Optical Properties of Meat

Howard J. Swatland

Meat is a translucent system. Between a colorimeter placed on a meat surface and the myoglobin dissolved below its surface is a microstructural labyrinth that modifies what a colorimeter measures and how myoglobin appears. This is the realm of refraction, reflectance, and interference, whose overall effect is light scattering—from the darkness of living muscle to the brightness of meat.

INTRODUCTION

Meat color is important to consumers and has been measured extensively (Mancini and Hunt, 2005), but explaining meat color is more complex than measuring meat color. Everyone in this audience has observed how the rate and extent of postmortem glycolysis may cause conditions ranging from dark-cutting beef to pale, soft, exudative (PSE) pork. But how does pH affect the appearance of meat—what is the underlying mechanism?

To answer this question, we need some fundamental points. If white light illuminating an object is absorbed, the object appears black; if most of the light is scattered, the object appears white; and if the light is reflected we see specular or mirror-like reflectance. If light penetrates an object to a limited depth before being returned to the observer, chromophores (pigments) may absorb certain wavelengths of light. Those not absorbed and returned to the surface give the object its hue (the redness of meat caused by myoglobin). Surface effects can be important, but the mechanism for pH-related paleness is within the translucent depth of the meat, where optical properties of meat microstructure are paramount.

The translucency of meat is anisotropic; it has directional properties, as discovered by Elliott (1967). Pork myofibers cut parallel to the plane of measurement appear paler than those cut perpendicularly. If you have no interest in explaining meat color and will only be making routine measurements with a commercial colorimeter, go no further: control the orientation of myofibers in your sample, and your results will be more reliable. And do not forget that myofibers are sectioned obliquely in chops and steaks cut perpendicularly to the vertebral column and that angles may change with animal growth (Swatland, 1994). As for explaining anisotropic translucency in meat, we still have far to go. Keep everything discovered about the proteomics of myoglobin in solution (Suman et al., 2007), but consider the context of meat microstructure (membranes and myofibrils) and their scattering, reflectance, refraction, and interference. There is a lot more to meat color than myoglobin. Even with a myoglobin solution in a spectrophotometer cuvette, the absorbance spectrum will change dramatically if we add scattering particles.

SCATTERING

When biochemists first took a serious look at meat quality, two biophysical mechanisms were proposed to explain how low pH increases reflectance: 1) light scattering may be increased by denaturation and precipitation of sarcoplasmic proteins (Bendall and Wismer-Pedersen, 1962), and 2) surface reflectance from myofibrils may increase (Hamm, 1960). Both ideas should be taken seriously, but they are difficult to separate and they were never tested experimentally (which is forgivable because the authors were biochemists rather than biophysicists). I make no claim to be a biophysicist, but I like to build microscopes and optical apparatus, and this has enabled me to tackle these ideas experimentally. I will attempt a simple explanation showing the apparatus and general results. The optical properties of bulk tissues are extremely complex, and there is no hope of me explaining them because my own understanding is inadequate. On the one hand, we may read that diffraction contributes to light scattering in muscle (Pollack, 1990); on the other hand, we may read that diffraction patterns are completely obscured by irregularities in the striation patterns of muscle (Huxley, 1990). Should we give up? I think not. A simple experimental approach is better than nothing and has the promise of leading to the development of new technologies. A key point about scattering in meat is that we cannot be sure where it originates at the microstructural level. Bendall and Wismer-Pedersen (1962) may have been correct about scattering originating from protein precipitates in PSE pork, but scattering also occurs in living tissues.

