface). Epimysium creates detectable laniary strata when we probe bulk meat. Thus, we detect major signals from strata (epimysium and fasciae) superim-

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doi:10.1016/j.meatsci.2007.04.013

posed on the background signals between the strata (endomysium and perimysium within muscles, and reticular fibres within adipose tissue). Intramuscular background signals correspond to the vast amount of information collected by shearing intramuscular cores. We will be questioning the relationships between major signals from laniary strata and the background signals between strata, and will then go on to look at complexities caused by cooking.

2. Comparing a muscle surface and its interior

When a probe is pushed into the eye muscle (*Longissimus thoracis*) of a beef rib roast, it passes through several grades of fibrous connective tissue (Swatland, 2006a). There is epimysium on the muscle surface, perimysium around bundles of muscle fibres and endomysium around individual muscle fibres. The aponeurosis is a very thick part of the muscle epimysium. Site 1 in Fig. 1 is where the maximum penetrometer force is encountered as the probe penetrates through the aponeurosis. Resistance to penetration is completely from collagen fibres. Site 2 is the minimum force immediately under the aponeurosis, with the probe tip passing through only muscle fibres and endomysium. Here resistance is limited to endomysial collagen fibres plus myofibrils, with the resistance from myofibrils being an interaction of sarcomere length and the extent of post-mortem autolysis. Site 3 is the maximum force anywhere intramuscularly when the probe tip encounters the thickest septum of perimysial connective tissue. The tip of our probe can detect both resistance to penetration and the optical properties of tissues at this point (Swatland, 2005).

The fluorescence data in Fig. 2 show where the probe tip passes through the *Longissimus thoracis* aponeurosis and underlying muscle. Fluorescence increases to a maximum where the probe penetrates the aponeurosis 2(1). Then there is a sharp decrease in fluorescence to the background level intramuscularly 2(2). A major connective tissue septum is encountered intramuscularly 2(3).

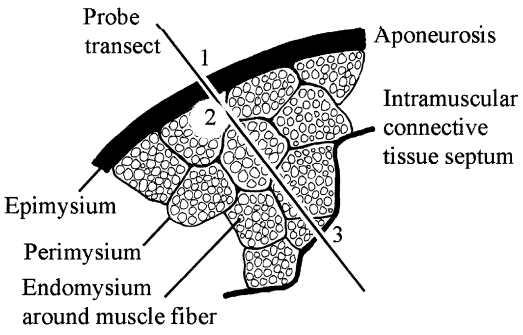

Fig. 1. Anatomy of a probe transect (from Swatland, 2006a). The aponeurosis is at point (1), the muscle immediately below the aponeurosis at point (2) contains only muscle fibres and endomysium, and a major seam of perimysium is at point (3).

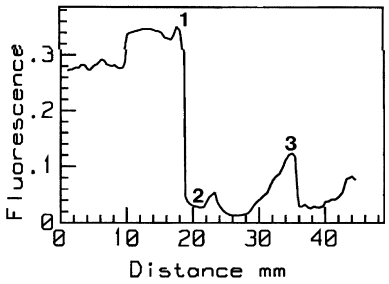

Fig. 2. Fluorescence signal (from Swatland, 2006a). After the probe enters the meat at distance 0 mm, it detects high fluorescence of the aponeurosis at point (1), low fluorescence of muscle immediately below the aponeurosis at point (2), and the intermediate fluorescence of a major seam of perimysium at point (3).

Looking at the corresponding penetrometer data, (Fig. 3), we see resistance to penetration increasing until the aponeurosis is penetrated. 3(1). Then there is a sharp decrease in resistance until the background level associated with endomysium and muscle fibres is reached 3(2). There is a resistance peak 3(3) corresponding to the perimysial septum identified by its fluorescence 2(3).

We all know meat is a highly variable commodity, and it is no surprise to find situations where the patterns of force and fluorescence do not match as clearly as in the example shown here. But these mismatches can be explained. Sometimes there may be an intramuscular fluorescence peak without any corresponding resistance. This may be where the probe window has passed tangentially by a septum of connective tissue, but has not actually penetrated through it. Conversely, there may be a peak of resistance without any increase in fluorescence. This may be where the probe tip has penetrated muscle fibres with strong myofibrils, either because of short sarcomeres or a lack of post-mortem autolysis. Ignoring these exceptions, we will collect our data using a split screen showing two matching data sets (with fluorescence peaks matching the pattern of penetrometer peaks).

