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MICROSCOPICAL OBSERVATIONS ON THE STRUCTURE OF BACON

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Abstract
Commercially processed salt-treated pig longissimus dorsi muscle, in the form of bacon slices, sometimes shows localized variations in the light-scattering properties of the tissue. The phenomenon is described as 'tiger-stripe'. A study of areas of tissue showing such variations, using electron microscopy, has revealed differences in the structure at the myofibrillar level. Areas which appear dark when viewed by incident illumination show ordered myofibrillar structure, whereas areas which appear light under similar viewing conditions appear to be disordered.

Introduction
The traditional Wiltshire process of bacon manufacture, as practised in the United Kingdom, involves the treatment of sides of pig meat with sodium chloride, together with sodium or potassium nitrite and, frequently, sodium or potassium nitrate. Polyphosphates and ascorbates are often used in modern cures. All these substances are incorporated in a solution (brane) which is injected under pressure into the side of meat using a multi-needle injection machine. An informative summary of the processes involved in curing and maturation during the manufacture of bacon is given by Lawrie (1985a). A more detailed account is to be found in the U.K. Bacon and Meat Manufacturers Association (BMMMA) Code of Practice (1978).

References in the literature to structural changes occurring in muscle tissue as a result of its conversion to bacon are scarce. However, the microstructure of raw bacon has been briefly described by Cassens et al. (1979), and of raw and cooked bacon by Stanley et al. (1980). Lewis (1974) described variations observed in sections of back bacon. Some myofibrils displayed recognisable features of sarcomeres, such as well-defined Z-lines and an array of filaments. Other myofibrils presented a granular appearance between rather indistinct Z-lines. According to Lewis, these two appearances are similar to those he observed in pork muscle following treatment with 10% salt plus polyphosphate and 10 or 20% salt alone. An additional variable was the brine to meat ratio, a high ratio (10:1) resulting in more structural disturbance than a low ratio (1:1). Lewis (1974, 1979) reported seeing contrasting structures in adjacent cells in commercially produced bacon but pointed out that such variation is not uncommon in any meat product which may be examined microscopically.

In previous work aimed at understanding the structural changes which occur when pieces of muscle are treated with salt, Voyle et al. (1984) reported observations made at the myofibrillar level in a model system. Blocks of pig LD muscle were soaked in a solution containing sodium chloride and sodium pyrophosphate at low (5.5) and high (8.0) pH values. A translucent zone extending to a depth of 3 mm from the surface of muscle...
the meat was formed in all the incubating media used. Evidence of swelling of myofibrils with some disruption of the Z-lines was seen in meat treated with either salt alone or salt plus pyrophosphate at pH 5.5. Treatment with salt plus pyrophosphate at both pH values resulted in partial or total removal of myosin from thick filaments close to the surface of the meat block but treatment with salt alone resulted in little or no extraction of thick filaments. Our attention has been drawn to a phenomenon occurring in commercially produced bacon, in which areas with contrasting light-scattering properties confer a striped appearance on the product. Light and dark areas of varying dimensions occur in the muscle tissue, an appearance which is most conspicuous in the LD muscle. The phenomenon has been referred to as ‘tiger-stripe’ (MRI Biennial Report 1981-83). A similar phenomenon has been observed in cooked, salt-treated beef. There is considerable interest in this phenomenon because of its possible influence on the acceptability of the product to the consumer. Using information already available regarding changes in myofibrillar structure resulting from salt treatment, it seemed desirable to investigate the structural basis for the difference in light-scattering properties found in the affected areas.

Materials and Methods

Six pouches of vacuum-packed bacon slices, and several loose slices, were obtained from a commercial processor. The pouches and the slices selected displayed a striped appearance consisting of a more or less parallel array of light and dark areas. The slices had been cut from sides of pig meat injected with curing brine using a multi-needle injection machine. The curing brine included sodium chloride, sodium nitrite and sodium nitrate in quantities similar to those recommended in the U.K. BMMA Code of Practice (1978). No polyphosphate was included. In this specification the target figures for the concentrations of the ingredients of the injection brine are:

- Sodium chloride 20%
- Sodium nitrite 0.08%
- Sodium nitrate 0.20%

The curing process is started within 24 h of slaughter and the brine temperature should be 2 - 4.5 °C. After injection the sides of pig meat are cured and matured according to the Wiltshire process.