When I examined normal (not PSE) pork in a goniospectrophotometer (Figure 1), the results were in complete

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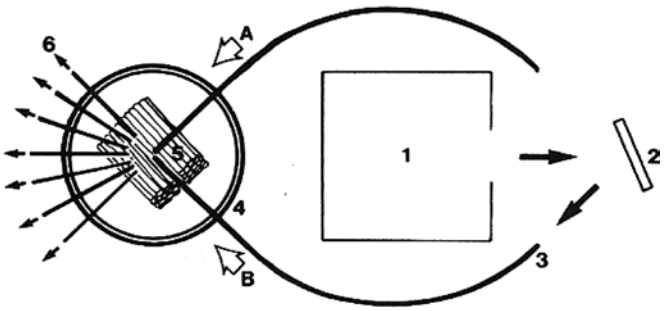

Figure 1. Scattering in pork measured with a goniospectrophotometer (Swatland, 1988). A xenon illuminator (1) reflects light into 1 of 2 fiber-optic light guides via a rotatable mirror (2) so that pork myofibers can be illuminated perpendicularly (A) or parallel (B) with respect to their long axes, and measurements of scattered light can be made from various angles using 6 optical fibers

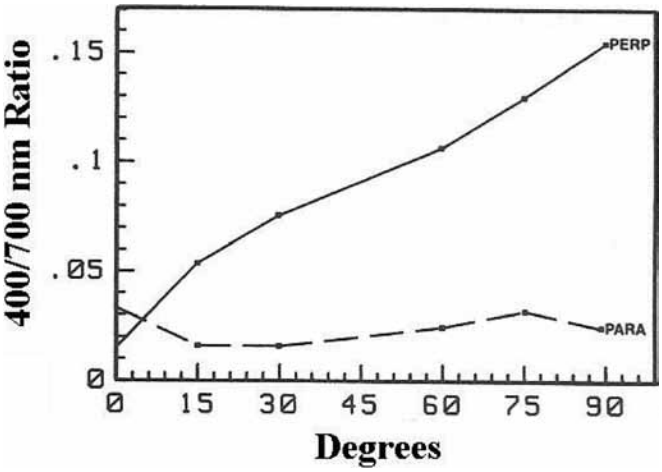

Figure 2. Reflectance ratio of 400/700 nm, with illumination perpendicular (PERP) and parallel (PARA) to myofiber long axes.

agreement with the observations of Elliott (1967). Let us back up a bit. Why is the sky blue? The answer, courtesy of British physicist Lord Rayleigh, is that short wavelengths are scattered far more than long wavelengths; thus, the sky overhead is blue, whereas sunrise and sunset appear red. Hence, a ratio 400/700 nm (violet/red) shows scattering. When myofibers are illuminated perpendicularly to their long axes (PERP in Figure 2), the scattering increases from measurements made in line with the illumination (0° in Figure 2), and it increases progressively as measurements are made to one side of the illumination (90° in Figure 2). But if myofibers are illuminated along their axes, the scattering is independent of the measuring angle; the 400/700 nm ratio is more or less constant (PARA in Figure 2). There are at least two possible explanations. First, perpendicular illumination will illuminate myofilaments along their length (thin myofilaments ≈1.2 to 1.3 μm; thick myofilaments ≈1.6 μm), whereas parallel illumination will illuminate myofilaments on their much smaller axial dimension (thin myofilaments ≈7 nm; thick myofilaments ≈15 nm). Note the orders of magnitude of difference between micrometers and nanometers. Second, myofibers themselves may act as optical fibers, conducting light by a series of internal reflections at the boundary between high (myofibrillar) and low (sarcoplasmic or intercellular fluid) refractive indices.

Bendall and Wismer-Pedersen (1962) were primarily concerned with increased scattering from the precipitation of sarcoplasmic proteins by low pH in hot muscle, superimposed on the scattering effects found in normal pork. Birth et al. (1978) confirmed this by scanning with a photodiode below pork chops illuminated from above by a red laser (Figure 3). Chops containing sections of longissimus dorsi were cut at a thickness of 25 mm with myofibers at an uncontrolled angle to the laser beam (probably about 45°). Illumination was at 632 nm and the photodiode scanned unilaterally (to only one side of the laser

