1989

The Structural Basis of the Water-Holding, Appearance and Toughness of Meat and Meat Products

Gerald Offer
Peter Knight
Robin Jeacocke
Richard Almond
Tony Cousins

See next page for additional authors

Follow this and additional works at: https://digitalcommons.usu.edu/foodmicrostructure

Part of the Food Science Commons

Recommended Citation
Offer, Gerald; Knight, Peter; Jeacocke, Robin; Almond, Richard; Cousins, Tony; Elsey, John; Parsons, Nick; Sharp, Alan; Starr, Roger; and Purslow, Peter (1989) "The Structural Basis of the Water-Holding, Appearance and Toughness of Meat and Meat Products," Food Structure: Vol. 8 : No. 1 , Article 17.
Available at: https://digitalcommons.usu.edu/foodmicrostructure/vol8/iss1/17

This Article is brought to you for free and open access by the Western Dairy Center at DigitalCommons@USU. It has been accepted for inclusion in Food Structure by an authorized administrator of DigitalCommons@USU. For more information, please contact rebecca.nelson@usu.edu.
The Structural Basis of the Water-Holding, Appearance and Toughness of Meat and Meat Products

Authors
Gerald Offer, Peter Knight, Robin Jeacocke, Richard Almond, Tony Cousins, John Elsey, Nick Parsons, Alan Sharp, Roger Starr, and Peter Purslow

This article is available in Food Structure: https://digitalcommons.usu.edu/foodmicrostructure/vol8/iss1/17
THE STRUCTURAL BASIS OF THE WATER-HOLDING, APPEARANCE AND TOUGHNESS OF MEAT AND MEAT PRODUCTS

Gerald Offer, Peter Knight, Robin Jeacocke, Richard Almond, Tony Cousins, John Elsey, Nick Parsons, Alan Sharp, Roger Starr and Peter Purslow

Muscle Biology Department,
AFRC Institute of Food Research - Bristol Laboratory
Langford, Bristol, BS18 7DY, UK

Abstract

A structural approach greatly clarifies which components of meat are responsible for its tenderness, water-holding and appearance, and the events occurring during processing.

In living muscle, water is held in the spaces between the thick and thin filaments. Changes in the content and distribution of water within meat originate from changes in this spacing. Myofibrils shrink laterally post mortem. The fluid expelled accumulates between fibre bundles and between fibres and is drained by gravity forming drip. In pale, soft and exudative meat, shrinkage of the myosin heads on denaturation increases myofibrillar shrinkage. In salt solutions used in meat processing, myofibrils swell laterally taking up water, probably by an entropic mechanism.

The colour of meat is determined by light scattering as well as by absorption. The light scatterers in meat are observed by scanning confocal light microscopy to be smaller than 1 μm and concentrated in the A-band regions. It is proposed that light scattering arises from the gaps between myofibrils.

The structures responsible for the toughness of cooked meat have been studied by observing its fracture behaviour. The perimysial connective tissue determines the breaking strength when the meat is pulled apart transversely. In longitudinal tensile tests, the initial event is the debonding of fibre bundles from the perimysium so that they contribute independently to load-bearing. At greater extensions, it seems likely that fibre bundles progressively fall leaving perimysial strands as the last structure to break. With aged muscle, the muscle fibres probably fail at smaller extensions and therefore contribute less to the breaking strength.

Introduction

In Western societies a larger amount is spent on meat and meat products than on any other food, and meat foods present a particularly wide variety of problems and challenges. Structural investigations on meat are very helpful because by identifying the spatial arrangement of components, they can identify the component responsible for a problem affecting meat quality and greatly clarify the mechanism responsible. These problems are caused by rather subtle changes in the colloidal character of meat and, although much is known about the factors producing them, the exact nature of these changes has until recently eluded a precise description; only now are meat scientists beginning to explain them. We are fortunate in being able to draw on a very large amount of structural and biochemical information provided by basic biological research on muscle and connective tissue. We are also fortunate in that muscle cells are very regular and this facilitates both the study of their structure and thinking about mechanisms.

In this brief review we have selected three topics, water-holding, appearance and texture. All levels of structure are informative, from single protein molecules up to coarser features that can be seen by eye. Correspondingly, a number of complementary structural techniques is proving valuable, including X-ray diffraction, scanning and transmission electron microscopy, phase-contrast, fluorescence, polarising, dark-field and scanning confocal light microscopy.

The Structure of Muscle

Before considering these three quality attributes, we will first briefly review the structure of muscle (for detailed recent reviews see Squire and Vibert, 1987). Surrounding each muscle is a thick connective tissue sheath, the epimysium, which is continuous with the tendon (Figure 1). The muscle is divided into muscle fibre bundles by the perimysial connective tissue network. The coarse or primary perimysial network, which is very obvious to the eye, is further sub-divided by thinner sheets of perimysial connective tissue, typically defining a bundle of muscle fibres of the order of 1 mm
Figure 1. Coarse structural organisation in skeletal muscle showing the connective tissue tracts of muscle. Top, whole muscle; bottom, a fibre bundle. (Adapted from Purslow (1987) by permission of von Nostrand Reinhold Company).

Figure 2. Scanning electron micrograph of an obliquely cut surface of beef sternomandibularis muscle. The fibre direction is from left to right. In the two fibres near the surface the enclosing endomysial sheaths have been largely removed revealing the closely packed arrangement of myofibrils within them. Bar = 20 μm.

across. The collagen fibres in the perimysium are crimped and arranged in a criss-cross lattice (Rowe, 1974). In muscle at rest length, the collagen fibres in the perimysium are arranged at an angle of about ± 55° to the muscle fibre axis but assume larger or smaller angles as the muscle shortens or lengthens. Individual muscle fibres are separated from one another by the endomysial connective tissue network (Figure 1).

The muscle fibres are multinucleated cells, typically 10 to 100 μm wide and as much as several centimetres long. They are filled with myofibrils, the contractile apparatus of muscle. These are long, thin (1 to 2 μm), roughly cylindrical organelles packed side by side (Figure 2). In white fibres, which are used for bursts of activity, few mitochondria are present and the myofibrils are separated only by the calcium-storing membrane-lined channels of the sarcoplasmic reticulum. In red fibres, which are used for sustained or repetitive activity, mitochondria also intrude between adjacent myofibrils.

Myofibrils have a regular striated appearance and, because they are packed in register, the whole muscle fibre appears striated (Figure 3). The wide protein-dense bands are called A-bands and each has a lighter zone, the H-zone, in its central part. In the middle of the A-band is a dense line, the M-line. The lighter bands are called I-bands and each is bisected by a Z-disc. The striations of the myofibril arise because the myofibril is made up of overlapping arrays of two kinds of filaments, thick and thin, which form a repeating pattern (Figure 3). In transverse sections through the region of overlap, the filaments may be seen to lie in a well-ordered hexagonal lattice (see Figure 4). Each thick filament, which is 1.6 μm long, is made up principally of about 300 molecules of the protein myosin. The myosin molecule has a long tail (156 nm x 2 nm wide) at one end of which two curved pear-shaped heads (19 nm long) are flexibly attached (see Figure 6). In the thick filament the tails pack together to form the
shaft, the tails in the two halves pointing in opposite directions. This leaves the heads on the surface where they can interact with the thin filament (see Figure 6c). The thin filament is made up principally of about 400 molecules of actin, the precise number depending on the species. Each actin molecule is dumb-bell-shaped and can bind one myosin head. The actin molecules pack in a helical manner with the long axis of each dumb-bell perpendicular to the filament axis. Other proteins, tropomyosin and troponin, bind to the actin filament and confer calcium-sensitivity on the filament.

