

Optical Prediction of Water-Holding Capacity

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Summary

The pH of meat has a strong effect on both light scattering (paleness) and water-holding capacity (WHC), using WHC imprecisely as a general term to describe fluid distribution and retention. Light scattering has many attributes in meat (in relation to wavelength, direction and polarization), and is easier to measure on a routine basis than pH. Thus, the indirect correlation of light scattering with WHC may allow us to predict WHC under industrial conditions, but advances in instrumentation are required before rigorous quality control standards can be imposed. The major problem is the non-linearity of pH effects. Predicting WHC is a lot more difficult than merely grading PSE objectively.

Introduction

Other presentations at this conference (Hamm, van Laack and Schmidt) describe the mechanism and characteristics of WHC in meat, and the objective of this paper is to explore the relationships between WHC and optical properties of meat. There are two goals: firstly, to develop instrumentation for the accurate prediction of WHC under industrial conditions (rapid, non-destructive, reliable and suitable for automation); and, secondly, to improve our understanding of how pH affects light scattering in meat.

We start with a brief review of detecting PSE pork with monochromatic light, because this is where the idea of using optical properties to predict WHC originates. Then follows a brief look at the effect of pH on WHC, and a consideration of spectrophotometry, which has more to offer than monochromatic measurements. Finally, other possibilities for future development are presented, such as goniophotometry and birefringence. En route, some basic questions are raised concerning the mechanisms by which pH affects both light scattering and WHC.

Monochromatic Measurements in Relation to pH

The paleness or darkness of meat, especially pork, is a good guide to its water-holding capacity, and experienced butchers must have known this for centuries, although they may have been more interested in paleness as a predictor of

keeping properties or the ease of penetration of dry curing salts. The search for rapid, non-destructive methods of predicting WHC started in the late 1930's, but it was not until the 1960's that German researchers started to exploit the correlation of paleness with WHC, or had access to commercially-available hand-held reflectance meters for measuring paleness. A general feature of most of these instruments is that they are or were monochromatic, making a reflectance measurement at a single color, usually a broad band of wavelengths.

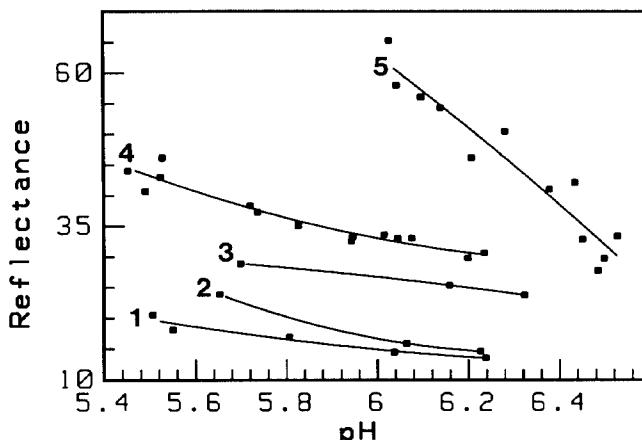
As research progressed, there was little experimental work to discover how low pH causes paleness, even though this period was marked by great progress in understanding how low pH decreases WHC, as in the work of Bendall, Hamm and Wismer-Pedersen. With a variety of photometers in use, each giving a different scale of readings, and many of them not originally designed for measuring meat, common trends among the results were difficult to establish. Line 1 in Fig. 1 shows results from MacDougall and Disney (1967) obtained with an Optica CF4DR instrument measuring reflectance at 525nm. Line 2 in Fig. 1 shows data from Barton-Gade (1981) and line 3 shows data from Lundström et al. (1979), both using a similar instrument (Zeiss Elrepho) at 535 nm. Line 4 in Fig. 1 (Schwörer et al., 1980) was obtained with an EEL 11 (Evans Electroelenium Ltd) reflectometer and a green filter (530-540 nm). These reflectance values vary when plotted against pH₁, probably because of differences in wavelength and method of standardization. The EEL reflectometer was first used by MacDougall et al. (1969), but the data of line 5 in Fig. 1 are from Evans et al. (1978) and Kempster et al. (1984). The scatter plot of data shown in Fig. 1 is for mean values (rather than raw data) and the relationship between reflectance and pH₁ appears to be stronger than it really is. Kempster et al. (1984) found that reflectance may be only poorly correlated with pH₁ ($r = -0.17$, $r^2 = 2.9\%$). Stronger correlations of reflectance with pH₁ may be detected in small studies under laboratory conditions (MacDougall and Disney, 1967). Essentially, all five lines shown in Fig. 1 are fairly close to being straight-line relationships, which contrasts to the marked curvilinearity of the relationships considered next and to the results of Warris and Brown (1987), who found a biphasic relationship between drip loss and reflectance. Below pH 6.1, decreasing the pH₄₅ had a small effect on drip loss and a large effect on reflectance, while effects were reversed above pH 6.1.

The Goefo reflectance photometer is or was used in Germany and Canada, and in the former country of Yugoslavia, to measure pork paleness. The Goefo is standardised against a dark plate instead of a white one, as in the results shown in Fig. 1. Thus, the current to the Goefo ammeter is directly proportional to pH₁ (Fig. 2) so that a low Goefo value indicates pale pork. At first, it appeared that Goefo mean values might

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Figure 1



Relationships of pH_i with monochromatic reflectance for longissimus dorsi muscles of Pietrain, Pietrain x Landrace and Landrace pigs using an Optica CF4DR (line 1, data from MacDougall and Disney, 1967); for Danish Landrace pigs using a Zeiss Elrepho (line 2, data from Barton-Gade, 1981); longissimus dorsi muscles of Swedish Landrace and Yorkshire pigs using a Zeiss Elrepho (line 3, data from Lundström et al., 1979); for longissimus dorsi muscles of Swiss Large White and Landrace pigs using an EEL 11 with green filter (line 4, data from Schwörer, 1982); and for longissimus dorsi muscles of English commercial pigs using an EEL smoke-stain photometer (curve 5, data from Evans et al., 1978, and Kempster et al., 1984).

be related to pH_i by a line similar to that derived from the first equation shown below. But this was not the case in larger data sets, as in data provided by K.H. Schepers (Bonn University) to the late J.R. Bendall, where the envelope enclosing the experimental points varies by as much as ± 15 Goefo units from a line of best fit (line 3 in Fig. 2). Goefo mean values appear to be related quasi-linearly with [H⁺] rather than with pH_i. From lines 1 and 3 of Fig. 2, respectively:

$$\text{amps} \times 10^{-6} = 70.9 - \text{antilog}_{10}(6.95 - \text{ph}_i)$$

$$\text{amps} \times 10^{-6} = 77.1 - \text{antilog}_{10}(7.073 - \text{ph}_i)$$

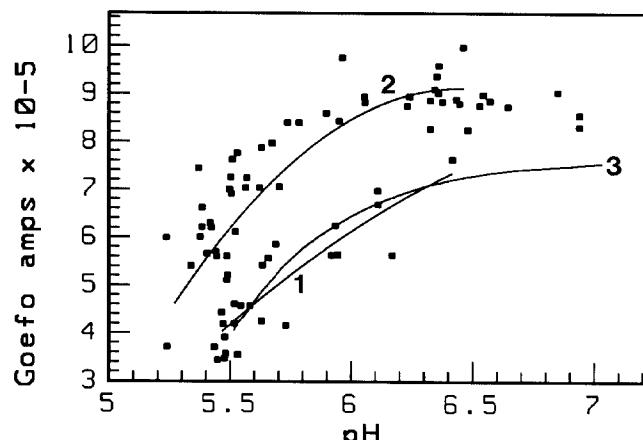
A similar but reversed equation applies to the reflectance data obtained by MacDougall et al. (1969) with a smoke-stain reflectometer.

$$\text{reflectance} = 39.8 + \text{antilog}_{10}(7.02 - \text{pH}_{90})$$

The scatter in all these equations is as high (or higher) as that found by Schepers.