In a typical set of Canadian prime rib roasts (well aged, well marbled Canada Grade AAA; $n = 13$), and taking the means of five measurements from each roast, fluorescence of the aponeurosis at site 1 is correlated with both

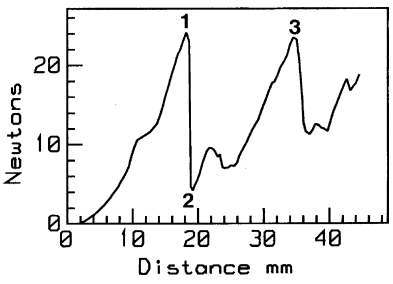

Fig. 3. Penetrometer resistance matching Fig. 2 (from Swatland, 2006a). After the probe enters the meat at distance 0 mm, it detects high resistance of the aponeurosis at point (1), low resistance of muscle immediately below the aponeurosis at point (2), and the resistance of a major seam of perimysium at point (3).

intramuscular fluorescence at site 2 ($r = 0.81$, $P < 0.0005$, $n = 13$) and with fluorescence of perimysial septum at site 3 ($r = 0.78$, $P < 0.005$, $n = 13$). Fluorescence at sites 2 and 3 is correlated ($r = 0.87$, $P < 0.005$, $n = 13$). Mean relative fluorescence intensities at sites 1, 2 and 3 are, respectively, 0.96 ± 0.40, 0.12 ± 0.08 and 0.28 ± 0.14 (relative to white paper standard = 1). With a paired t-test, differences are significant, $P < 0.001$.

For the matching penetrometer data, the force required to penetrate the aponeurosis at site 1 is correlated with both intramuscular resistance at site 2 ($r = 0.51$, $P < 0.05$, $n = 13$) and with perimysial septum at site 3 ($r = 0.63$, $P < 0.025$, $n = 13$). No relationships are detected in this set of samples between penetrometer forces at sites 2 and 3 ($r = 0.37$, NS, $P > 0.05$, $n = 13$). Mean values for penetrometer forces at sites 1, 2 and 3 are, respectively, 27.7 ± 4.8 N, 8.5 ± 2.1 N and 18.0 ± 3.9 N. With a paired t-test, differences are significant, $P < 0.001$. Pooling all the data (five measurements from 13 roasts), force at site 3 is correlated with both force at site 1 ($r = 0.39$, $P < 0.0005$, $n = 65$) and at site 2 ($r = 0.29$, $P < 0.01$, $n = 65$).

Despite these obvious relationships within the data sets for fluorescence and penetrometer force, the relationships of fluorescence and penetrometer force are difficult to detect – if they exist. No significant relationships of fluorescence with penetrometer force are detectable at sites 1 ($r = 0.03$, NS, $P > 0.05$, $n = 13$) or 3 ($r = 0.14$, NS, $P > 0.5$, $n = 13$). Although, fluorescence is correlated with force at site 2 ($r = 0.52$, $P < 0.05$, $n = 13$).

What do these data show us? Firstly, both fluorescence and penetrometer data show there are obvious relationships between connective tissues on the muscle surface and those within the muscle. This is to be expected because both are part of the same system developed by the animal to transmit the forces of muscle contraction to the skeleton. Secondly, the perimysial effect on meat toughness detected by Brooks and Savell (2004) may now be quantified. The strength of the strongest perimysium in aged beef roasts is more than twice the sum of endomysial plus myofibrillar strength (18.0 ± 3.9 N for perimysium and 8.5 ± 2.1 N for endomysium + myofibrils). As expected, the aponeurosis is stronger than anything else in the *Longissimus thoracis* (27.7 ± 4.8 N). But, remember, penetrometer data are difficult to standardize. A sharp probe gives lower values than a blunt probe. We can only make relative deductions: the aponeurosis in aged roasts is stronger than the strongest perimysium, and the strongest perimysium is stronger than endomysium plus myofibrillar structure ($P < 0.001$).

This helps explain why penetrometer data are so difficult to use on-line for the prediction of toughness in a whole carcass. The more senior members of this audience will doubtless remember several probe systems failing to live up to their initial expectations. Although we see here that the strength and composition of epimysium, perimysium and endomysium are all interrelated, we can also see the risk of a large sampling error. Epimysial connective tissues are much stronger than perimysial and endomysial connective tissues, and chance encounters with epimysial tissues will certainly increase sampling error. Thus, it is essential to standardize the anatomical location of probe transects and this is extremely difficult to achieve with either human operators or robotics.