Muscle contraction is triggered by the release of calcium ions from the sarcoplasmic reticulum in response to a nervous impulse. The calcium ions bind to the thin filament switching it on in such a way that myosin heads can attach to the thin filament and generate force, probably by undergoing a change of shape. Shortening of the muscle occurs by successive cyclic interactions of the myosin heads resulting in increased overlap of thick and thin filaments. The energy for contraction comes from the hydrolysis of ATP catalysed by the myosin heads.

After the death of an animal, the muscle continues to hydrolyse ATP at a slow rate. For a time, the levels of ATP are maintained by the breakdown of glycogen to lactic acid, resulting in a fall in pH from 7 to around 5.5. Eventually the glycogen stores are depleted and the ATP concentration falls to zero. At this time the heads attach strongly to the thin filaments (see Figure 6b) causing the muscle to be rigid and stiff. This condition is known as the rigor state.

**Water-Holding of Meat**

Lean meat immediately after slaughter contains about 75% water. But, depending on the properties of the meat and how it is treated, it may gain or lose water, (see reviews by Hamm, 1960, 1986; Offer and Knight, 1988a,b). This is important economically since meat is sold by weight. The content of water and its distribution within the meat also determines its quality. For example, it is possible to have two pieces of meat with initially the same water content, one dark and dry in appearance, and the other pale and rapidly exuding water (Wismer-Pedersen, 1959).

Water can be lost from raw meat by evaporation from the surface, or by the exudation from the cut surfaces of drip. This is a concentrated solution of intracellular muscle proteins, including myoglobin, which gives it its red colour. The amount of drip is increased in pale, soft and exudative (PSE) meat. The PSE condition results from the denaturation of proteins caused by the attainment of a low pH while the carcass is still warm (Wismer-Pedersen, 1959). This can occur if the rate of breakdown post mortem of glycogen to lactic acid is fast, the final pH is particularly low, or chilling is slow. Meat loses even more water (up to 40% of the weight of the meat) on cooking and there is a correspondingly large shrinkage.

**Figure 4. Hypothesis to explain the origin of changes in the water-holding of raw meat. A myofibril is seen in transverse section in a shrunken state on the left-hand side and a swollen state on the right-hand side. The diagram is simplified and does not depict the depolymerisation of the thick filament that occurs in high salt concentrations. (Reproduced from Offer et al. (1984), by permission of the Royal Society of Chemistry).**

Conversely, when meat is treated during processing with NaCl, or NaCl together with polyphosphates, water is taken up. Polyphosphates are added for three reasons. Firstly, they reduce the concentration of NaCl required for water uptake. Secondly, they help solubilize myosin which forms a sticky coat on the surface of meat pieces and sets as a gel on heating, thereby binding meat pieces together (see Jolley and Purslow, 1988). Thirdly, polyphosphates greatly reduce the loss of water on cooking.

Since myofibrils occupy about 80% of the volume of living muscle fibres, most of the water in the muscle cell is present in the myofibrils in the spaces between thick and thin filaments (Figure 4). Only a small fraction of this water (perhaps about a tenth) is actually at any instant bound to protein (see Offer and Knight, 1988a). The simplest hypothesis to explain changes in the water held by meat is to suppose that they have their origin in changes in the volume of the myofibrils. In the raw state, where the filament lattice is regular, we suppose that uptake of water occurs by the entry of water into the myofibrils as they swell laterally by an expansion of the filament lattice. Conversely, we suppose that loss of water occurs by the expulsion of water from the myofibrils as they shrink laterally when the filaments get closer together (Figure 4).

**Drip Loss**

X-ray diffraction has the dual advantages of being non-invasive and being capable of determining the centre-to-centre spacing between neighbouring thick filaments with a high precision. Figure 5 shows the filament lattice spacing in beef rectus abdominis muscle as a function of time post mortem. The spacing does not change much with time initially, but at rigor onset there is a rapid decline to a new lower spacing, about 4.4% smaller than the original.
Lateral shrinkage of a myofibril post mortem. (a) Living muscle. The myosin heads are detached and the filaments are widely spaced. (b) Normal rigor muscle. The myosin heads have attached to the thin filaments drawing the filaments closer together. (c) PSE rigor muscle. Shortening of the myosin heads prior to rigor has caused the filaments to be drawn still closer together at rigor onset. At the bottom of the figure two myosin molecules are shown on the same scale, one normal and the other denatured under PSE conditions. The heads of the latter are shorter.

(Knight, unpublished results). This corresponds to a 9% decrease in the cross-sectional area, and therefore volume, of the myofibrils. The decrease is partly due to a fall in pH and partly due to the attachment of myosin heads at rigor onset (see Offer and Knight, 1988b) (Figure 6a,b).

Myofibrillar shrinkage is faster in pig muscles than in beef corresponding to the shorter times to rigor onset in pig (Knight, unpublished results). Preliminary results show that in PSE meat the myofibrils shrink about twice as much as in normal meat accounting for the higher drip in this state (Knight, unpublished results). What is responsible for this greater shrinkage? Penny (1967a) and Stabursvik et al. (1984) showed that myosin was denatured in PSE muscle. We have recently found by negative staining that if myosin is exposed to conditions (pH 6.0, 35°C for 5 or 10 minutes) similar to those experienced in PSE meat and which cause substantial loss of myosin ATPase (Penny, 1967b), the head length decreases from 19 nm to 17 nm (Sharp, Walker and Offer, unpublished results). This is sufficient to draw the thick and thin filaments even closer together at rigor onset and give rise to increased expulsion of water (Figure 6c). Calculations show that this small change in head length is sufficient to account for the increased myofibrillar shrinkage and therefore the increased exudation in the PSE state.

We can now ask where the water expelled from the myofibrils accumulates. Ideally, to observe this, we need a structural technique with a moderately high resolution (say 5μm), which could non-invasively image water compartments in a whole muscle. Perhaps in the future nuclear magnetic resonance imaging may prove helpful, but in the shorter term we have studied water compartments in sections of muscle fixed at various times post mortem and embedded in paraffin wax (Offer and Cousins, unpublished results). We find that in beef sternomandibularis muscle at 2 h post mortem there are no large channels in the meat; the fibres fill the endomysial network and the fibre bundles fill the perimysial network (Figure 7a). At 6 h post mortem, however, gaps of variable width appear between fibre bundles (Figure 7b). At this stage there are few gaps between fibres. Finally, after 24 h post mortem, gaps also appear between fibres (Figure 7c), as was first shown by Heffron and Hegarty (1974).

Evidently the fibres shrink as their constituent myofibrils shrink and the water that is left behind accumulates first around the perimysial network and later around the endomysial network, giving rise to two extracellular water compartments.

Finally, we can ask which water compartment is the source of drip, along what channels does it flow to the surface, and what force drives it out? A slice of meat held with the fibres vertical produces much more drip on the lower than the upper surface (Offer, 1984a). Similarly, if the slice is placed with the fibres horizontal, the lower part of the cut surface can be seen to be much wetter than the upper. If a piece of meat is placed in an organic solvent of the same density as meat so that hydrostatic pressures due to gravity inside and outside the meat are equalised at every level, drip is
Water-holding, appearance and toughness of meat

producing a small hydrostatic pressure on that region. If the column is small, say 1 cm diameter, the solution passes through the meat parallel to the fibre direction. Washing out myoglobin and other soluble proteins from a cylinder of the meat, and a circle on the opposite face of the meat appears nearly colourless (Starr, Almond, Knight and Offer, unpublished results). If the solution contains fluorescently-tagged bovine serum albumin and the opposite face is observed with a binocular microscope equipped with fluorescence optics, the first appearance of fluorescence is quite local and confined to the primary perimysial network (Figure 8b) together with regions of the secondary perimysial network opening from it. With time, fluorescence emerges over a larger area but still is confined to the perimysial network (Figures 8c-f). This confirms the existence of continuous longitudinal channels through the meat between the fibre bundles. We suppose that drip arises predominantly by the action of gravity draining the fluid in these channels to the cut surfaces.