Correlations of monochromatic reflectance with pH₉₀ or with pH_i, based on straight-line regressions seldom are strong enough to be of any industrial value (details given in Table 3 of Bendall and Swatland, 1988). Values for r² only exceed 45% in two small and one larger study, although slightly stronger relationships might exist with a polynomial fit. MacDougall and Disney (1967) and Barton-Gade (1981) found that reflectance at 525-535 nm is affected by myoglobin, which was and still is a problem in attempting to predict WHC from light scattering. Barton-Gade estimated that reflectance falls by about 5% for each extra 8 mg of total heme iron, and found that reflectance increases by about the same amount for an extra 1.7% of fat.

Figure 2



Relationships of pH and monochromatic reflectance measured with a Goefo photometer for longissimus dorsi muscles of German Large White, Landrace and commercial pigs relative to pH_i (line 1 with scatter plot, data from Blendl and Puff, 1978, and Schmitten et al., 1984); Canadian Landrace, Yorkshire and Duroc pigs relative to pH from 45 min to 24 h post-mortem (line 2 with scatter plot, data from Swatland, 1981); and German pigs relative to pH_i (line 3 only, data from Schepers, personal communication).

The pigment effect may not affect reflectance at 640 nm (Eikelenboom et al., 1974). The effect of myoglobin on Goefo reflectance probably contributed to the differences between lines 1 and 3 versus line 2 in Fig. 2 since line 2 included many slow-growing animals with high myoglobin levels (as well as including measurements made perpendicularly to the axes of the muscle fibers).

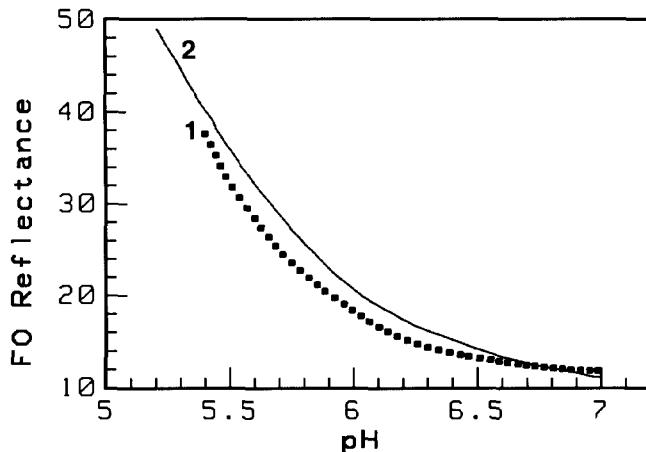
A monochromatic fiber-optic probe was developed by MacDougall and Jones (1975) to measure the internal reflectance or interactance of meat. It has a peak sensitivity at 900nm, where absorbance by red haem pigments is minimal. Bendall and Fenzl (Bendall, personal communication) made fiber optic reflectance measurements during the development of rigor in *longissimus dorsi* muscles of halothane-negative Large White pigs, near to where samples had been taken for measurements of pH and other biochemical parameters. These results (line 1 of Fig. 3) show that fiber optic reflectance increases slowly as the pH falls from 7 to 6.3 and then more rapidly from there down to the pH value of about 5.4, where it reaches a maximum value.

The change in fiber optic reflectance caused by the decline in pH is described by the equation:

$$\text{fiber optic reflectance} = 11.2 + \text{antilog}(6.82 - \text{pH})$$

None of the muscles in these experiments had a pH_i of less than 6.3, and it is extremely unlikely that protein denaturation was the cause of the increase in fiber optic reflectance towards the ultimate pH of approximately 5.4. Somers et al. (1985; line 2 of Fig. 3) showed that the relationship of fiber optic reflectance with pH_i was similar to that of fiber optic reflectance with declining pH.

Figure 3



Relationships of pH and monochromatic fiber optic reflectance as pH falls post-mortem in seven Large White carcasses (line 1, Bendall, personal communication) and for pH_u of longissimus dorsi muscles of 1584 Irish pigs (line 2, Somers et al., 1985).

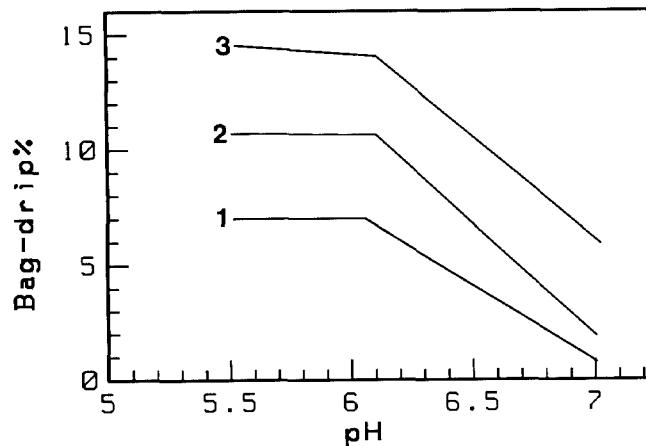
A large increase in fiber optic reflectance occurs in PSS pigs with $\text{pH}_u < 5.8$ and $\text{pH}_u\text{-index} > 60\%$ (Cheah et al. 1984). In halothane-positive pigs, fiber optic reflectance in the *longissimus dorsi* muscles at 24 h post-mortem ($\text{pH}_u \approx 5.4$) rises to 53.4, compared with 32.9 for halothane-negative pigs, and 34 for the Large White pigs at comparable pH_u values of 5.4 to 5.5 (Bendall, personal communication). Protein denaturation may occur in halothane-positive pigs, but is unlikely to be the cause of paleness in halothane-negative pigs (Penny, 1969).

In summary, research with monochromatic systems shows that light scattering can be used to assess the level of PSE in pork and, indirectly, the WHC of the meat, but the latter relationship is not particularly reliable. Two main problems are the confounding effect of myoglobin and possible non-linearity between reflectance and WHC. With regard to the mechanism of pH-related light scattering, the early research shows that it involves something in addition to protein precipitation. For example, differences in protein denaturation are unlikely to explain the difference between normal and dark-cutting beef.