Photographs of affected slices were taken using incident illumination and a black background, or with transmitted light reflected from a white background. Photographs were also taken of the entire contents of a pouch, the slices being arranged serially.

Samples were removed from both a light and a dark area of the LD muscle in one bacon slice from each of the vacuum-packed pouches. These were prepared for examination by transmission electron microscopy. Pieces of tissue measuring 5 x 5 mm were taken from each slice having a thickness of 3 mm. The samples were fixed for 18-24 h in 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer at pH 6.8. Fixation was followed by washing in the same buffer prior to further trimming of the blocks of tissue to final dimensions of 2 x 2 x 3 mm. The tissue blocks were fixed for a further 2h in 1% osmium tetroxide in cacodylate buffer, followed by an overnight wash in the same buffer. Dehydration through graded alcohols from 70% to 100% (absolute) alcohol was followed by impregnation and embedding in LR white resin (London Resin Co., Basingstoke, Hants., UK.) and polymerization at 60 °C for 24h. Six blocks from each sample of tissue were prepared, making a total of thirty six blocks each of tissue from light and dark areas. Sections were cut from a minimum of three blocks from each sample at a thickness of 50-60 nm, using a glass or a diamond knife. The sections were mounted on copper grids and stained with uranyl acetate and lead citrate and examined using a JEOL 1200EX electron microscope.

Results

Macroscopic appearance

Individual slices of bacon showed an uneven distribution of light-scattering properties, particularly in the LD muscle, when viewed under incident illumination. In some slices there was an array of alternate light and dark stripes which were approximately perpendicular to the layer of back fat. In other slices high light-scattering properties occurred particularly in the LD muscle. In slices where stripes were clearly visible, the centre-to-centre spacing between stripes of similar light-scattering properties was about 17 mm. Figure 1 shows odd-numbered slices from a commercial vacuum-packed pouch of twelve slices. In slices where stripes were clearly visible, the array of parallel stripes of low and high light-scattering properties in the first slice gave way to increasingly irregular and larger areas of high light-scattering properties so that at the end of the series the majority of the area occupied by the LD muscle in the bacon slice has a light appearance.

Whereas the sites where injection needles entered the meat were not conspicuous in the packed slices of bacon in our sample, some of the loose slices showed regularly spaced marks which were assumed to be caused by the needles. Such marks may be seen in Figure 2a and b (arrows), where they appear to coincide with dark areas in the muscle tissue.

Figures 2a and 2b show the same slice of bacon under incident and transmitted illumination with dark and light backgrounds respectively. Areas which appear dark under incident illumination (Figure 2a), appear light when viewed with transmitted illumination (Figure 2b). This change in appearance indicates a difference in translucency between adjacent areas in the slice of bacon, the areas appearing dark under incident illumination being relatively more translucent than those which appeared light under
Microstructure of bacon

Figure 1 A series of bacon slices from a commercial vacuum-packed pouch showing change in the shape of light and dark areas. Slices were viewed by incident illumination.

Figure 2a A slice of bacon viewed by incident illumination.

Figure 2b The same slice viewed by transmitted illumination. Areas marked * are examples of the reversed light scattering patterns in the 'striped' areas of the longissimus dorsi (LD) muscle. Arrows indicate injection sites.
the same conditions of illumination.

Microscopic appearance

Sarcomere lengths were found to be in the range of 1.1 to 1.3 μm in our samples. Under these conditions it was difficult to recognise discrete A and I bands. The thin filaments normally seen between the A-band and the Z-line were completely overlapped by the thick filaments of the A-band. Furthermore, there was substantial double-overlap between the thin filaments within a sarcomere. These short sarcomeres may be explained by the fact that while the LD muscle was in the pre-rigor condition following slaughter, the carcass was suspended from the Achilles tendon and the muscle was not under tension. Although this constraint made it difficult to determine the fate of thick and thin filaments with precision, substantial differences were observed in the fine structure of myofibrils from areas of contrasting translucency in the LD muscle. Dark areas: Sections of tissue removed from areas which appeared dark under incident illumination consistently showed recognisable features of myofibrillar structure, as illustrated in Figure 3. Myofibrils were clearly defined and Z-lines showed the demarcation of sarcomeres. At a higher magnification overlapping thick and thin filaments could be recognised (Figure 4). No evidence of the removal of thick filaments from the A-band was observed, a finding which is consistent with our previous work (Voyle et al., 1984). Discontinuity within some Z-lines was detected however, possibly due to myofibrillar swelling.