Meat Processing

We have tested the hypothesis that water uptake in meat processing is due to lateral expansion of myofibrils. This was done by observing the effect on individual myofibrils of successive irrigations of salt (Offer and Trinick, 1983; Knight and Parsons, unpublished results) by the technique of Hanson and Huxley (1955). Phase-contrast light microscopy allows the myofibrils to be viewed in their hydrated state without staining. In our earlier work the myofibrils were attached to cover-slips but in more recent work they have been suspended between the bars of an electron microscope grid. When rabbit or beef myofibrils are treated with a series of NaCl solutions of increasing concentration and buffered to pH 5.5 to simulate rigor conditions, lateral swelling of the myofibrils starts in 0.6 M NaCl and is accompanied by extraction of myosin from the centre of the A-band, approximately the H-zone (Figure 9). Further swelling occurs in 0.8 and 1 M NaCl. The response of different myofibrils is somewhat variable, but the average swelling in 1 M NaCl is 96% ± 4% for rabbit myofibrils and 94% ± 6% for beef myofibrils (Knight and Parsons, unpublished results). This is more than sufficient to explain the uptake of water during meat processing.

If pyrophosphate is included in the irrigation medium, extraction of myosin occurs at lower NaCl concentrations and the extraction is more complete. In the presence of 10 mM pyrophosphate and 1 mM MgCl₂, very little happens when the myofibrils are irrigated with 0.3 M NaCl, but in 0.4 M NaCl the A-band is completely or nearly completely extracted commencing from both edges eventually leaving strings of I-segments (Offer and Trinick, 1983). In the presence of pyrophosphate, swelling is markedly less than that produced in NaCl alone (42% in rabbit, 14% in beef) (Knight and Parsons, unpublished results).
When meat is processed, it is often treated with small volumes of very concentrated brines so that initially myofibrils may be exposed to high concentrations of NaCl. During curing, for example, near the sites of brine injection there will initially be a very high salt concentration which progressively diminishes, whereas further from these sites the concentration of NaCl increases progressively from zero. It is therefore important to know how myofibrils respond to very high NaCl concentrations. While naively one might think that higher NaCl concentrations would have a greater effect than lower salt concentrations, this is not so. As the concentration of neutral salts like NaCl increases, initially there is an increasing tendency for protein assemblies to dissociate (the 'salting-in' range) but at higher concentrations this tendency diminishes (the 'salting-out' range). Callow (1932) found that the water uptake by meat in brines was maximal at 1 M NaCl and no water was taken up at concentrations above about 4 M NaCl. Correspondingly, myofibrils do not swell and show little change in structure when treated with 5 M NaCl buffered to pH 5.5, although at lower

---

**Figure 8.** Demonstration of longitudinal channels through meat. A 12 cm head of a solution of fluorescently-tagged serum albumin has been applied to one surface of a 1.5 cm transverse slice through beef semitendinosus in the rigor state and the emergence of this solution on the opposite face followed with time. (a) Opposite face observed by visible light showing perimysial network. (b-f) observed with fluorescence optics (b) 8 minutes (c) 16 minutes (d) 20 minutes (e) 24 minutes (f) 32 minutes after application of hydrostatic head.
Water-holding, appearance and toughness of meat

Figure 9. Swelling of rabbit myofibrils in salt solutions. The same myofibril is seen throughout by phase-contrast microscopy. (a) After preparation in pH 7 preparation medium. (b) After irrigating with 0.1 M NaCl, 1 mM MgCl₂, 10 mM sodium acetate, pH 5.5. (c) - (i) after further 3 minute irrigations with NaCl at the molar concentrations shown on the left hand side together with 1 mM MgCl₂, 10 mM sodium acetate, pH 5.5. Sarcomere length 2.3 μm. (Reproduced from Offer and Trinick (1983) by permission of Elsevier Applied Science).

concentrations (e.g., 1 M) they swell and the A-band is partly extracted (Knight and Parsons, 1988).

We may conclude that during the curing process the sites where swelling and protein solubilisation first occur are not immediately adjacent to the injection sites but at some distance from them. The difference in the time-course of changes in NaCl concentrations in different parts of the meat is likely to be the source of the approximately periodic stripes often present in bacon slices (Voyle et al., 1986).

We commented above on the variability of the response of myofibrils to salt. In a field of myofibrils prepared from rabbit plantaris muscle irrigated with 0.45 M NaCl plus pyrophosphate at pH 5.5, some myofibrils are extracted and swell, others are only slightly extracted and do not swell (Figure 10) (Knight and Parsons, unpublished results). This is due to the difference between fibre types. Myofibrils prepared from muscles with a high proportion of white fibres respond to a lower salt concentration than those from muscles like the soleus with a high proportion of red fibres. By antibody labelling of myofibrils from a muscle such as rabbit plantaris, which comprises a mixture of fibre types, we have shown that myofibrils from fibres containing slow myosin require a higher concentration of salt for extraction than myofibrils from fibres containing fast myosin (Knight and Parsons, unpublished results). The suitability of meat for processing may therefore be influenced by variation in the content of fibre types from muscle to muscle and animal to animal.

Mechanism of Swelling. In a previous publication we supposed that chloride ions binding to the filaments increased their negative...
charge, causing increased long-range electrostatic repulsive forces and therefore swelling (Offer and Trinick, 1983). However, at high salt concentrations, the excess of counterions balancing the negative charge on the filaments is localised so closely to the filament surface that the charge on the filaments would be effectively screened. This screening effect would more than outweigh the effect of increased charge due to Cl- binding (Ledward, 1983; Offer, 1984b; Offer et al., 1989).

In collaboration with Dr B Millman and Dr B Nickel of Guelph University, we have proposed a new kind of hypothesis in which salt-induced swelling is entropically rather than electrostatically driven (Offer et al., 1989). This is illustrated in Figure 11. It is well known that moderately high salt concentrations (0.5 to 1 M) depolymerise thick filaments into myosin molecules. In the region where thick and thin filaments overlap, these myosin molecules would tend to remain attached to actin filaments (Figure 11b), although in the H-zone they would be free to diffuse away. The myosin tails, being flexibly attached to the heads, would tend to explore space but this motion would, we suppose, in the unswollen lattice be severely restricted by the presence of neighbouring thin filaments and the myosin molecules attached to them (Figure 11b). If the lattice swells (Figure 11c) the tails would have greater freedom of motion and therefore higher entropy. Since systems tend to move to a state where they have highest entropy, there would be a marked tendency for the lattice to expand, which, compared with the electrostatic swelling pressure, would diminish only slowly with expansion.

On this hypothesis, depolymerisation of the thick filaments liberates the myosin tails necessary for the driving force to be developed. By contrast, dissociation of the myosin from actin would reduce the swelling pressure by reducing the amount of bound myosin. This effect would be particularly great if there were an excess of brine such as in the irrigated myofibrils, since much of the myosin would then escape.