WHC in Relation to pH

Obviously, if light scattering is to be used to predict WHC by exploiting the fact that both are determined by pH, then it is necessary to understand the relationship between pH and WHC. This is a difficult undertaking that, hopefully, other speakers will explain in detail. There are many different protocols for measuring WHC, but the majority are based on only a few basic possibilities: (1) squeezing out fluid with a press, (2) centrifugation and (3) weight loss by gravity acting on sliced muscles. WHC generally decreases if the pH is low or the rate of pH decline is rapid, but the relationship is not exactly linear, and with many methods there seems to be a point of inflection at around pH 6.1. The details are given by Bendall and Swatland (1988, pp. 101-109), careful reading of which will soon reveal a serious problem between results obtained with different methods.

Figure 4



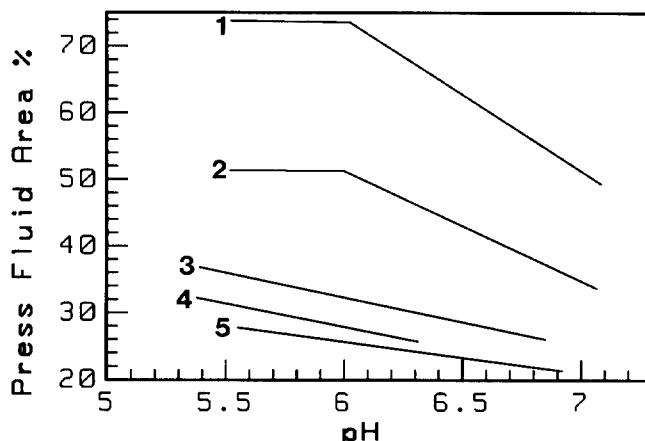
Relationships of pH, with bag-drip% for boneless pork chops at 1 d post-mortem versus pH, (line 1, data from Cheah et al., 1984), for whole chops at 1 day post-mortem (line 2, Large White and Landrace x Large White pigs with $\text{pH}_u > 6.0$, Bendall, personal communication), and for whole chops at 3 days post-mortem (line 3, data from Warriss, 1982).

Bag drip methods using gravity weight-loss from meat slices in an inflated bag to control evaporation (Fig. 4, lines 1 to 3) have a marked inflection at pH 6.1, so that fluid losses increase rapidly as the pH drops from 7 to 6.1, but show little increase below pH 6.1. This can be seen in some press methods where the loose fluid is measured by filter paper area (Fig. 5, lines 1 and 2), while others lack an inflection and have a linear relationship to pH over a wide range (Fig. 5, lines 3 to 5). These latter data are compatible with relationships of fibrillar centrifugation WHC with pH (Fig. 6, lines 2 and 3) but not when there is a point of inflection (Fig. 6, line 1). Thus, bag drip increases rapidly from pH 7 down to 6.1, then has little further increase at lower pH's, whereas fibrillar WHC may have the opposite pattern, showing little change from pH 7 down to 6.1, then a rapid decrease at lower pH's.

Whatever the cause of this conflict, it does not bode well for any optical method that attempts to predict WHC via a mutual dependency of both light scattering and WHC on pH. This problem prompted us (Irving et al., 1989) to use low-angle x-ray diffraction to look at the effect of pH on the negative electrostatic repulsion between filaments which, as other speakers will explain in detail, is the basis of WHC. The results, Fig. 7, follow a similar pattern to that for WHC as seen in Fig. 6, line 1, although the inflection is at pH 6.5 rather than at pH 6.1. Reference to the error bars in the original publication (Irving et al., 1989) allows a reasonable confidence in the existence of a point of inflection, and a similar effect has been observed in muscles of other species. But why is the major component of bag drip loss to be found above pH 6.1 (Fig. 3) if fibrils do not start to release fluid from between their filaments until below this pH 6.5 (Fig. 7)?

To answer this question requires some consideration of downstream fluid compartments from the primary source of fluid from within the filament lattice. In our experiments, we have measured filament separation by x-ray diffraction and the interfiber space by differential interference contrast mi-

Figure 5

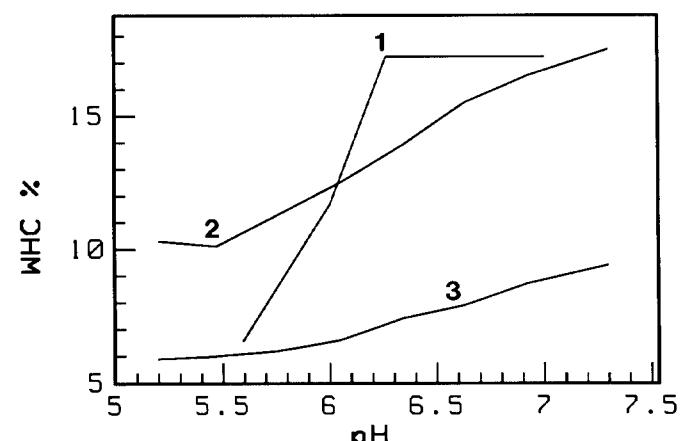


Relationships of pH with various pressed fluid methods, versus pH, in longissimus dorsi muscles of English Large White pigs (line 1, Bendall, personal communication), versus pH, in longissimus dorsi muscle of Danish Landrace pigs (line 2, Wismer-Pedersen, 1959), versus pH, (line 3, Scheper, 1975), versus pH, for Swiss Landrace and Large White pigs (line 4, Schwörer, 1982), and versus pH_v (line 5, Scheper, 1975).

croscopy, at approximately constant sarcomere lengths measured by laser diffraction. Assuming from stereological grounds that these areas are proportional to the volumes of their corresponding fluid compartments, filament lattice spacing (converted to lattice area to be comparable with other areas) was negatively correlated with interfiber area ($r = -.67$, $P < .01$, Fig. 8) and interfiber area was negatively correlated with centrifugation WHC ($r = -.75$, $P < .005$; Fig. 9). Please note that a volume measurement (WHC) has been correlated with an area, and that the relationship is slightly stronger ($r = -.78$) if this is corrected. However, no significant relationship ($P > .05$) was detected between filament lattice area and WHC ($r = .28$), which demands an explanation.