How about using fluorescence probes to predict toughness? All the positive tests for predicting toughness from fluorescence were based on intramuscular transects (Swatland, 1995). Thus, we see fluorescence correlated with force at site 2 involving only muscle fibres and endomysium ($r = 0.52$, $P < 0.05$, $n = 13$). But we can now see why researchers who tested fluorescence probes without solving the anatomical problems of sampling were doomed to failure. Despite a network of significant interrelationships within strength and fluorescence data among different strata of connective tissues, a key relationship fails in the data presented here. No relationship is detectable between the fluorescence of the aponeurosis and its strength. The most likely explanation is elastic deformation. If a fluorescent septum is wrapped around an optical window, then linearity of fluorescence with strength is unlikely – a saturation effect. Thus, fluorescence probes need to cut through small structures, generating a fluctuating signal whose peaks can be counted and measured. Endomysial and minor perimysial fluorescence signals are related to meat toughness (Swatland, 1995), but not if we add in random large peaks from connective tissues outside the muscle of interest.

3. Cooking the aponeurosis on the muscle surface

We know a lot about meat cooking (Tornberg, 2005), and how collagen fibres contract to squeeze out fluid from muscle fibres (Bendall & Restall, 1983). Collagen fibres may be completely gelatinized if meat is thoroughly cooked, especially with moist heat, but this may not happen when steaks or roast are lightly cooked. Thermal denaturation of intramuscular collagen typically occurs at 53–63 °C (Martens, Stabursvik, & Martens, 1982) but denaturation may occur at 70 °C in extramuscular collagenous structures such as ligaments (Vangsness et al., 1997). What happens to the aponeurosis on the surface of the *Longissimus thoracis* when it is cooked? Collagen contracts and melts during thermal denaturation (Chien, 1975), but are optical and rheological changes simultaneous? To answer these questions, strips of aponeurosis were removed from beef roasts ($n = 15$) similar to those used in the last experiment. Strips were heated gradually in distilled water while recording their length and reflectance (Swatland, 2006b).

Heating first caused a gradual increase in reflectance (mean 0.026 ± 0.021, averaging reflectances at 400, 500, 600 and 700 nm), reaching half maximum at 59.9 ± 5.6 °C and maximum at 67.1 ± 5.5 °C (Fig. 4). The decrease in reflectance associated with gelatinisation did not occur until later, and the initial increase in reflectance was thought to be caused by protein denaturation.

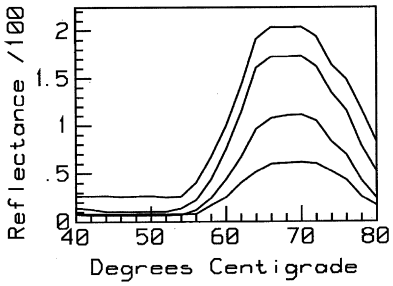

Fig. 4. Reflectance changes in a strip of *Longissimus thoracis* aponeurosis when heated (lines from top to bottom, reflectances at 400, 500, 600 and 700 nm, respectively; from Swatland, 2006b). From 40 °C to 56 °C there is no change in reflectance, but then reflectance increases to peak at around 68 °C.

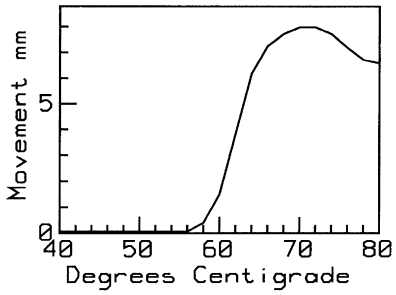

Fig. 5. Contraction in a strip of *Longissimus thoracis* aponeurosis when heated, matching Fig. 4 (from Swatland, 2006b). The contraction seen as movement on the *y*-axis starts around 56 °C and peaks around 70 °C just before gelatinisation releases the strip from its clamps.

Simultaneously, as strips of aponeurosis were heated they also contracted (Fig. 3). Half maximum contraction was reached at 62.8 ± 3.4 °C and the maximum was at 69.2 ± 3.2 °C. These temperatures did not differ significantly from the corresponding temperatures to reach half, and maximum increases in reflectance ($P > 0.05$). The mean contraction was $33.8 \pm 15.8\%$ of starting length (3 cm). The lengthening of aponeurosis strips at temperatures >70° was an artefact caused by the clamps failing to hold strips securely after they softened. Unclamped strips in the water bath remained at their contracted length while hot.