The effectiveness of polyphosphates in promoting water uptake and protein solubilisation in meat processing is in part due to the small increase in pH alkaline polyphosphates produce (Lewis et al., 1986; Trout and Schmidt, 1986) but there is also a specific effect (Bendall, 1954). It is very well established that pyrophosphate in the presence of magnesium ions weakens the association of actin and myosin heads (see Offer and Knight, 1988a). It is also known that pyrophosphate assists chloride in causing the depolymerisation of thick filaments (Harrington and Himmel, 1972). This gives us a basis for understanding the effects of pyrophosphate. Because depolymerisation is promoted by pyrophosphate, extraction of myosin and swelling occur at a lower concentration of NaCl than in its absence. But because pyrophosphate promotes dissociation of myosin from actin, maximum swelling in the presence of pyrophosphate would be expected to be lower than in its absence, especially in systems, like irrigated myofibrils, where myosin would be lost. In systems where the brine:meat ratio is much smaller, such as occurs in meat processing, the swelling with pyrophosphate would be greater since more myosin would remain bound to actin.

The importance of myosin to swelling has been tested by irrigating myofibrils, from which myosin has been previously extracted, with a solution of myosin. The thin filaments bind the

---

**Figure 11.** Mechanism of swelling of myofibrils in salt. (a) Longitudinal section through the overlap region of a myofibril showing two thin filaments and one thick filament with its myosin heads attached to the thin filament. (b) As (a) but showing the depolymerisation of the thick filament as a result of treatment with NaCl before swelling has occurred. The motion of the myosin tails is restricted. (c) After swelling of the filament lattice, the myosin tails are able to move through a larger angle. (Reproduced from Offer et al. (1989) by permission of Marcel Dekker.)
myosin and the myofibrils swell substantially. Thus the hypothesis appears plausible and we will be testing it critically.

Effect of the Endomysium on Swelling. Having discussed the swelling of myofibrils in salt solutions, we can now consider the swelling of whole fibres, and consider the influence of the connective tissue. The degree of swelling of dissected muscle fibres in salt solutions is in our hands very variable from fibre to fibre. Wilding et al. (1986) had shown that, if the endomysial sheath surrounding a fibre is damaged at one point, much more swelling takes place there and they concluded that the endomysium acts as a mechanical restraint to swelling. It seemed possible that the variability we observed was due to some fibres being dissected with an endomysial sheath and some without. Whether or not a fibre has a sheath can be determined by dissolving out muscle proteins from the muscle fibre with SDS. After irrigation of a sheathed fibre, the endomysial sheath is left, but with an unsheathed fibre the fibre preparation dissolves. We found that the sheathed fibres swelled only a little in salt, and the unsheathed fibres swelled much more (Knight et al., 1989; Offer et al., 1989). This reinforced the conclusion of Wilding et al. that the endomysium could act as a mechanical constraint and also showed that fibres can be dissected with or without a sheath.

We further found that the remarkable increase in swelling over a narrow period of time post mortem observed by Wilding et al. could be entirely explained by the change with time of the ease of stripping of the endomysium; there was no other effect of time (Knight et al., 1989). Stanley (1983) showed that emptying of the contents of muscle fibres when exposed to calcium ions requires an extended period of conditioning. All this suggests that the optimum time post mortem for processing of meat may depend on proteolytic weakening of the connection between muscle fibres and connective tissue allowing stripping of the endomysial sheath during comminution, and therefore greater myosin extraction and water uptake.

Appearance of Meat

The colour of meat is determined not only by the quantity and oxidation state of the pigment myoglobin, but by the light scattering properties of the meat (MacDougall, 1982). In samples with a high light-scattering ability, such as PSE meat, light does not penetrate far into the meat before being scattered; hence there is relatively little absorption by myoglobin and so the meat appears pale. In contrast, dark, firm and dry (DFD) meat, formed when the final pH is high, say >6.0, scatters light only to a small extent. Incident light is therefore able to penetrate the meat for a substantial depth and is strongly absorbed by myoglobin. Such meat therefore appears dark. We shall now discuss the structural features responsible for this light scattering.

It has long been appreciated that there is a broad relationship between the appearance of the meat and its water-holding characteristics (Hamm, 1960), although drip loss and light scattering do not accurately parallel one another (Warriss and Brown, 1987). We have supposed that the light scattering property of muscle resides in the myofibrils and depends on their degree of expansion (Offer and Trinick, 1983). Recent experiments have enlarged and modified this view. Jeacocke (1984) showed that the light-scattering ability of muscle increases substantially at rigor onset, presumably due to the shrinkage of myofibrils occurring at this time. He also showed that the light-scattering ability is little affected by treatment of muscle with detergent, suggesting that membranes and membrane-lined organelles contribute little to the light-scattering by muscle.

Dark field microscopy provides a rather direct way of investigating light scatter since the image is constructed from light scattered by the object. Dark-field images of isolated myofibrils in suspension indicate that the A-band, and to a lesser extent the Z-disc, are bright sources of scattered light (Jeacocke, unpublished results). The intensity within the A-band is not uniform; the intensity distribution is consistent with an origin in light scattered principally at the surfaces between the A-band and the suspending medium and at the A-I junction (Figure 12a). In general, light scattering occurs at interfaces between two phases that have different refractive indices. The observations on myofibrils are therefore explained by the steps in refractive index occurring at their surfaces and at the A-I interfaces. Light scattered from a suspension of myofibrils is maximal at a pH of about 5 and decreases at lower or higher pH's (Jeacocke, unpublished results). This is consistent with light scattering depending on myofibrillar volume which would be expected to be minimal at pH 5, the isoelectric point of myosin and actin.

In muscle the light scattering properties of myofibrils are modified because they are packed alongside one another in register with a small but variable gap between neighbours (Figures 12b, c, d). If neighbouring myofibrils touched (Figure 12b), there would be no step in refractive index at their junction and therefore no light scattering at this interface. A small gap, say greater than a tenth the wavelength of light (Figure 12c), would be sufficient to create substantial scattering and the degree of scatter would increase with the width of these gaps. The step in refractive index would be greatest in the A-band and hence light scattering would be greatest at the intermyofibrillar gap between A-bands.

The confocal scanning light microscope produces reflectance images of skeletal muscle which differ substantially from those produced by conventional transmission light microscopy (Jeacocke, unpublished results). The particular advantages of this technique is that artefacts due to fixation, dehydration and embedding are avoided and that the optical section providing the image can be as thin as 0.8 μm, less than the diameter of a myofibril. This allows the light
scattering features in a thin layer within a sample of meat to be viewed without superposition effects from structures above and below the section. Figure 13 shows the appearance of a thin optical section within a fibre bundle from rabbit psoas muscle. The most obvious feature of this image is the highly speckled appearance. Small, bright sources of light substantially smaller than 1 \( \mu \text{m} \) in width are distributed throughout the section but are particularly numerous along transverse bands about 1 \( \mu \text{m} \) wide. These bands often extend right across the fibre without dislocations and are arranged with the

Figure 13. Scanning confocal light micrograph of part of a fibre within a fibre bundle from rabbit psoas muscle in rigor showing light scattering features. The figure shows a longitudinal optical section (approximately 0.8 \( \mu \text{m} \) thick) taken on a Lasersharp MRC-500 instrument with a 60 x 1.4 NA oil-immersion objective and operated in the reflectance mode. Bar = 20 \( \mu \text{m} \).

same periodicity as the sarcomere. Comparison between reflectance and fluorescence confocal images of skinned muscle in which the myosin has been labelled fluorescently suggest that each speckled band coincides axially with the central region of an A-band. The speckled appearance varies along the length of a fibre and also between fibres. In PSE meat the speckles are larger and there appear to be more of them. The speckled appearance is not substantially altered by treatment with detergents, suggesting that membrane-bound organelles, such as mitochondria and the sarcoplasmic reticulum, are not directly responsible for the scattering.