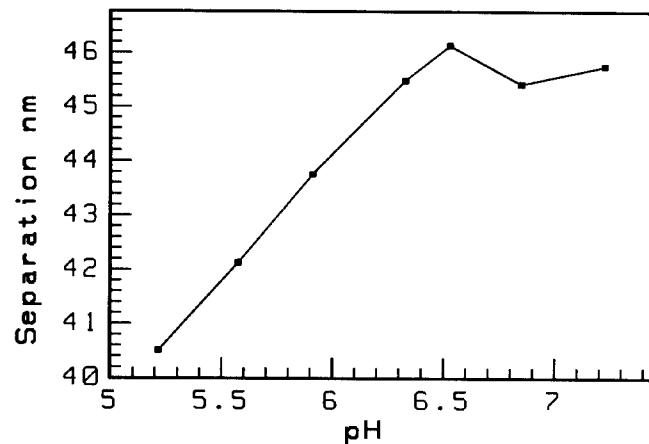
The primary reservoir of releasable muscle fluid from pork is probably from within the filament lattice of the live stress-resistant pig, although I am concerned that stress-susceptible pigs might have some other compartment which is unusually swollen, ultimately to release fluid from the meat. But in rat *extensor digitorum longus* (Zierler, 1973), the total water content (0.80 ml/g wet muscle weight) is partitioned between extracellular space (0.13 ml/g), sarcoplasmic reticulum volume (0.13 ml/g) and sarcoplasmic volume (0.54 ml/g). By extrapolation to zero time post-mortem using the data from Swatland and Belfry (1984) for porcine *longissimus dorsi* muscle, it seems that 20% or less of the sarcoplasmic volume is interfibrillar space (if rat and porcine muscles are comparable), and that the minimum intrafibrillar (interfilament) space is in the order of 0.43 ml/g. Corrections are needed for variations in intramuscular fat content, but the interfilament space looks like the major primary reservoir of fluid released from pork as a result of a post-mortem decline in pH. The interfilament compartment is highly dependent on pH (Diesbourg et al., 1988) whereas a pH-dependency has yet to be demonstrated for the other compartments but could exist. The sarcoplasm between the fibrils and the extracellular fluid between the fibers also may contribute some fluid to be released, but

Figure 6



Relationship of pH with fibrillar WHC (line 1, Penny, 1969, for muscles with pH, 7.0 to 5.4 homogenized in pH 7 buffer; line 2, Bendall and Wismer-Pedersen, 1962, for normal muscles with pH, 6.3 adjusted with NaOH or HC1 to pH 7.2 to 5.2; and line 3, Bendall and Wismer-Pedersen, 1962, for muscles from PSS pigs with pH, 5.6 with pH adjusted as in line 2).

Figure 7



Effect of pH on the lateral separation of filaments in pork longissimus dorsi measured by low-angle x-ray diffraction (Irving et al., 1989).

the relative amount is unknown. Swollen remains of mitochondria and sarcoplasmic reticulum in the interfibrillar compartment might conceivably trap fluid rather than release it as a consequence of a post-mortem decline in pH. Thus, when interfilament area is negatively correlated with interfiber area (Figure 8), perhaps much of the fluid released from the filament lattice has been transferred to, and is still present, in the interfiber space at the time of sampling (when frozen in liquid nitrogen).

Likewise, the interfiber and interfascicular space, rather than the interfibrillar space, could be the reservoir from which fluid may be extracted by high speed centrifugation. Centrifugation causes a decrease in filament separation of only 2.6 nm ($P < .01$), corresponding to a 12% decrease in filament lattice volume. This amounts to a decrease in interfilament space of

less than 14%, whereas fluid losses from centrifugation may range from 17% to 49%. In other words, centrifugation pulls out fluid from other fluid compartments in addition to that from the filament lattice of fibrils, and the decrease in filament lattice area caused by centrifugation is only weakly correlated with WHC ($r = .22$). Thus, differences between samples in WHC may be caused by compartments downstream from the filament lattice, possibly the reservoir between fibrils and demonstrably the reservoir between fibers. Thus, the relationship between filament separation and WHC is indirect. The only relationship between filament lattice separation and WHC might be via their mutual relationship with interfiber space.

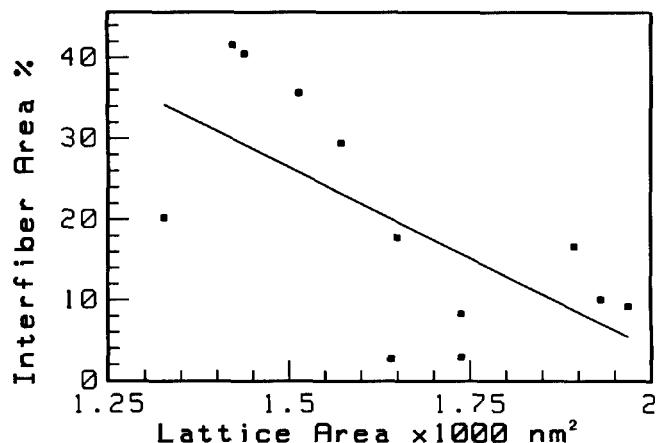
Thus, although the filament lattice probably is the major source of meat exudate, under typical commercial conditions much of its fluid already may have been lost and may be awaiting release from downstream compartments. X-ray diffraction measurements on the fairly consistent population of pigs slaughtered at the University of Guelph (in which PSE is rare unless specially created) show that our pork carcasses lose the fluid from their filament lattice in the first few hours after slaughter, with little subsequent change, and perhaps even a slight re-uptake of fluid (Fig. 10). By about 6 hours post-mortem, transmission electron microscopy shows that the released fluid has swollen the sarcoplasmic compartment between the fibrils to about its maximum (Fig. 11). In summary, we can demonstrate that the spatial sequence of fluid release in normal pigs is from the filament lattice, to the interfibrillar sarcoplasm, to the interfiber space, and, finally, as exudate lost through the interfascicular space. Thus, apparently conflicting data relationships seen in Figs. 4, 5, 6 and 7 could be an illusion. Consider the analogy of rain-bearing clouds in a major storm, first creating a deluge high in a watershed, and then swelling the volume of wetlands and rivers. To estimate the flood risk down on the delta, we need to look at the pattern of the water volume moving downstream, and instantaneous correlations of precipitation on the hills with flooding in the delta might be weak or zero.

In summary, the source of the exudate is traceable by sequential relationships down the pathway from the filament lattice to the meat surface, but direct correlations of the first and last compartments may be very weak if no account is made of the lapse of time. In other words, looking at a real-time effect of pH (eg. changing the pH of samples for x-ray diffraction) is not the same as examining the correlation of drip loss with ultimate pH.

Spectrophotometry

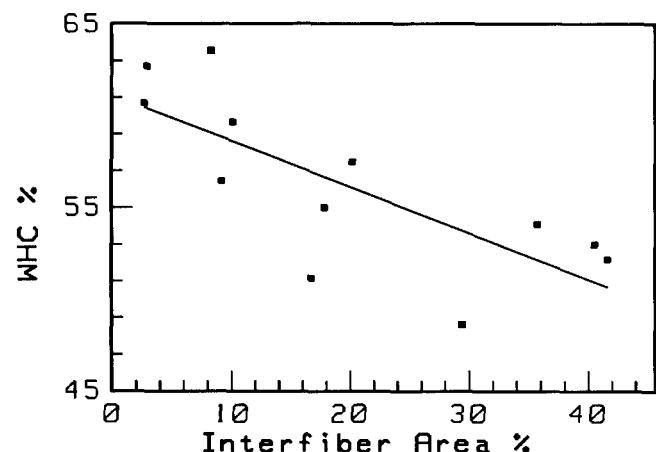
The use of optical fibers to obtain reflectance measurements from within a sample of meat, pioneered by MacDougall and Disney (1967), was a logical continuation of earlier work using glass light pipes with an internally reflecting silvered surface. Spectrophotometry through optical fibers, instead of monochromatic measurements, was the next logical step in the development of this technology (Swatland, 1982). Meat spectrophotometry goes back a long way, at least in Canada (Winkler, 1939), but recent advances in optoelectronics were required before it was possible to make hand-held instrumentation for industrial use. Theoretically, there are several advantages of spectrophotometry over monochromatic measurements.