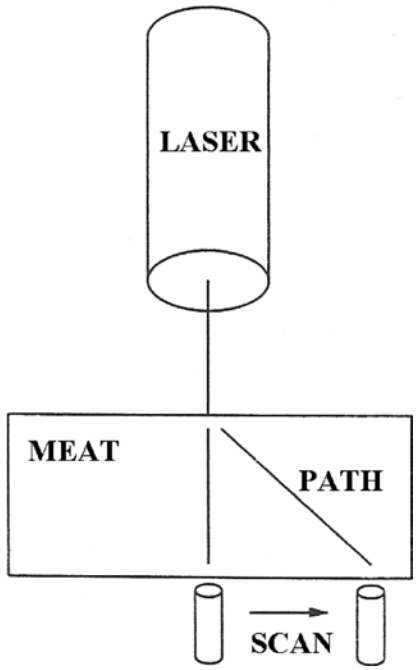

Figure 3. Measurement of light scattering in pale, soft, exudative pork by scanning a photodiode below a pork chop illuminated from above by a red laser (Birth et al., 1978).

beam) beneath the meat sample with a 2-mm-diameter measuring aperture. Over a certain range, the logarithm of the photodetector response was approximately linear with respect to the optical path length through the meat, which is how the slope of the intensity-distance relationship was used as a measure of scattering. For normal pork, the laser beam was scattered slightly to form a small bright spot on the lower surface of the pork chop; for PSE pork, the beam was scattered widely to produce a large dull spot.

With a view to developing methods that are industrially applicable, the laser-scattering method invented by Birth et al. (1978) is of great interest. With the advances in digital imaging since the 1970s, we might now capture an image above the sample and make the same measurement of scattered laser light. However, when laser scanning was tested for detecting PSE turkey breasts, some other factors became apparent (Swatland, 1991). Following the method of Birth et al. (1978), the length of the light path through the meat was calculated trigonometrically from the position of the photodetector and the depth of the meat sample, and the photodetector response was transformed to a logarithm. However, complete bilateral scans passing across the area of meat illuminated by the laser showed strong optical asymmetry caused by the myofiber orientation distorting the scattering pattern. Even worse, curvilinear responses were difficult to reduce to a coefficient using a linear regression of a logarithm, and they were strongly dependent on sample thickness, which was difficult to control. These problems are not insoluble, but neither can they be ignored: scattering in meat depends on wavelength and myofiber orientation (Figure 4).

A final point about scattering not generally appreciated relates to cooking the meat. When red meat develops a brown color, research attention has focused on the chemical development of brown pigments, but the changes in chromaticity coordinates x and y are quite minor. The major change is a massive increase in paleness from scattering (CIE Y in Figure 5).

REFLECTANCE

The idea that the paleness of PSE meat originates from the increased lateral surface reflectance of myofibrils (Hamm, 1960) has received very little attention for the last 50 years, but this is a difficult possibility to test. X-ray diffraction shows that low pH decreases the lateral negative electrostatic repulsion between myofilaments so that they move closer together, shrinking myofibrillar diameters and releasing fluid (Diesbourg et al., 1988; Irving et al., 1989; Swatland et al., 1989). As Hamm (1960) suggested, this is highly likely to increase the refractive index of myofibrils and, hence, to increase Fresnel reflectance. This should be detectable by polarized light; the problem is how to interface the apparatus to bulk meat. A solution is shown in Figure 6. Unfortunately, this gave little or no evidence of Fresnel reflectance from myofibrils (Swatland, 1998a). In fact, when myofibrillar proteins were dissolved in a sodium chloride solution, the polarization of light reflected from within bulk meat increased, indicating that it originated from membranes now in a clear solution (Swatland and Barbut, 1999). Why the concern with working on bulk meat? Myofibrillar reflectance can be seen by microscopy using a variety of methods, but either they change the refractive index of the medium above the myofibrillar surface, or they use a method to enhance the reflective boundary, as in scanning confocal light microscopy (Offer et al., 1989). There is no doubt the myofibrillar surface can be made to reflect light, but does this prove myofibrillar reflectance is the cause of pH-related paleness in meat when the myofibrillar surface is embedded in sarcoplasm?