Assuming that the speckled character is not an artefact of coherent light illumination, we may conclude that light scattering from muscle is predominantly not from molecular features (for example, cross-bridges) but rather from much larger structures. It seems possible that the speckled appearance arises from variations in the closeness of packing of adjacent myofibrils. On this basis the light scattering sources would correspond to small regions where the gap between adjacent myofibrils was wider than elsewhere. Adjacent myofibrils are joined together at Z-disc (and M-line) level by proteins such as desmin (Granger and Lazarides, 1978) (Figure 12d). The speckles may therefore arise from light scattering at the boundaries of intermyofibrillar compartments bounded by these structures. It is at present unclear whether the T-tubules and the terminal cisternae of the sarcoplasmic reticulum, which are present as a collar around each myofibril at the ends of the A-bands, play any

Figure 12. Hypothesis explaining the cause of the light scattering in meat. (a) Isolated myofibril. (b-d) Neighbouring myofibrils in muscle. In (b) the adjacent myofibrils touch. In (c) there is a small space between neighbouring myofibrils. In (d) structures linking myofibrils are also shown but not the transverse tubules and sarcoplasmic reticulum which are also present in the gaps between myofibrils.

G. Offer et al.
role in producing light scattering, for example by acting as spacers between myofibrils. Further work is required to test these notions and to determine the precise nature of the scatterers.

**Toughness**

The most important attribute of the eating quality of meat is its tenderness. When we chew meat, it is pulled apart, and the difficulty with which this is achieved is perceived as the sensation of toughness. A variety of mechanical tests has been applied to meat, but tensile tests, in a direction either parallel or perpendicular to the fibre axis, are the simplest to interpret (Bouton et al., 1975). The longitudinal breaking strength of raw meat correlates reasonably well with the sensory perception of toughness of cooked meat (Stanley et al., 1972) although no such comparison seems to have been done on the longitudinal breaking strength of cooked meat.

A particularly useful approach is to examine the structural events occurring when samples of meat are extended to breaking point (Carroll et al., 1978; Purslow, 1985, 1987). In a tensile test, the progressive breakdown of the structure leading to its final fracture will depend not only on the stiffness, breaking strength and breaking strain of its components, but on their spatial arrangement and the connections between them.

When a transverse slice of cooked meat is pulled apart in a direction perpendicular to the muscle fibre axis, the first event seen is the opening up of cavities throughout the slice in regions between fibre bundles (Purslow, 1985, 1987) (Figures 14a,b). Histological investigation shows that the site of fracture lies between the perimysial network and the endomysia of fibres on the surface of a bundle (Purslow, 1987). A striking demonstration that the cleavage pathway is between perimysium and endomysium is obtained by viewing the cleaved surfaces by scanning electron microscopy (Purslow, 1987). Evidently the weakest component of the cooked meat, and therefore the first to break, is the junction between the endomysium and perimysium. As the load is further increased, some of the cavities join up in a fracture path which runs along the boundaries between fibre bundles, with strands of perimysium, presumably originating from nodes of the perimysial network, bridging the gap at intervals and carrying the load (Figure 14b,c). When a still higher load is applied, further extension occurs and these perimysial strands rupture (Figure 14c,d). Thus, in this transverse direction, the breaking strength (maximum stress) of the meat is simply determined by the amount and strength of the perimysium, the last structure to break.

We shall now consider what happens when strips of raw and cooked meat are subject to tensile testing parallel to the muscle fibre direction. In raw meat, after an initial stiff phase for small extensions, the material becomes more compliant, and only when the meat is stretched to twice its original length, does the stiffness rise steeply due to stretching of the perimysium (Davey and Dickson, 1970). Cooking dramatically changes the shape of the load-extension curve. After cooking at 80°C, the initial stiffness is much higher, but after loads of approximately 1 to 1.5 kg/cm² and extensions of 5 to 20% (the yield point), the stiffness falls markedly (Bouton et al., 1975; Locker et al., 1983). The breaking strength of meat cooked at 70 or 80°C is appreciably greater (1.5x) than that of raw meat (Bouton et al., 1975; Locker et al., 1983), whereas breaking extensions have been reported to decrease slightly on cooking (Locker et al., 1983) or to increase (Bouton et al., 1975). Cold-shortened samples have a far greater breaking extension than an unshortened control, and stretched muscles have a smaller breaking extension (Bouton et al., 1975). For meat cooked at 80°C the peak force is largest for cold-shortened samples, followed by stretched samples and is least for unshortened controls.

If cooked muscle is stretched to about 65% extension, then the tension is removed, the strip returns almost to its original length (Locker et al., 1983). However, on reloading the strip, the load-extension curve is markedly different: the load required for small extensions is much smaller, although as would be expected, the load required to return to a 65% extension is the same as for the first run. The form of the curves is consistent with elastic structures progressively failing at extensions right up to 65% and not only at or near the yield point. Ageing for 7 d at 2°C prior to cooking almost halves the initial stiffness without affecting the stiffness at higher extensions (Locker et al., 1983). The breaking strength is slightly reduced. After cooking for 3 h at 100°C, which causes the solubilisation of a substantial fraction of the collagen as gelatin, the yield point survives but the longitudinal breaking strength is greatly reduced (Locker et al., 1983). This suggests that connective tissue contributes substantially to the breaking strength but is not implicated in the structural changes responsible for the yield point.

The precise sequence of structural events that occurs as the cooked meat is stretched is not yet clear, although we do know many of the elements of the overall picture. At the macroscopic level, the first clearly observable event upon extending unaged cooked meat is the separation (debonding) of fibre bundles from each other (Purslow, 1985, 1987) (Figure 15b), showing that the endomysial-perimysial junction is a weak component in this testing direction, as well as in the transverse direction.

The result is that each fibre bundle and the perimysial network become isolated from one another and independently bear the tension. The fibre bundles and perimysium can then be regarded as elements acting in parallel. In this case, the element with the least extensibility (least breaking strain) is the first to break and the load is then thrown onto the remaining elements. Structures progressively break down and the most extensible component finally remaining determines
Fracture behaviour of a transverse slice of cooked meat when it is pulled apart in a direction perpendicular to the fibre axis. The stippled dark grey areas represent the fibre bundles and the dark lines show the perimysial network. (a) Prior to the application of tension the fibre bundles are joined to the perimysium by tenuous connective tissue threads. (b) After extension, cavities develop due to the partial separation of fibre bundles and perimysium. (c) On further extension, a fracture pathway connects some cavities but is bridged by perimysial strands. (d) Complete fracture.

the load and extension at failure. Let us consider the extensibility of these two elements. Muscle fibres isolated from cooked muscle can be stretched by greater amounts before they break than can raw fibres, in some cases to more than 100%, although considerable variability is observed (Wang et al., 1956; Hostetler and Cover, 1961; Jeacocke, unpublished experiments). Ageing the meat decreases the extensibility of the cooked fibres. Perimysium isolated from cooked muscle is also very extensible. Breaking extensions are very variable, but in some cases exceed 150% (Lewis and Purslow, unpublished results).

Thus when a cooked muscle strip continues to be stretched longitudinally, after the point where debonding of fibre bundles from the perimysium occurs, both the perimysial network and the fibre bundles will contribute to the load-bearing at substantial extensions. It seems most likely that fibre bundles progressively fail as independent units (Purslow, 1985) (Figure 15c), finally leaving perimysial strands as the last structures to break (Carroll et al., 1978 (Figure 15d). However, it is not known whether peak loads have been passed at the stage when only perimysial strands remain. In other words it is not yet clear what contribution fibre bundles make to the longitudinal breaking strength (maximum stress).