Light areas: Thin sections of tissue from areas which displayed high light-scattering power under incident illumination showed a loss of ordered structure. There was an accumulation of amorphous material such that, in general, myofibrils were no longer clearly defined and structural features normally recognised in sarcomeres were no longer apparent. The appearance shown in Figure 5 is representative of the areas of high light-scattering power in the LD muscle.

Gaps were sometimes observed between blocks of amorphous material such as have just been described. These gaps occurred at intervals of about 1 μm, a periodicity which is similar to the sarcomere length in these samples. It is reasonable to suppose that the break has occurred at the junction between the Z-line and thin filaments, since this is the site of similar breaks observed in conditioned or aged meat (Davey and Dickson, 1970).

Discussion

An increasingly familiar phenomenon, known as 'tiger-stripe', has been observed in pre-packed sliced bacon prepared from Wiltshire sides subjected to multi-needle injection of curing brine. About 80% of the production of sweet cure bacon of one producer, and smaller amounts of mild and ordinary Wiltshire bacon have been found to be affected. In addition, a major retail group in the United Kingdom is known to have returned a sizeable batch of back bacon to the processors because of its apparently unacceptable appearance. Observations made in local supermarkets showed that 'tiger-stripe' can be detected readily in packs of sliced bacon in display cabinets. The pattern observed did not always present as a periodic array of stripes, the size and shape of the areas of high light-scattering power being somewhat variable. Figures 1 and 2a and b illustrate this variability. The purpose of this investigation was to seek a structural explanation for the differences in light-scattering power between the light and the dark areas.

Contributory factors associated with the manufacturing process, which we consider are likely to be involved, include the number and location of injection sites, the composition of the brine, and the spread of the brine through the muscle by mechanical movement and diffusion. It is clear from our results that these factors give rise to changes in the fine structure of the muscle tissue and that these changes alter the light-scattering properties of the tissue. The low light-scatter in regions close to the injection sites is explained by their ordered structure, while the high light-scatter in the areas in between is explained by their amorphous granular structure.

Number and location of injection sites

In an earlier experiment conducted in this laboratory a brine containing 16% sodium chloride and 4% sodium tripolyphosphate was injected by hand into a block of porcine muscle. Around the injection site there was a marked increase in the translucency of the tissue. Similarly, we have previously reported (Voyle et al., 1984) that a translucent zone was formed at the surface of blocks of pig LD incubated in various salt solutions whether or not these contained polyphosphate. It was interesting, therefore, to observe that the injection sites near the LD muscle included in the slice of bacon illustrated in Figure 2 appear to coincide with the stripes of low light-scattering power. The regular spacing between such stripes seen in some slices supports the view that they result from the introduction of brine into the tissue through the array of needles in the injection machine.

The spacing of the needles is important when considering the distribution of brine through the tissue. It would be expected that the closer the needles are together the more efficient would be the distribution of brine. The U.K. BMMCA Code of Practice (1978) does not specify what the distance between needles should be, simply saying that 'the sides should be covered by a regular pattern of injection points'. Pearson and Tauber (1984), in describing the curing method used in North America, say that pickle is well distributed through the meat (bellies and hams) and curing is rapid because a large number of needles spaced close together are used. Pockets of brine formed initially around the injection site would be expected to spread mechanically, due to the pressure applied during the injection process. It is to be expected that the main pathway for the
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Figure 3 Thin section of myofibrils from an area which appeared dark by incident illumination.

Figure 4 Thin section at a higher magnification from an area similar to that shown in Figure 3, showing thin (arrows) and thick (arrow heads) filaments.

Figure 5 Thin section from an area of LD muscle which appeared light by incident illumination. Normal structural features are obscured.

distribution of the brine would be longitudinally between muscle fibre bundles. From these pockets the curing agents would spread by diffusion into the fibres.