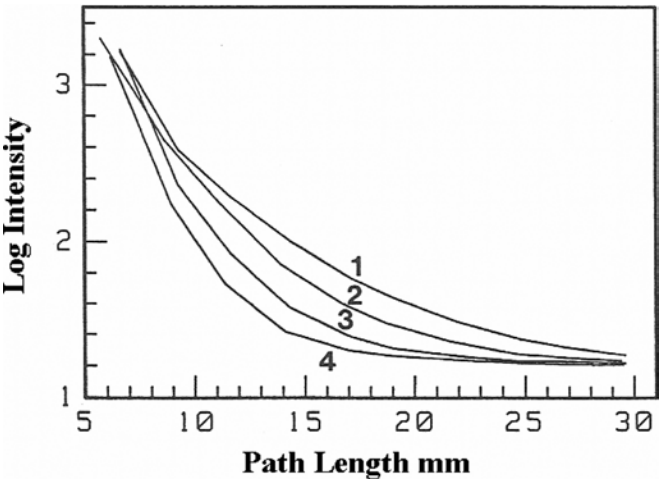

Figure 4. Laser scans through turkey breast meat using red (lines 1 and 2) and green (lines 3 and 4) lasers, perpendicular (lines 1 and 3) and parallel (lines 2 and 4) to the long axes of myofibers (Swatland, 1991).

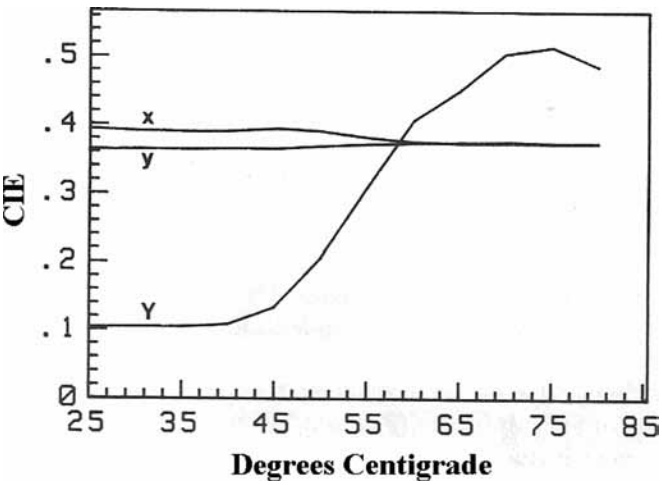

Figure 5. Changes in Commission Internationale de l'Éclairage (CIE) chromaticity coordinates inside mutton during cooking, using a weighted ordinate transformation of data collected by fiber optics (Swatland, 1989b).

In summary, the idea that pH-related paleness originates from protein precipitation (Bendall and Wismer-Pedersen, 1962) could be true in cases of severe PSE meat but is unlikely to explain the pH-related differences in paleness between normal and dark-cutting meat. With continuous flushing of disks of normal pork with phosphate buffer, paleness can be controlled by changing the pH of the buffer; paleness can be increased or decreased in cycles