This kind of model can explain in general terms the differences between the longitudinal and lateral breaking strengths of meat and the effect of ageing. In unaged meat the longitudinal breaking strength of cooked meat is more than ten times higher than the lateral breaking strength (Bouton and Harris, 1972; Purslow, 1985). This can be understood if in unaged meat the fibre bundles make a substantial contribution to the longitudinal breaking strength and none to the transverse breaking strength. As meat is aged, the lateral breaking strength is unaltered (Bouton and Harris, 1972; Purslow, unpublished experiments). This
indicates that although the perimysium may be subtly altered during conditioning (Stanton and Light, 1987), its strength after cooking is unchanged. However, the longitudinal breaking strength declines considerably during ageing (Bouton and Harris, 1972; Purslow, unpublished experiments). This can be understood if the relative contribution of the fibre bundles to the longitudinal breaking strength declines with age. This may come about because when the meat is aged, fibre bundles tend to fail at smaller extensions and therefore contribute less to the maximum stress.

It is of considerable interest to know what happens in the myofibrils when a cooked fibre is stretched. In muscle cooked at temperatures above 60°C, the thick filaments fuse to form an apparently amorphous A-band (Schmidt and Parrish, 1971) probably consisting of a myosin gel (Ishitoroshi et al., 1982; Hermansson and Langton, 1988) in which gap filaments are embedded. The thin filaments in the I-band lose their identity on cooking above 60°C (Schmidt and Parrish, 1971) but it is not yet clear whether the actin and regulatory proteins form a gel. When unaged cooked meat is stretched to breakage, Locker and Wild (1982) considered that there was no visible damage to the integrity of the myofibril. At extensions up to 30%, the I-band increases in length, but at extensions up to 60% the A-band also stretches. Unfortunately no information is available for larger extensions. It appears that up to 60% extension at least, the length changes in the muscle strips are accommodated by a uniform, proportional increase in the length of all sarcomeres. However, the point does not seem to have been rigorously tested. Sarcomeres from muscle that had been stretched to breaking point and allowed to recover appear similar to those from rest length muscle (Locker and Wild, 1982) but the available resolution may be inadequate to detect breaks in the structure. On stretching up to 30%, filaments of unknown origin, possibly gap filaments, appear in the I-band. At 50% extension, the stretched A-band develops a speckled appearance due to fragments superimposed on an array of fine filaments, which have been assumed to be gap filaments, although this
remains to be proved.

When cooked beef muscle that had been aged 7d at 2°C is stretched to the breaking point, the extension occurs by the stretching of the I-bands alone; the A-bands are unaltered (Locker and Wild, 1982).

At present, it is therefore far from clear what structural events are responsible for the yield point, what structures in the A and I bands are load-bearing and where in the sarcomere failure occurs. It is also unclear when the fibre contents fall, whether structural continuity is provided by the endomysium; Street and Ramsey (1965) show that, at least in red muscle fibres, following rupture of the fibre, the endomysium is capable of maintaining the integrity of the fibre at nominal loads of 3.7 kg/cm², a value close to the breaking strength of cooked meat.

Clear evidence is needed on the relative contributions of fibre bundles and perimysium to the stress-strain curves of muscle aged for different times, stretched or shortened to different extents and cooked under different conditions. We especially need to establish at what point different structural elements fail in a longitudinal tensile test.

The structural approach therefore is valuable in focussing attention on the components that determine the strength of the meat and enables the wealth of biochemical information on the nature of the collagen making up the connective tissue, and in particular its cross-linking, to be integrated into the mechanical picture. It is also useful in providing a framework within which to investigate the effects of other processes which affect toughness, such as cooking, ageing and cold-shortening.

References


Water-holding, appearance and toughness of meat


Discussion with Reviewers

R. Hamm: The authors explain the high drip loss of PSE pork by denaturation of myofibrillar proteins, particularly by changes in the head moeity of the myosin molecule. On the other hand, Honikel and Kim (1986) concluded from their results that the fast release of drip post mortem from PSE muscle must be mainly due to changes in the muscle cell membranes, and not more than 25% might be due to denaturation of myofibrillar protein. Do you agree that increased permeability of cell membranes contributes considerably to the wateriness of PSE pork?

Authors: No, we are not convinced by their arguments. Honikel and Kim (1986) found that in pig psoas muscles considered to be PSE in that they had a pH < 5.8 at 45 min post mortem, only 25% of the myosin was denatured as judged by loss of ATPase and/or by differential scanning calorimetry. But their argument, that wateriness cannot be due to myosin denaturation because it is so limited, is logically flawed.

PSE is not an all-or-none phenomenon, Drip loss increases progressively with decrease of pH. In studies on pig longissimus dorsi muscles, maximum drip loss occurred at a pH of 6.0 or below (Warriss and Brown, 1987), although the extent of denaturation, and its dependence on pH, would be expected to depend on the chilling rate and may well depend on the muscle, for example due to fibre type differences. Honikel and Kim excised their muscles soon after death and then kept them at 35°C. The conditions experienced by these muscles were therefore less severe than would have been encountered had the muscles remained on the carcass, where in the PSE state the temperature can exceed 40°C at 1 h post mortem. It is not therefore surprising that the degree of myosin denaturation they observed was lower. By contrast, Stabursvik et al. (1984) showed that in muscles kept in a PSE carcass, a larger fraction (about 50%) of the myosin was denatured.

Honikel and Kim did not measure the drip from their muscles and were therefore not in a position to prove that their muscles gave the maximum amount of drip that pig psoas muscles are capable of. In other words they did not establish that their muscles were in an extreme state of
PSE, and therefore could not expect the degree of myosin denaturation to be maximal. Even if it were established that, in PSE muscles giving maximum drip, the degree of myosin denaturation was only about 25%, this would not demonstrate that myosin denaturation was unimportant. We do not know the dependence of myofibrillar shrinkage on the degree of myosin denaturation. There is no reason to think that maximum shrinkage requires all the myosin to denature. It could be that when only a fraction of the heads has denatured, their tendency to shorten over-rides the undenatured heads and causes the lattice to shrink maximally. What is urgently needed is to determine the degree of myosin denaturation and myofibrillar shrinkage for a wide range of pH values.

With regard to membrane damage, it is known that if a muscle is cut very soon after death and centrifuged, very little water is lost (Ling and Walton, 1976); in other words its water-holding capacity is high. Thus despite the cell membrane having been severed, water is well retained by the cell contents (by the myofibrils in our view). This shows that breakage of the cell membrane by itself is not the cause of drip. The fact that drip is a solution of sarcoplasmic proteins (Haggard et al., 1960; Surge, Warriss and Jolley, personal communication), suggests that even in normal rigor the cell membrane becomes leaky. Our own experiments have shown that in PSE muscle the myofibrils have shrunk by an amount that can explain the increased drip and there does not seem to be a need to invoke any other explanation.

G.R. Schmidt: The authors mention that the amount of drip in PSE meat is due to a rapid breakdown of glycogen post mortem, or due to slow chilling of the carcass. Could there be other processes in the muscle which are genetically controlled which could affect the water holding capacity and appearance of the muscle? Do these genetically-controlled factors need to be specifically associated with glycogen breakdown and rate of chilling?