Figure 8



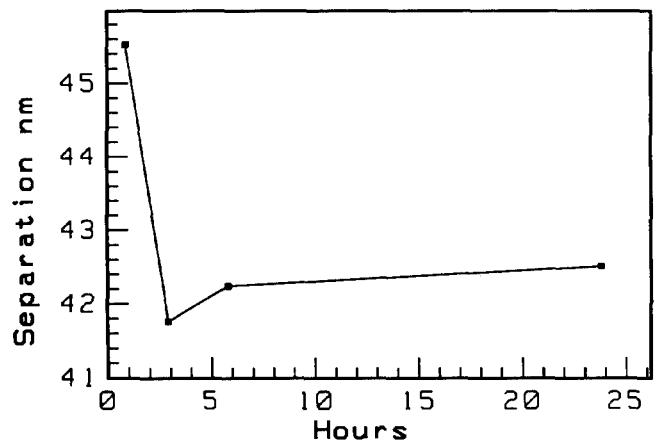
Relationship in pork loins between filament lattice area measured by x-ray diffraction and the area between muscle fibers measured by differential interference contrast microscopy (Swatland et al., 1989).

Figure 9



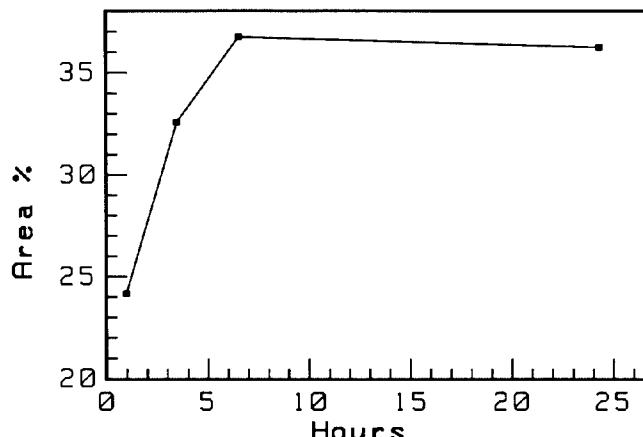
Relationship in pork loins between the area between muscle fibers measured by differential interference contrast microscopy and centrifugation WHC (Swatland et al., 1989).

Figure 10



Decrease in lateral separation between filaments measured by x-ray diffraction in pork loins post-mortem (Diesbourg et al., 1988).

Figure 11



Increase in the sarcoplasmic area between fibrils measured by electron microscopy in pork loins post-mortem (Swatland and Belfry, 1985).

(1) The possibility of measuring myoglobin separately from pH-related light scattering, thus removing the confounding effects of age-related myoglobin discovered by MacDougall and Disney (1967) and Barton-Gade (1981). This is particularly important in grading veal paleness (Swatland, 1985a).

(2) The possibility of using different regions of the spectrum to build a compound prediction equation for WHC.

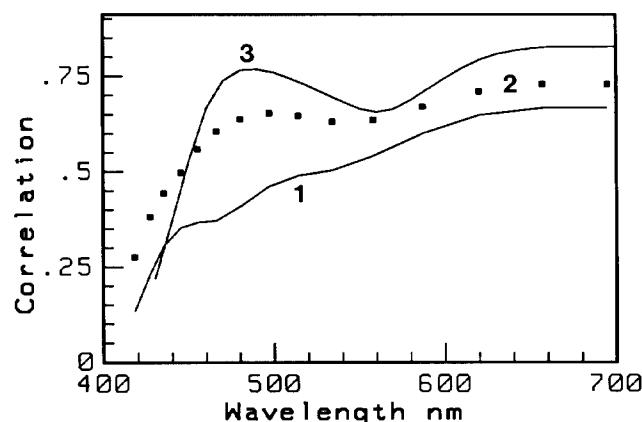
(3) The possibility of using different regions of the spectrum to make other measurements of value in meat science, such as the yellowness (Swatland, 1988a) or softness of fat (Irie and Swatland, 1992a).

All these possibilities have been demonstrated in laboratory research but, for a variety of economic and political reasons, have not yet made the technology transfer into industry. Monochromatic measurements are far easier than spectrophotometry, so that monochromatic apparatus has fewer components and less complexity than spectrophotometric apparatus, thus reducing the cost and increasing the durability.

The Canadian Colormet meat probe (Swatland, 1986) was a multipurpose reflectance spectrophotometer (400 to 700 nm) engineered by Ken Butt of Instrumar Ltd to measure a number of parameters of interest in meat science. Ten years ago, it was at the leading edge of technology, with a xenon flash unit synchronized with a photodiode-array spectrometer and connected through a stainless-steel fiber optic probe. For a sophisticated and relatively rugged device that cost only approximately \$3,000, it is unfortunate that it failed commercially, especially since a similar instrument is being redeveloped in Japan at the present time (Tsuruga et al., 1994).

Results obtained with the Colormet meat probe explain some of the conflicting results obtained earlier with monochromatic devices, because different regions of the spectrum may be seen to have different relationships with pH and with filament separation. Fig. 12 shows the spectral distribution of correlation coefficients of *longissimus dorsi* reflectance with *gluteus medius* reflectance (line 1), of *longissimus dorsi* reflectance with pH_o (line 2), and the absolute value of r (which is negative) for the correlation of *longissimus dorsi* reflectance with x-ray diffraction measurements of interfilament spacing (line 3). **Note how interfilament spacing and light scatter-**

Figure 12



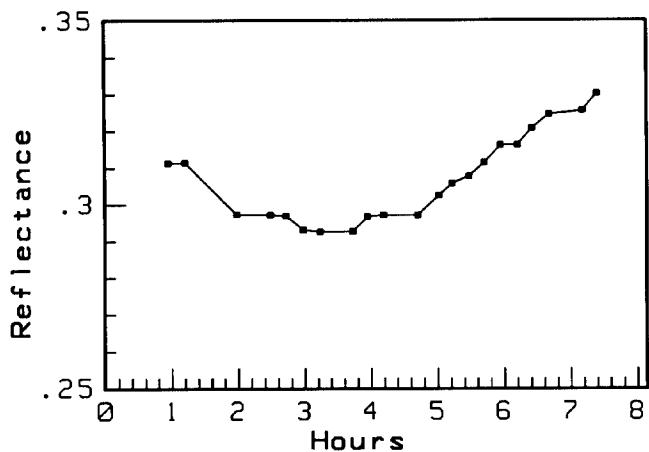
Spectral distribution of absolute values for correlation coefficients of fiber optic reflectance in *longissimus dorsi* with fiber optic reflectance in *gluteus medius* (line 1; Swatland, 1986), with *longissimus dorsi* pH_o (line 2; Swatland, 1986), and with *longissimus dorsi* filament separation measured by x-ray diffraction (line 3, negative correlation from unpublished data of Irving, Swatland and Millman). All samples were from commercial pork loins with a range from mild PSE to mild DFD.