It has been reported (MRI Biennial Report, 1981-83) that the levels of water and curing salts are similar in the dark and light areas, although in the hand-injected material referred to previously the translucent zone around the injection site had a higher water content than the more remote light zone. It is likely, however, that the structural changes which give rise to the 'tiger-stripped' phenomenon have occurred before homogeneity in salt distribution is attained, i.e. that change in structure depends on the time course of the change in the concentration of salt and, if present, of polyphosphate, in the tissue.

In taking packed bacon slices from the production line of the processing unit it was not possible to choose samples from precisely identified injection sites. It is clear, however, that in further work samples for structural and other forms of analysis should be selected from sites in the proximity of the injection site as well as from positions a known distance away.

Composition of brine

Target values for the percentage composition of injection and immersion brines used in the Wiltshire tank cure method of bacon manufacture have been listed in the U.K. BMMA Code of Practice (1978) and by Wilson (1981) who also discusses the role of the various constituents of the brine. Aspects of product quality affected include preservation, flavour, water retention and colour. In the context of our investigation the role of sodium chloride is most important. The recommended concentration of salt in brine used in the Wiltshire tank cure method lies in the range of 18-22%, with a target figure of 20%. This is much higher than the final concentration of salt in the meat of about 2-5%.

The influence of salt concentration on water retention and protein solubilization in meat has been discussed at length by a number of workers (Callow, 1929, 1931; Hamm, 1960, 1975; Kotter and Fischer, 1975; Offer and Trinick, 1983; Lawrence, 1985b). Callow (1929, 1931) and Kotter and Fischer (1975) showed that the salt concentration (about 5%) which leads to optimal water uptake is also associated with solubilization of myosin. Offer and Trinick (1983) observed that concentrations of salt of around 3-4% caused substantial swelling of isolated myofibrils, and that this swelling reached a maximum when a substantial part of the A-band had been extracted. Callow (1929, 1931) studied the effect on pig muscle of solutions of sodium chloride over a very large range of concentrations. It is noteworthy that in solutions containing more than 21% salt there was actually a loss of water from the meat and markedly less protein solubilization. It should not be assumed, therefore, that a high concentration of salt, such as would be expected to be present initially around the injection sites, necessarily would cause greater structural...
changes than a lower salt concentration.

A further factor in determining the translucency of the meat is the pH. It should be appreciated that polyphosphate, when present, may cause this to rise in the region of injection sites due to its high buffering capacity.

Changes in the morphology of myofibrils in the presence of concentrated salt solutions, first reported in isolated myofibrils by Hanson and Huxley (1955), may contribute to the changes in appearance of gross tissue, it is probable that extraction of proteins from myofibrils may also play a role, as reported by Offer and Trinick (1983) and Voyle et al. (1984). There was clear evidence of the removal of protein from the A-band when pyrophosphate was present. In the absence of pyrophosphate, A-bands remained intact, an observation which is similar to that made in the present work on bacon slices cured with salt only. In areas of low light-scattering power thick filaments, and to a lesser extent, thin filaments were visible. The high degree of overlap between the thick and thin filaments limited the visibility of the latter.

The most conspicuous change in the appearance of myofibrils was the amorphous granularity associated with those areas of tissue which displayed a high degree of light-scattering. There are several possible explanations for this, one possibility is that denaturation of filaments, such as has been observed in cooked meat (Voyle, 1974) may have occurred. Such a change would result in the fusion or destruction of the filaments normally observed in cross-sections. Another possibility is that sarcoplasmic proteins have been precipitated, even denatured, and deposited on the myofibrils thus obscuring the detail of their fine structure. A further possible explanation is that sarcoplasmic (soluble) proteins or solubilized myofibrillar proteins have been cross-linked to other structures by the action of the fixing agent, glutaraldehyde. However, this action does not explain the initial variation in light scattering which is clearly seen prior to fixation.

Further investigation is required in order to establish which of these explanations is correct. Further work is also needed to determine how the time course of changes in salt concentration in the presence or absence of polyphosphate, and changes in pH, cause a difference in myofibrillar structure between areas close to injection sites and the intervening areas. These investigations will best be made on a model system initially, before returning to factory-processed material.

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References


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Discussion with Reviewers

G.R. Schmidt: Could part of the stripes visualized in the meat product be due to localized pH and ionic strength environments? Could some of the increased disruption of the proteins be due to the physical disruptions supplied by the injecting needle?