Authors: There are four circumstances in which PSE meat has been reported to form:

1) In animals that are stress-susceptible due to a genetic defect, there is a high rate of post mortem glycolysis.
2) Even in stress-resistant animals, excessive stress at slaughter can also trigger a high rate of post mortem glycolysis.
3) Certain pig carcasses have been shown to exhibit a near-normal rate of glycolysis but a larger-than-normal extent of glycolysis, so that the final pH is low (~5.1) (Lawrie et al., 1958; Monin and Sellier, 1985).
4) If the chilling rate is excessively slow, the temperature in the deep muscles of a carcass can remain high for a long time post mortem (MacDougall, 1982). The formation of PSE meat when the post-mortem temperature is kept high can be demonstrated by holding meat post mortem at 37°C (Wisme-Pedersen and Briskey, 1961).

The common feature of these is that the muscles experienced a low pH while the carcass is still warm. As far as we are aware, all cases of PSE can be explained by the simple hypothesis that it is due to the time-dependent denaturation of protein occurring under these conditions. Of the causes given above, (2) and (4) are due to bad handling of animals or carcass, rather than a genetic defect. The high rate of glycolysis in stress-susceptible pigs is well established as a genetic defect. The cause of the low ultimate pH in (3) is not known and has been rather little studied, but might be the result of another genetic defect, for example causing an increase in the amount of muscle glycogen at slaughter.

While paleness in meat might come about simply by a reduced myoglobin level (which might be genetically controlled), our current understanding is that the combination of pale, soft and exudative characteristics is caused only by thermal denaturation of proteins. It is difficult to envisage any other set of circumstances, genetically controlled or otherwise, that would bring about this thermal denaturation, and which would be the cause of PSE.

However, the severity of the PSE state resulting from a particular time-course of temperature and pH might be influenced genetically. For example, the myosin isofoms present in different muscle fibre types might have slightly different susceptibilities to denaturation. Apart from this, we think it unlikely that there are genetic differences in myosin between breeds which affect stability, but this cannot be ruled out.

R. Hamm: The authors propose a new hypothesis in which salt-induced swelling is entropically, rather than electrostatically, driven, and the depolymerisation of the thick filaments is the decisive factor. It was relatively easy to explain the effect of different anions and cations on the swelling of muscle and the water-holding capacity of meat by changes in electrostatic interactions between protein molecules. How can the new hypothesis explain these differences: by different effects of the ions on the depolymerisation of the thick filament? Is it possible that the association of the tail moieties of myosin is caused by electrostatic forces and that short-distance electrostatic effects of ions result in more or less depolymerisation of the filament depending on the type of ion, rendering possible entropic swelling? In this case, both electrostatic and entropic effects would participate in the swelling.

Authors: Neutral salts have their effect on protein systems by affecting the stability not only of electrostatic bonds but also of hydrogen bonds and hydrophobic bonds (see von Hippel and Schleich, 1969). When the concentration of the salt is raised, hydrophobic groups make increasingly unfavourable interactions with the solution, whereas charged groups or polar groups make increasingly favourable interactions. The net result of increasing the concentration of the salt is first to weaken interactions between...
Water-holding, appearance and toughness of meat

process where swelling and protein solubilisation occur extensively at the sites of needle injection. This phenomenon causes the appearance of periodic stripes in bacon slices and we also observe this in roast beef and hams from time to time. Do the authors have recommendations for processors to prevent the formation of these periodic stripes of variable protein solubilisation in meat products?

Authors: We have investigated the nature of these periodic stripes in bacon and shown that near the sites of brine injection there is actually rather little change in structure; the myofibrils are still clearly seen and thick and thin filaments are still apparent (Voyle et al., 1986). It is further from these injection sites where myofibrillar structure is grossly disrupted. We suggested that although the final salt concentration is likely to be uniform throughout the meat, the difference in behaviour might be due to the different time-courses of salt concentration at the two locations. Near the sites of brine injection, the salt concentration will rise very fast to a high concentration and then slowly fall to the final concentration. Further from the injection site, the salt concentration will be zero for some time after injection and then slowly rise to the final concentration as salt diffuses in from the injection sites. Experiments with myofibrils show that prior exposure to high salt concentrations reduces both swelling and extraction at moderate concentrations (Humphrey and Parsons, 1988), suggesting that salt-induced denaturation may have a role in the formation of the stripes.

Since the periodicity of the stripes is the same as the interval between needles, it would seem reasonable to attempt to combat the problem by using a smaller separation between needles.

R. Hamm: Is it possible to explain the effect of pH, in the absence of salt, on the swelling and water-holding capacity of meat only by the electrostatic hypothesis, or does pH exert an influence on the depolymerisation of the thick filaments?

Authors: Although pH undoubtedly has a strong influence on the myosin molecule-myosin filament equilibrium (Josephs and Harrington, 1968), we doubt whether, in the absence of added salt, there is significant depolymerisation of myosin filaments in the pH region of greatest interest, pH 5.5 to 7.0. Rather, we suppose that raising the pH increases the negative charge on the filaments, thereby increasing the electrostatic force between them and expanding the lattice. This is well shown by Matsuda and Podolsky (1986). In their work, raising the pH from 5.5 to 6 expands the lattice considerably, but raising the pH further (to 7) had little further effect. This latter result can be explained on the electrostatic mechanism by charge saturation (Offer and Knight, 1988a), but would be hard to explain by depolymerisation.

We consider that the filament lattice spacing of myofibrils, and therefore the water-holding capacity of meat, depends on a number of factors: pH and ionic strength affect

protein molecules (or subunits). This is the salting-in effect and one of its manifestations is to increase the solubility of the protein. For example, raising the NaCl concentration to 0.6 M at pH 5.5 will cause depolymerisation of a myosin filament, and thereby bring myosin into solution. However, above a certain limit, raising the concentration of neutral salt has the opposite effect and interactions between protein molecules (or subunits) are enhanced. This is the salting-out effect, where the solubility of the protein decreases to such an extent that it may precipitate. Thus at very high NaCl concentrations (~4 M), myosin filaments are not depolymerised and muscle and myofibrils do not swell (Callow, 1932; Knight and Parsons, 1988) and it is possible to precipitate myosin at such high salt concentrations (Edsall, 1930).

Although this behaviour applies to all cations and anions, they differ markedly in these abilities. Some ions, for example CNS\(^-\) or I\(^-\), are very effective at dissociating protein assemblies at low concentrations and will even cause protein subunits to unfold. They increase the solubility of proteins very greatly but maximum solubility requires relatively high ion concentrations and the salting-out effect may not be observed at practically realisable concentrations. Other ions, such as sulphate, are much less effective at salting-in, but achieve their maximum effect at relatively low concentrations so that salting-out can be readily demonstrated at concentrations where salts cause swelling of myofibrils by disrupting protein structure. On our hypothesis, the relative effectiveness of different ions in processes such as salting-out are expressed in the lyotropic or Hofmeister series. The relative effectiveness of ions, which is similar for several systems, has no obvious relationship to the sign or magnitude of the ionic charge suggesting that their action is not simply a question of suppressing electrostatic interactions (von Hippel and Schlicht, 1969). Professor Hamm (1960) showed that the same order applied for water uptake by muscle. We think this is entirely consistent with the view that ionic effects cause swelling of myofibrils by disrupting protein structure. On our hypothesis, the relative effectiveness of different salts in causing swelling would be seen as being due to the differences in their abilities to depolymerise thick filaments.

It is not necessary to suppose that the interactions between the myosin tails in the filament are exclusively electrostatic in character; hydrogen bonds and hydrophobic bonds may also be involved. It is possible that salt depolymerises myosin filaments by increasing the interactions which polar, rather than charged, groups make with the medium. In the sense that electrostatic effects may be responsible for thick filament depolymerisation, we would agree that electrostatic effects may play a role in swelling, but it seems to us likely that the swelling pressure generated by the addition of salts is not electrostatic.