ing are strongly correlated. In this example, where the optical measurement was internal reflectance measured via optical fibers, reflectance of red light provides a good measure of pH-dependent state of the filament lattice with the least error originating from intermuscular variation. Low correlations around 555 nm in lines 2 and 3 probably originate from variance in myoglobin concentration between carcasses. For Fig. 12, the range in pork quality was from mild PSE to mild DFD, and was representative of commercial pork from halothane-negative pigs. Stronger correlations might have been obtained if a greater range in PSE to DFD had been used, but I prefer to work with a typical commercial range of material.

The rate at which light scattering increases after slaughter is important because it determines when measurements can be made, and with what reliability. When optical apparatus to measure pork paleness is maintained at a constant position on a static carcass (so as to avoid sampling error), paleness may show a transient **decrease** before its ultimate **increase** (Fig. 13; Swatland, 1985b). Given that the time of occurrence of the reflectance minimum differs between animals, this is a major problem in the prediction of ultimate WHC from optical measurements made soon after slaughter. Do muscle fibers initially take up extracellular fluid as their internal osmotic pressure rises at the start of post-mortem glycolysis?

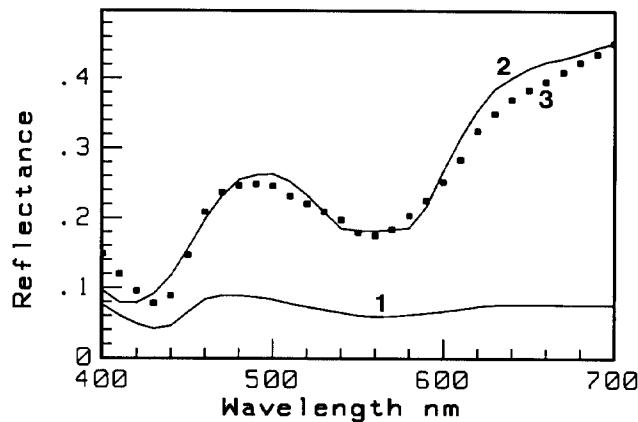
One of the problems of measuring meat spectra through fiber optics is the difficulty of relating them to conventional surface reflectance spectra, because internal and surface reflectance spectra differ in shape (Fig. 14). Some specific differences, especially at the Soret absorbance band of myoglobin, may be attributed to the higher degree of myoglobin oxygenation or oxidation on the meat surface; but also there are general wavelength-related differences originating from within the optical system, at the meat interface, and from scattering within the meat. These more general features affect the 400/700 nm reflectance ratio which is correlated with the water space of the filament lattice (Irving et al., 1989) and with

Figure 13



Transient decrease in reflectance in the adductor muscle of a pork carcass post-mortem (Swatland, 1985b).

Figure 14



Reflectance spectra of pork measured via optical fibers (line 1) and from the meat surface (line 2). Line 3 is a transformation of line 1 by the third power of wavelength, showing a fairly close match to line 2 (Swatland, 1989b).

subjective assessments of paleness (Swatland, 1988b).

Elliott (1967) showed that surface reflectance is higher when muscle fibers are parallel to the surface than when they are perpendicular. However, the effect is not uniform from 400 to 700 nm, and the 400/700 nm reflectance ratio is lower when fibers are parallel to the surface than when they are perpendicular. From Elliott's (1967) results, it appears that the 400/700 nm reflectance ratio is directly related to optical coupling efficiency and, hence, inversely related to the overall intensity of reflectance. Optical coupling efficiency is a concept used in fiber optic technology and some aspects may be applicable to other complex interfaces, such as the meat surface. With a high coupling efficiency, most of the light passing to the meat may be transmitted deep into the meat with little returned to the optical system. With a low coupling efficiency, less light penetrates deep into the meat, because much of it has been returned to the optical system by reflection or scattering in the meat close to the optical window.

Experimental studies (Swatland, 1989b) support this hypothesis. Internal reflectance spectra of pork *longissimus dorsi* muscles measured with a Colormet meat probe were compared with surface reflectance spectra (illumination at 45° and measurement at 90°). Oxymyoglobin was detected on meat surfaces, but not internally. With both methods, reflectance at 400 nm was low (0.5 to 0.1) but, at 700 nm, internal reflectance (0.05 to 0.10) was lower than surface reflectance (0.36 to 0.52), and surface spectra were related to internal spectra by the third power of wavelength. Changes in refractive index of the medium linking optical fibers to white standards caused major changes in reflectance spectra. Thus, the 400/700 nm reflectance ratio may be directly proportional to the coupling efficiency between optical fibers and muscle fibers, but inversely proportional to the overall intensity of reflectance.

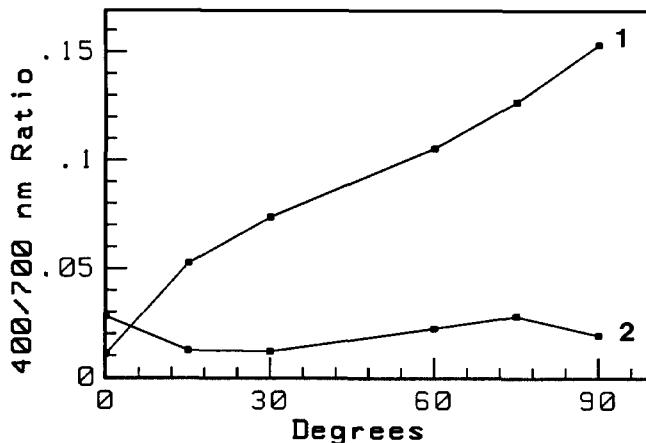
Comparing the lines for surface and fiber optic reflectance in Fig. 14, it is evident that the 400/700 nm reflectance ratio is inversely proportional to the overall intensity of reflectance. With a high reflectance measured on the meat surface, the 400/700 reflectance ratio is about 0.20 for normal pork. With a low reflectance measured internally, the ratio is about 0.96. Step-index optical fibers conduct many modes simultaneously and light strikes the distal perpendicular end-face at many angles. Total internal reflection at the end face occurs when the angle of incidence is large and when the medium beyond the face has a low refractive index. Of the light rays that escape from the end of the fiber, the probability of recapture (sterance) after reflection or scattering in the external medium is dependent upon a number of factors, but mainly wavelength and refractive index of the immediate external medium. Ideally, therefore, the refractive index of the medium linking an optical system to a white plate at the time of standardization should be close to the refractive index expected in the sample and, empirically, internal reflectance spectra collected via optical fibers may be transformed to conventional surface reflectance spectra by a power function of wavelength. Internal Fresnel reflectance from optical fibers in meat probes is described in detail elsewhere (Swatland, 1991) and is an effect that should not be ignored by those who build fiber-optic meat probes or attempt to understand the results obtained from them.