Collagen fibres show both Mie and Rayleigh scattering (Saidi, Jacques, & Tittel, 1995). Mie scattering is from particles >20 μm in diameter and involves visible light at all wavelengths, whereas Rayleigh scattering is from much smaller structures and primarily involves violet and blue light (white clouds in a blue sky are caused by Mie and Rayleigh scattering, respectively). Normally we only see the aponeurosis before and after cooking – not during cooking. It starts as a bright, white, glistening structure and may finish as a dull, translucent layer of gelatine. Thus, it was not anticipated the reflectance of the aponeurosis would initially increase before it subsequently decreased as gelatinisation occurred. In retrospect, this makes sense. Increases in light scattering accompany protein denaturation in many food systems, as when egg albumin changes from transparent to white on heating. An increase in light scattering is also the predominant internal optical change occurring when meat is cooked (Swatland, 1989a). The outside of the meat, of course, is darkened by drying and a variety of chemical changes and its reflectance decreases as it is cooked.

In beef *semitendinosus* during cooking, the gelatinisation of the perimysium may contribute to tenderness below 60 °C (Christensen, Purslow, & Larsen, 2000). But optical measurements of perimysial gelatinisation in beef indicate 67 °C as the point of gelatinisation (Swatland, 1989b), closer to the reflectance and contraction peaks seen for aponeurosis in Figs. 4 and 5. Perhaps we are seeing differences caused by methodology, but there might be other factors involved, such as differences caused by mechanical restraint.

4. Importance of mechanical restraint

This just about brings me up to date (February 2007) with published experiments, but leaves us with a myriad of complex possible interactions, and the floor will soon be open for discussion and the floating of new ideas. One intriguing factor many of us have ignored is the importance of mechanical restraint on laniary septa during cooking. I came across this by accident. I was testing a new computer-operated polarising microscope I had just built. I found a strong signal from raw aponeurosis – as one would expect, because it has a highly anisotropic structure causing strong birefringence. I cooked the samples and re-measured them, but their flatness had to be maintained, so I clamped them between two metal plates during cooking. To my surprise, far beyond the normal temperature of denaturation, the samples maintained their degree of internal structure and optical properties. I was expecting fully gelatinised samples with an isotropic structure and no birefringence, as occurs when perimysial septa are cooked (Swatland, 1989b).

A literature search showed this effect is well known. Mechanical restraint preventing heat-induced contraction protects collagen from thermal denaturation (Wells, Thomsen, Jones, Baek, & Humphrey, 2005). This was confirmed for bovine aponeurosis by cutting parallel strips (length 10 cm, width 5 mm, depth 3 mm) and clamping them at one end and at half way along their length. Thus, after they had been heated in distilled water to 70 °C, it was possible to compare shear forces between restrained and unrestrained parts. Using a Warner–Bratzler shear test (500 N load cell, Dillon BFG-500 N, Weigh-Tronix, Fairmont, Minnesota; compression tester, Dillon SnapShot, capacity 1 kN, range 29 cm, velocity 4 mm s^{-1}), restrained parts of strips were much tougher (31.9 ± 16.6 N) than unrestrained parts (19.3 ± 7.0 N; $P < 0.005$ with paired *t*-test, $n = 14$ pairs).

This could be an important factor in explaining events occurring as a roast is cooked. For example, an aponeuro-

sis near a cut meat surface might have minimal gelatinisation because it is exposed to dry heat, whereas deeper in the roast formation of steam might accelerate gelatinisation – but not if the aponeurosis is mechanically restrained. Does this help explain the popularity of boned and rolled roasts? Bones connected to laniary septa might retard gelatinisation and reduce overall tenderness.

5. Conclusion

The classical approach to measuring meat toughness in cores of meat taken from within muscles and carefully stripped of extraneous connective tissue is important, but is only part of the story. We also need to look at the stratification of tissues in whole cuts of meat. This may help us understand consumer responses to whole steaks and roasts, as well as the problem of sampling error when making online measurements from intact carcass. Is it possible to breed or feed our meat animals to have easily gelatinised laniary septa? Consumers would be delighted if we could.

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