Authors: In unpublished trials we have found that striping is more pronounced when polyphosphates are present in the brine, but as in the samples we have described, the phenomenon also occurs in the presence of salt alone. We have drawn attention to the specific effects of polyphosphates as well as to the local rise in pH that will occur near the injection site in the presence of polyphosphate but we do not rule out the effect that this ingredient would have on ionic strength.

It is possible that some mechanical disruption may be due to the injection needles, but we have shown that myofibrillar structure is more, not less ordered in the regions where injection occurs.

H.J. Swatland: Is there any relationship between the periodicity of the 'tiger-stripe' and the fascicular arrangement within the longissimus dorsi (LD) muscle? How are the 'tiger-stripe' arranged in a reconstruction of the muscle from its serial slices?

Authors: Close examination of the surface of bacon slices which include the LD muscle shows bundles of muscle fibres surrounded by perimysial connective tissue, sometimes made more conspicuous by the presence of intramuscular fat. These bundles range from 0.5 mm to about 4 mm in diameter, this variation clearly representing the fascicular arrangement of the fibres within the muscle. However, the stripes, as observed in Figure 2, appear to run independently of the perimysial network. Our Figure 1 gives some indication of the arrangement of the stripes in a serial reconstruction over a limited distance.

C.E. Carpenter: Why is it likely that structural changes occur before homogeneity in salt distribution is attained? Data on the time course has not been presented.

G.R. Schmidt: Is there any reason to believe that in injected products the material becomes completely homogeneous, even if permitted to stand for a considerable length of time after injection?

Authors: As salt diffuses from the regions near the injection sites the salt concentration will rise gradually. It is known that when a critical salt concentration is reached, extraction of proteins and myofibrillar swelling occur on a relatively rapid time scale. Offer and Trinick (1983-text ref.) demonstrated this to be of the order of a few minutes.

Although the advantages of the multi-needle injection method of introducing brine into meat are said to include more efficient and uniform distribution of brine throughout the tissue, we are not aware of any data demonstrating that complete homogeneity is achieved. However, 'tiger-stripe' is clearly seen in bacon samples several days, even months, after injection when
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salt distribution must be much closer to homogeneity than immediately after injection.

G.R. Schmidt: Does the application of mechanical energy such as tumbling or massaging facilitate the uniform distribution of brine ingredients in a meat product?
Authors: Yes, (text ref. Lawrie, 1985a) but this is more frequently used in the production of cured hams. Mechanical action tends to damage fat and cause it to separate from the lean. It is, therefore, not often used for bacon processing.

C.E. Carpenter: Why should a highly ordered structure show less light scatter? Glass is amorphous yet is almost transparent.
Authors: Highly ordered, crystalline materials do not scatter light appreciably except at their surfaces. This is because scattered radiation from the unit cells in the material interferes destructively. For normal crystals whose unit cells have dimensions very much less than the wavelength of light, constructive interference cannot take place with visible light and thus diffraction, that is scattering at a series of defined angles, does not occur. With other, less well-ordered, materials the degree of scatter depends on the inhomogeneities in the material. What is important here is both the magnitude of the inhomogeneities and the distance scale over which they occur. For appreciable scattering to take place, the inhomogeneities should occur over distances approaching and exceeding the wavelength of light. Although glass, like water, is not uniform at the molecular level, over a scale approaching and exceeding the wavelength of light the fluctuations are small and only a small degree of scatter occurs. By contrast, if a piece of glass is shattered into many small particles very large fluctuations occur and the system is highly scattering.

Muscle is composed of myofibrils in which the repeating unit, the sarcomere, is large enough so that at a few discrete angles of incidence constructive interference can arise and diffraction can be observed. At other angles of incidence this does not occur and the light scatter arises only because of non-periodic features of the muscle structure, e.g. particulate material such as mitochondria, and from the small differences that arise between sarcomeres. Should this regularity of the structure be altered in any way, either by physical disruption or the creation of particulate material, the inhomogeneity would arise and the light scattering would be expected to increase.

C.E. Carpenter: From the data presented absorbancy, which has no effect on light-scatter, cannot be excluded as a source of translucency differences.
Authors: We agree that this is a possibility.