In summary, light scattering is closely related to interfilament spacing, which is determined mainly by pH. When internal reflectance is measured via optical fibers, the information content on filament spacing is towards 700 nm. But, as was argued earlier, interfilament spacing may not have a strong instantaneous correlation with WHC (because of streaming from one compartment to another: Filament lattice → sarcoplasm → interfiber → interfascicular → surface).

Light Scattering and Goniophotometry

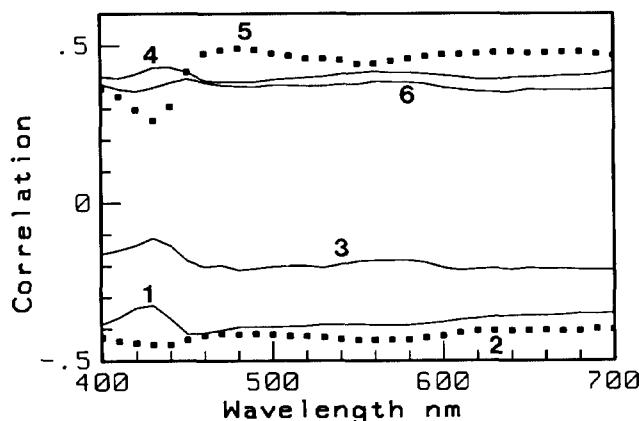
Goniophotometry, measuring light through different angles, is another way of measuring light scattering. The Kubelka-Munk function for light scattering in translucent systems was first used for meat in attempts to develop rapid methods for the measurement of myoglobin and its derivatives. Some investigators rashly assumed that the scattering coefficient (*S*) of meat is some type of constant, whereas it may readily be shown that it is strongly affected by pH and may vary from sample to sample and with the wavelength of light. In the

Figure 15



Effect of the angle of measurement (degrees) on the 400/700 nm ratio of transmitted light in pork with a perpendicular junction between muscle fibers and optical fibers (line 1) and with optical fibers meeting muscle fibers coaxially or parallel (line 2). From Swatland (1988c).

Figure 16



Correlations of fiber optic reflectance with WHC of unfrozen pork (line 1), WHC after freezing and thawing (line 2), slicing losses of unfrozen pork (line 3), slicing losses after freezing and thawing (line 4), drip loss in unfrozen pork (line 5), and drip loss after freezing and thawing (line 6). From Irie and Swatland (1993).

Kubelka-Munk function, $K = \text{absorbance coefficient}$. Snyder (1965) concluded that K/S ratios should not be used to plot whole spectra without finding S at each λ , since scattering tends to be inversely proportional to λ^4 . Thus, at each λ , K and S must be determined separately and it is difficult to use the Kubelka-Munk function,

$$K/S = (1 - R)^2 / 2R.$$

Wavelength-related light scattering is detectable in meat when the interface between muscle fibers and optical fibers is perpendicular. More light at 700 nm is transmitted forward through the meat than is scattered perpendicularly, whereas light at 400 nm tends to be scattered more evenly in all directions (Fig. 15). Thus, by measuring internal reflectance as a

function of orientation relative to the muscle fibers, the measurement of light scattering can be enhanced. This approach was pioneered by Birth et al. (1978) using a red laser to measure light scattering in pork chops. With illumination of the upper surface of the sample,

$$\log M_T = A - Br$$

where M_T is the radiant exitance on the lower surface, A is the intercept of regression, B is the slope of regression and r is the path length through the meat. Referring back to the Kubelka-Munk relationship considered earlier,

$$B = \log 2 (S + K)$$

The slope of the regression was not given any special name but it might reasonably be called a spatial measurement of scattering. In other words, as well as measuring light scattering by looking at absolute values of reflectance, also it is possible to assess light scattering from measurements made at different positions relative to the angle of illumination. Measuring both wavelength and position may enhance the prediction of commercially important features such as fluid loss and processing characteristics. We would not choose to do this if it was not necessary, because it introduces greater levels of cost and complexity into the apparatus, but without some further advances in this direction, the optical prediction of WHC and drip losses is not reliable enough to be of much industrial value (Fig. 16).

Multidimensional matrices are somewhat difficult to present visually. Here we will use wavelength across the x-axis, with position of measurement relative to the point of illumination up the y-axis. The data we are interested in can be shown in either of two ways. With a pseudo-gray map, the probability of a pixel being turned on is proportional to the statistical parameter of interest. For example, Fig. 17 shows the absolute value of the t-statistic (range, -3.66 to -1.43) for a correlation of transmitted light with bag drip loss over 3 days from pork *longissimus dorsi* after freezing and thawing. The optical measurements were made 24 hours after slaughter, but the bag drip was not measured until much later. In Fig. 17, a grid with opposite logic to the pixel pattern has been superimposed to show the wavelength increments (from 400 to 700 nm in 10 nm increments) and position increments (from -30 to +30 mm in 5 mm increments relative to the point of illumination at 0 mm). The monochromatic spatial measurement of scattering proposed by Birth et al. (1978) is contained within the wavelength-position (WP) matrix as

$$\begin{aligned} P &= 13 \\ \beta_1 &= \log_{10} WP_{P,24} / \sqrt{(25^2 + (5 \cdot ABS(P-7))^2)} \\ P &= 1 \end{aligned}$$

The pseudo-gray map shows that the information content of the matrix is focused at 400 nm in the optical axis, directly in line with the point of illumination.

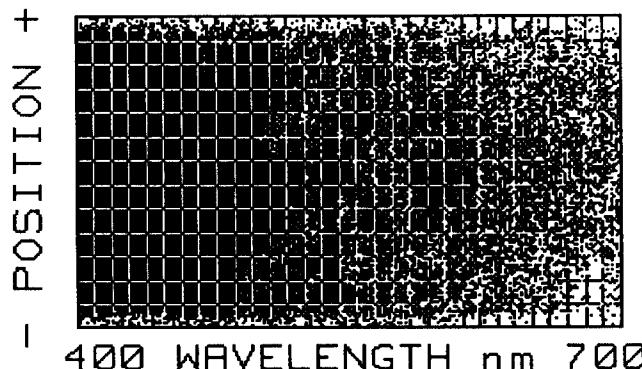
Finding the information content down at 400 nm in Fig. 17 may seem odd, after we have seen the information content towards 700 nm in Fig. 12. Remember that most of the early work reviewed at the start of this paper was directed at making an objective measurement of PSE pork, where **subjective paleness** is the dominant criterion. Next it was shown