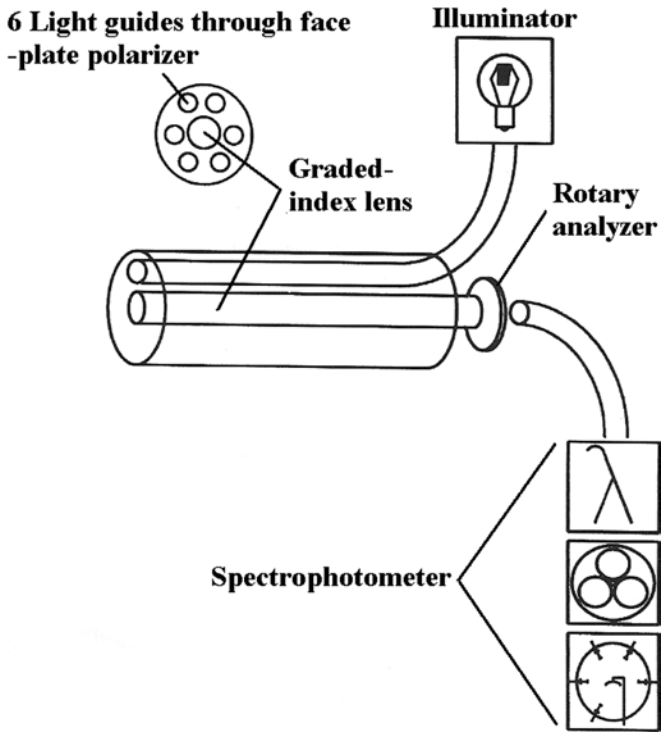

Figure 6. Polarimetry probe for bulk meat, with light to the meat passing through a face-plate polarizer with a central hole for a graded index lens to keep the polarization of light returning from the meat, thus allowing space for a rotary analyzer (Swatland, 1998a).

(Swatland, 1995). To be the cause of pH-related paleness in this situation, precipitated proteins would have to resist flushing, and precipitation would have to be reversible; both are possible, but unlikely. Similarly, the idea that pH-related paleness is caused by lateral myofibrillar reflectance (Hamm, 1960) has not yet been proved. It is disturbing to leave pH-related paleness without explanation; it is profoundly important in meat science, and everyone has seen it. Fortunately, as discussed below, there is another possibility, although not immediately obvious.

REFRACTION

An interesting point to know about white paint is that we pay a premium for very bright white paint containing inclusions with a high refractive index. How might this apply to meat? Consider the possibility, shown in Figure 7, of how a ray of light from above the meat might be refracted back to an observer. This possibility agrees with the anisotropy of meat translucency because if the light ray passes along the length of the myofibrils, there will be minimal refraction back to the observer. Light passing along the length of a myofiber may travel by internal reflections within the myofibrils, as well as in the sarcoplasm between the myofibrils, where there is considerable absorbance by mitochondria, depending on the histochemical fiber type (Swatland, 2004). Thus, the possibility in Figure 7 reveals two contributions to the optical anisotropy of

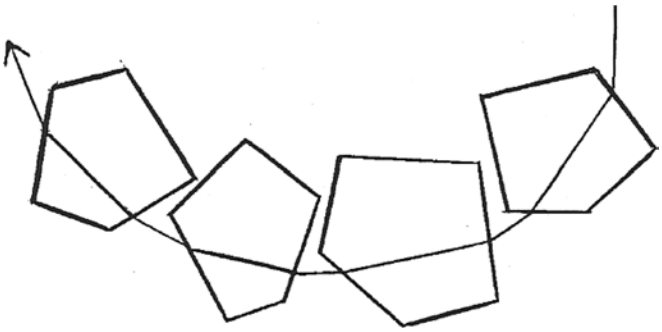

Figure 7. Diagrammatic transverse sections of myofibrils showing how a ray of light from above the meat might be refracted back to an observer.

bulk meat (lesser myofibrillar refraction and greater sarcoplasmic absorbance along myofibers than across myofibers). But what is the evidence for myofibrillar refraction? At this point, we change to using a second meaning for anisotropy, relating to polarized light and myofilament orientation. Bulk meat is optically anisotropic at the macroscopic level in a colorimeter, whereas with a polarizing microscope, we can also detect the anisotropy giving rise to the names for the bands along the myofibrils, the A band (anisotropic) and the I band (isotropic).