G.R. Schmidt: The authors mention the curing
the electrostatic repulsive force between filaments; the structural integrity of the myofibrillar components affects the mechanical constraints on swelling; the presence of osmotic agents such as sarcoplasmic proteins, partially or completely excluded from the lattice, lowers the chemical potential of the water outside the myofibrils relative to that inside and thereby causes shrinkage. In addition, entropic mechanisms, such as we have outlined, may come into play under conditions where the degree of molecular freedom would increase if there were an expansion of the filament lattice. At present, we can envisage such a mechanism operating only when the myosin filaments depolymerise and we think this would occur only in the presence of salt and polyphosphate, or with acid marinades (Ofer and Knight, 1988a).

G.R. Trout: In the section on water-holding, no mention is made of the effect of heating and the interaction between heating, pH, salt type and concentration on the microstructural changes and their relationship to water-holding. Would you comment on the importance of these factors in light of the fact that the major objective of studying the effects of salts, such as sodium chloride and sodium tripolyphosphate, on water-holding is to determine how they affect the water-holding ability of the cooked meat product? Authors: We quite agree that it is very important to understand how NaCl and polyphosphates affect the water-holding ability of cooked meat products. But meat is treated with these salts in the raw state and we do need to know what is happening in this system first before tackling the experimentally harder problem of the structural changes occurring when salt-treated meat is cooked.

We think it likely that polyphosphates in the presence of NaCl decrease cooking loss by their action in solubilising myosin (Ofer and Knight, 1989a). When myosin molecules are heated, they form a gel with good water retention (Tsai et al., 1972). The solubilised myosin is presumably capable of permeating the entire muscle, that is to invade the I-bands of the muscle fibres from which it was previously absent, and also to invade the extra-cellular space. If this is correct, when the salt-treated meat is cooked, a myosin gel will be formed throughout the meat and is likely to play a major role in water retention (Bendall, 1954; Sherman, 1961; Hellendoorn, 1962; Kotter and Fischer, 1975; Ofer and Knight, 1989a). We can explain the effect of polyphosphate in the presence of NaCl on cooking loss, since polyphosphates cause dissociation of actomyosin and more myosin will be solubilised. Greater solubilisation will also occur at higher pH.

G.R. Schmidt: The authors discuss extensively a previous paper by Ofer and Trinick (1983) in which myofibrils are irrigated with an extraction medium. Generally, this research differs from applied meat processing in two ways. There is no mechanical action applied to facilitate the disruption of the myofibrils and the pH utilized is 5.5, whereas in meat processing the pH is normally 6.0. Would altering the mechanical action and the pH affect the interaction between the proteins and the irrigation medium? Authors: In those experiments we were not trying to duplicate precisely the conditions occurring in meat processing, but rather by simplifying the system to gain insight into the mechanisms involved in water uptake and myosin extraction. We chose a pH of 5.5 to mimic the conditions in fresh meat. We wanted to explore the specific effects of NaCl and polyphosphates without complicating matters by altering the pH as well. However, Dr. Schmidt is right to emphasise the small rise in pH that occurs when alkaline polyphosphates are used and it would be desirable to determine more precisely what effect this might have.

A rise in pH would be expected to lower the NaCl concentration required to depolymerise the thick filament (Josephs and Harrington, 1968), and hence assist both swelling and myosin extraction.

With regard to mechanical action, it is at present far from clear precisely how much disruption of muscle structure occurs in different products. At one end of the spectrum, in a traditional ham, gross disruption of the muscle fibre probably occurs only at the surface of the meat and, in a British-style sausage, muscle fibres probably also survive intact. In a frankfurter, it seems that even the myofibril is grossly disrupted, although to what level is unknown. However, regardless of the size of the meat piece or fragment of myofibril, the underlying molecular interactions between the ions of the medium and the myofibrillar proteins are presumably the same as with isolated proteins.

G.R. Schmidt: The authors discuss the ageing of the endomysium and indicate that aged muscles empty the contents of their muscle fibres when exposed to the proper ionic environment more readily than fresh muscle. If this is so, how does this phenomenon interact with the property of myosin being more extractable pre-rigor than, obviously, the endomysium is still in a very strong state? Authors: The work we have discussed was performed on rigor muscle. The pre-rigor state has quite different properties. We have found (Elsey and Knight, unpublished experiment) that it is quite easy to draw muscle fibres, free of endomysium, from a transversely cut surface of pre-rigor muscle (Schoenberg and Eisenberg, 1985), but this is difficult from rigor muscle. Perhaps, therefore, a high incidence of stripping accompanies comminution pre-rigor, but this needs to be tested. We suppose that removal of the endomysium from around a muscle fibre would promote extraction of solubilised myosin, in both pre-rigor and rigor muscle, by removing an impediment to outward diffusion.

The ATP present only in pre-rigor meat produces a high degree of dissociation of myosin from actin, permitting a quicker diffusion of myosin molecules once added. NaCl has
depolymerised the thick filaments. This factor will influence the outward movement of myosin even in fibres still enclosed in endomysium.

G.R. Schmidt: The authors discuss the changes that take place in muscle when myofibrils in a cooked fibre are stretched. The authors do mention gap filaments. What do the authors speculate is the contribution of titin to the longitudinal strength of cooked meat?

Authors: We discussed this important question recently (Offer et al., 1988). Titin is a very large protein (molecular weight 3 million) and titin molecules are very thin filaments about 4 nm wide and up to 1 μm long (see reviews by Squire et al. (1987) and Offer (1987)). Recent location studies using monoclonal antibodies to titin have shown that a single titin molecule spans all the way from the Z-disc to the M-line (Whiting et al., 1989). There is evidence that titin is the primary component of the gap filaments, the structures spanning the gap between A and I-bands in highly stretched muscle.

When muscle is cooked, the thick filaments fuse together and the thin filaments disintegrate, although the A and I bands persist (Schmidt and Parrish, 1971). Leet et al. (1977) have shown that, unlike the thick and thin filaments, gap filaments survive cooking and have argued that gap filaments alone provide the tension-bearing elements in the cooked myofibril.

It has been suggested that since titin is rapidly degraded by proteolysis on ageing, it could not support load (King and Kurth, 1980), but there is no reason why a break in the primary structure should necessarily cause the titin molecules to lose their secondary or tertiary structural integrity. Myosin filaments form a strong gel on heating, so that, in the A-band region, load is probably borne both by the myosin gel and by titin filaments. However, the structure formed from the thin filaments on cooking is not known, and it is possible that, at the high concentrations present in muscle, the thin filament components also form a gel. Thus we do not know for sure that titin is the only component in the I-band that supports tension and thus whether titin alone determines the breaking strength of the cooked myofibril.

G.R. Schmidt: The authors comment that there is a variability of response of myofibrils to salt. They attribute this difference to muscle fibre types. Is it possible that the protein titin could be involved in this phenomenon?

Authors: Paterson et al. (1988) have found that titin is extracted from myofibrils under conditions of salt concentration that extract myosin and cause swelling. They have proposed that loss of titin may be a cause of swelling, so it is conceivable that variable loss of titin could underlie variable swelling. Variable loss of titin might arise either from variable conditioning or to differences in fibre type. It remains to be shown whether titin and/or nebulin do indeed constitute constraints on swelling of the myofibril, and whether extraction of titin correlates with the amount of swelling.

Additional references