A ruler partly immersed in water appears to bend (refraction) at the water surface, where the speed of light decreases as it passes from air to water. Water is isotropic (i.e., it has no directional properties) and thus has a single refractive index. But the alignment of charged groups on myofilament surfaces interacts with the electric vector of light so that myofibrils have two refractive indices (birefringence); light passing along the myofilaments is slower than light passing across the myofilaments. A polarizing microscope has a polarizer in the illumination pathway so that only plane polarized light illuminates the specimen, whereas above the specimen is another polarizer, called the analyzer (because it is rotatable and may be used to make measurements). If the analyzer is rotated perpendicularly to the polarizer, then the microscope field is dark, except if a birefringent specimen can rotate the plane of polarized light. Thus, the A bands of the sarcomere transmit more light (i.e., are brighter) than the I bands of the sarcomere (Figure 8). This is the opposite of a stained myofiber viewed with an ordinary microscope, in which myosin in the A band takes up more stain than actin in the I band so that the A band is darker than the I band. Figure 8 reveals that the I band is not completely isotropic; it simply has lower anisotropy than the A band. Similarly, the Z line can have slightly greater anisotropy than the I band, and the lowest anisotropy in the A band corresponds to the H zone. So where is all this leading? With a flash of as yet unfulfilled hope, you might guess that polarized light offers a chance for measuring sarcomere length and detecting cold shortening. With less need for hope and more certainty, you may be interested

to learn that polarized light microscopy may be used to measure the difference between the two refractive indices of the myofibril (Figure 9). In numerous experiments with both manual and automated microscopes, and with numerous types of meat, the result is always the same: birefringence increases as pH declines toward the isoelectric point (Figure 10).

So why do I think the effect in Figure 10 contributes to pH-related paleness in meat (Swatland, 2008)? Starting with samples of PSE chicken breast meat and comparing them with normal meat, 1) meat with low pH appears pale because it scatters more light back to the observer than meat with high pH, 2) meat with high pH appears dark because it transmits more light into its depth than meat with low pH, 3) the effect of pH is detectable at the cellular level across individual myofibers, and 4) pH affects myofibrillar refraction within myofibers. Under the microscope in this experiment, there was no visible protein precipitate when using differential interference contrast of transmitted light, nor was there any bright reflectance from myofibrils when using vertical illumination from above the specimen.

INTERFERENCE

Cutting across the grain of beef semitendinosus sometimes reveals rainbow colors—iridescence (Lawrence et al., 2002; Kukowski et al., 2004; Fulladosa et al., 2009; Realini et al., 2011). Iridescence has not yet been reported in myofibers cut along their length, so iridescence has some connection with light passing along the length of myofibers; iridescence appears when myofibers are sectioned transversely. Bundles of myofibers may all exhibit the same color, but a range of colors is often seen in different myofibers adjacent to each other. In other words, the myofibers are optically isolated, and each myofiber may exhibit its own color. Under a polarizing microscope, the interference colors are not altered by rotating a polarizer in the illumination pathway or by rotating the analyzer in the measuring pathway. But when the analyzer is perpendicular to the polarizer, iridescence is completely extinguished, as is Fresnel reflectance from the meat surface. The reflectance spectra of interference colors in individual myofibers show interference peaks (Figure 11); major peaks are strongly dependent on the angle of measurement. Figure 8 shows that A bands have a higher refractive index than I bands. Does reflectance from A bands with interference from reflectance from A bands at different depths account for iridescence?

What is the importance of this rarity (rainbow colors on the meat counter)? Although iridescence may appear only when myofibers with a favorable degree of hydration and sarcomere length are cut neatly in cross section, all myofibers may have interference along their length as a source of scattering. This will weaken, but not cancel, the macroscopic anisotropy in meat translucency, as summarized in Table 1, which is presented to show how the optical anisotropy of meat translucency may impinge on the

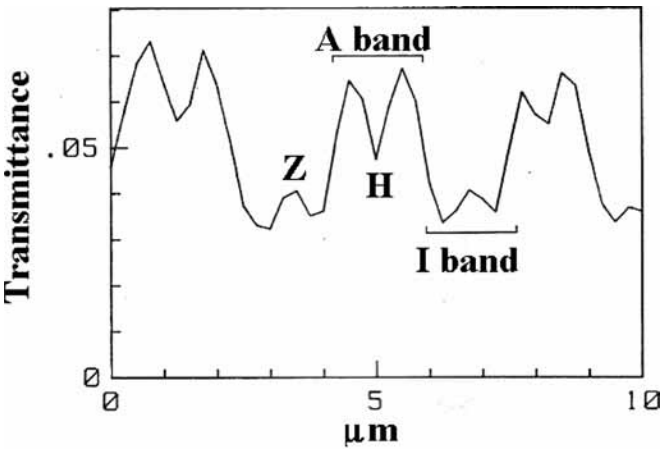

Figure 8. Scanning along three sarcomeres of a pork myofiber with a polarizing microscope (Swatland, 1989a).

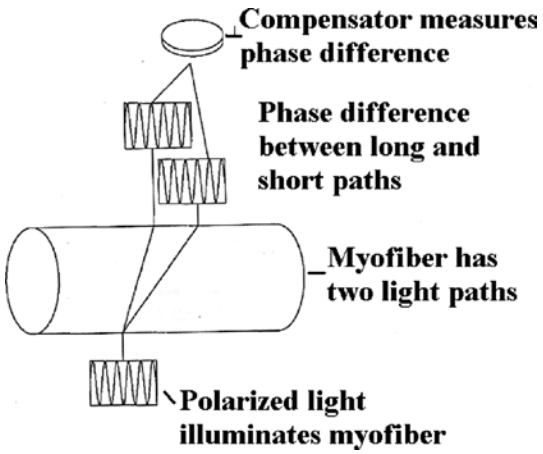

Figure 9. How a de Sénarmont compensator measures birefringence.

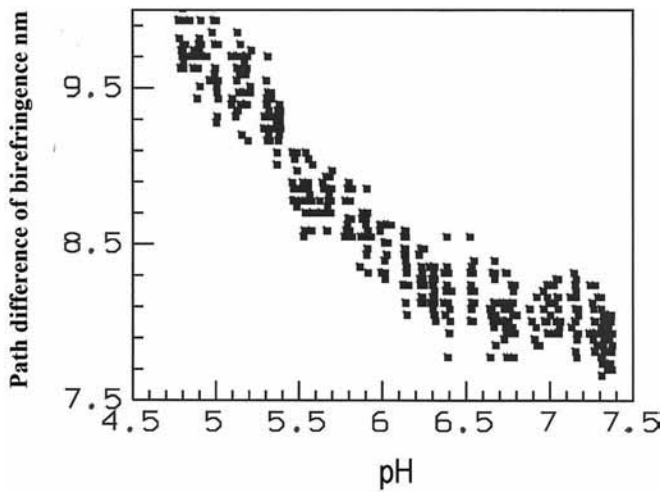

Figure 10. Path difference of birefringence increases as pH declines.

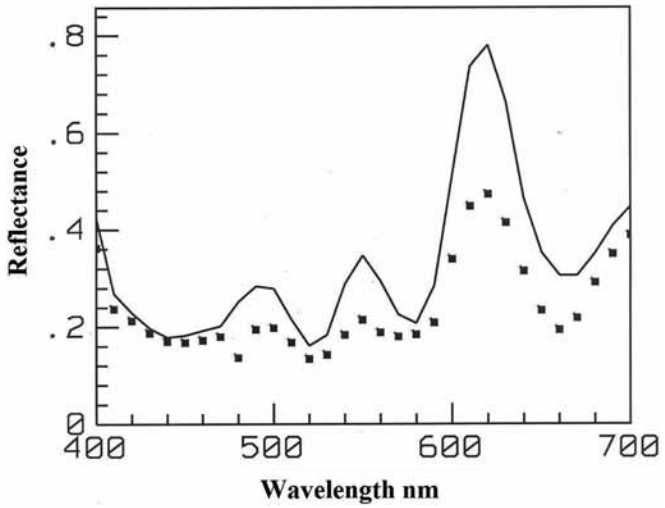

Figure 11. Reflectance spectrum of second-order yellow interference in myofibers from cooked beef iliocostalis (Swatland, 2011). The line is a mean (n = 10) with the standard deviation subtracted (■).

mainstream meat science topics of myoglobin chemistry and meat colorimetry.

What is the possible relationship between light scattering causing paleness and the interference of reflected light causing iridescence? How might paleness and iridescence be interrelated? The answer may depend on the number of reflective layers. When only a few A bands are involved, interference colors may appear (Figure 11), but when many reflective layers are involved, the colors may become a misty white of scattered light. If scattering from myofibrillar refraction is less than normal because of high pH, this may allow iridescence to appear. Iridescence is lost when scattering is strong. Myofibrillar reflectance as proposed by Hamm (1960) may need some amendment. Probably it is not the lateral surface of the myofibril that scatters light by reflectance, but the A bands reflecting light passing along myofibers.

LOOKING FORWARD

A full consideration of the optical properties of meat would require a whole textbook, which would not sell many copies, so only a few points that might interest a polite audience have been considered here. Methods such as the infrared analysis of meat (Swatland, 1985) have now become routine, and fluorescence (Swatland, 1987) also may be heading in this direction. Whether a method becomes popular depends largely on the commercial availability of instrumentation; this, in turn, depends on the number of potential applications. Infrared has many applications in medicine and agriculture, so many instruments are adaptable for use with meat, and fluorescence is catching up fast. What other multipurpose instruments are already commercially available and waiting to be applied by meat scientists?

The most promising is hyperspectral or multispectral polarization imaging. For each of the pixels in a digital image, information may be obtained on the wavelength and plane of polarization of reflected light by using an acousto-optical tunable filter (AOTF). The AOTF is a piezoelectric transducer coupled with a crystal of tellurium or silicon dioxide to create acoustical waves altering the refractive index of the crystal at a high frequency. This functions like a diffraction grating in spectrophotometry, but instead of a slow, mechanical rotation of a grating, the wavelength is changed almost instantaneously by the electrical frequency applied to the piezoelectric transducer. As with meat colorimetry, results from an AOTF system will depend on myofiber orientation.

Making guesses about future technologies requires a broad look at the way things are moving right now. The primary restraint at present is how to access a muscle. A few muscles are exposed on the carcass, but measurements made on them have errors from wet or dry surfaces, aerobic exposure leading to oxygenation or oxidation of myoglobin, and irregular myofiber orientation. More reliable results may be obtained with a fiber-optic or photodiode probe pushed through a muscle to obtain measurements, or from digital imaging of a muscle cross-sectional area such as the rib eye. Both probes and digital imaging allow the acquisition of a multidimensional matrix. For example, if the baseline measurement is reflectance, we can add an extra dimension by including different light paths through the meat (Swatland and Irie, 1991)—in Figure 3, for example, by illuminating with white light and scanning with a spectrophotometer instead of using a

Table 1. Summary suggesting how the optical anisotropy of meat translucency relates to mainstream topics of myoglobin chemistry and meat colorimetry

Light along myofibers	Light across myofibers
Goes deep into meat	Strong scattering back to meat surface
Long light path for absorbance by mitochondria and for selective absorbance by myoglobin	Short light path with minimal absorbance by mitochondria and selective absorbance by myoglobin
Dominant scattering from A-band reflectance plus interference	Dominant scattering from lateral refraction through myofibrils
Effect of low pH—not measured yet	Effect of low pH—increases myofibrillar refraction

laser and a photodiode. Many different dimensions can be added. For a probe giving a vector of depth measurements, we may add reflectance and fluorescence (Swatland, 2000), mechanical resistance to penetration (Swatland, 2005), optical path length (Swatland, 1999b), or electrical impedance (Swatland, 1999a). For digital imaging based on the x:y coordinates of an image, we may add reflectance wavelength and polarization using an AOTF. Thus, a likely direction for progress is in the acquisition of multidimensional signals, and this makes a whole realm of statistical methods available for extracting key information. Treatment of matrices with Boolean algebra allows memory, learning, and probability-based decisions (Swatland, 1998b). This is my guess regarding how the optical properties of meat might one day become important for meat scientists. Right now, is this just a hobby for an emeritus professor?

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