that paleness and WHC are not linearly related via their mutual dependency on pH, and then it was argued that the filament lattice, although the source of most of the exudate, may not have a strong instantaneous relationship with exudate. None of this necessarily means that red light towards 700 nm is the best indicator of WHC, and Fig. 17 shows the opposite: for transmitted light, the information on WHC is towards 400 nm. Whether or not low wavelengths (which are far more likely to be scattered than long wavelengths) are able to penetrate through the meat seems to be the best indicator of WHC and related fluid characteristics. If reflectance spectra describing pH-related paleness are examined carefully, it may be seen that they do not respond uniformly from 400 to 700 nm. Relative to high-pH dark meat, meat with a low pH which is pale generally has an increase in reflectance towards 700 nm, but sometimes it also has a decrease towards 400 nm (Swatland, 1988b), and it is this latter effect which may have the greatest importance for predicting WHC. Thus, adding in information at lower wavelengths can increase the accuracy of objective measurements of PSE, reaching $R = 1$ under laboratory conditions with a wide range from PSE to DFD (Swatland and Irie, 1992). But predicting WHC is a lot more difficult than simply grading PSE objectively!

Having seen the t-statistic for a correlation of transmittance with WHC in Fig. 17, we may now examine the same relationship displayed in a second way, as a contour map (range, $r = -0.47$ to -0.20 , contour interval = 0.1) in Fig. 18. So far we have not gained much in terms of accuracy of prediction ($r = -0.47$), but we can see the potential for multiple regression analysis. In this data set (Swatland and Irie, 1991), optical measurements were correlated ($P < 0.01$) with 1 day drip losses of unfrozen pork ($R = 0.72$), and with fluid loss when sliced for Japanese cooking ($R = 0.82$).

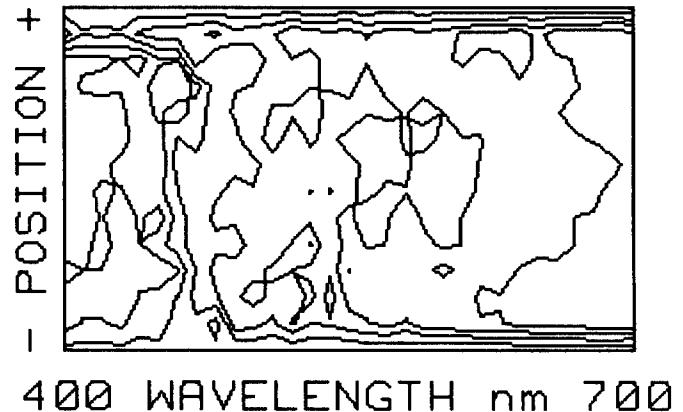
For those who have not been following recent technical advances in optoelectronics, one further piece of information may be needed to see where all this technology is headed. A photodiode array is a chip with a matrix of minute photocells that can be scanned very rapidly. Coupled to a flat ribbon of optical fibers that splay out to different positions in the meat, it should be possible to grab a matrix, similar to that shown in Fig. 17 or 18, very rapidly. Unfortunately, the idea is now gathering dust for lack of research funding.

Figure 17



A pseudo-gray map for the correlation of transmittance with drip loss from pork loins. The probability of a pixel being turned on is proportional to the absolute value of the t-statistic (Swatland, unpublished data).

Figure 18



A contour map for the relationship shown in Fig. 17, plotting the strength of the correlation coefficient with a contour interval of $r = 0.1$ (Swatland, unpublished data).

In summary, goniophotometry shows us that optical information on WHC may also be obtained at low wavelengths, towards 400 nm. Thus, the information content on WHC depends on the way optical measurements are made (transmittance, internal reflectance or surface reflectance), on wavelength, on the angle and length of the light path relative to the point of illumination, and on the coupling between optical systems and muscle fibers. Although interfilament spacing may be the dominant factor in meat paleness, we must cast a wider net to catch all the information needed to predict WHC.

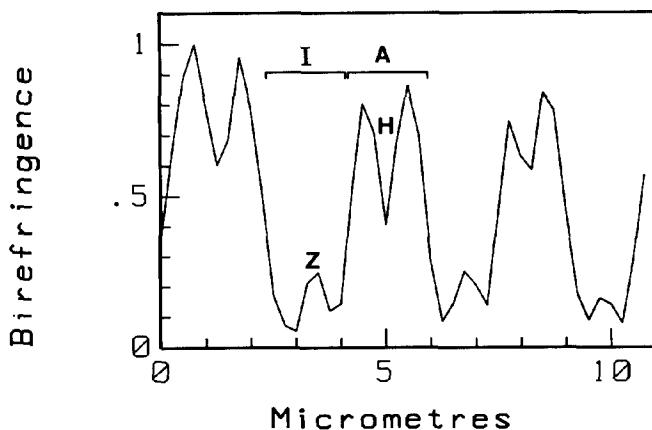
Birefringence

In all that we have covered so far, we have been using non-polarized light. Now we consider optical factors in the meat that depend on the plane of polarization.

The relationship between pH and light scattering extends over a wide pH range, including the near neutral pH range of the living animal. Thus, dark-cutting beef has very little scattering, like living muscle. Many factors could be involved over this wide range of pH, but their relative importance is unknown. Bendall & Wismer-Pedersen (1962) proposed that light scattering at a low pH is caused by protein denaturation. Hamm (1960), supported by Offer & Trinick (1983), proposed that shrinkage of fibrils at a low pH increases the refractive index difference between fibrils and the sarcoplasm, thus increasing scattering from the fibrillar surface. Both ideas seem reasonable. Evidence of scattering from the fibrillar surface was reported by Offer et al. (1989) using scanning confocal light microscopy, but no evidence of light scattering from molecular features within the fibril was detected.

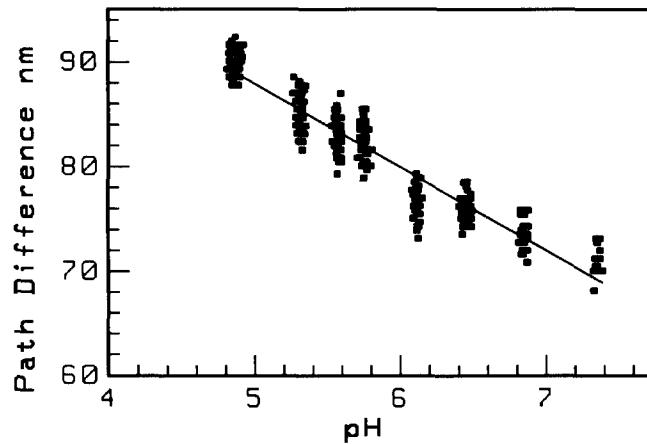
However, fibrils account for much of the volume in meat, so that even small optical changes could have a major impact. We have seen in Fig. 12 that there is a strong relationship between light scattering and interfilament spacing measured by x-ray diffraction. Increased fibrillar refractive index caused by low pH might increase scattering by increasing the angular deflection of light passing through fibrils. But how can we use polarized light?

Figure 19



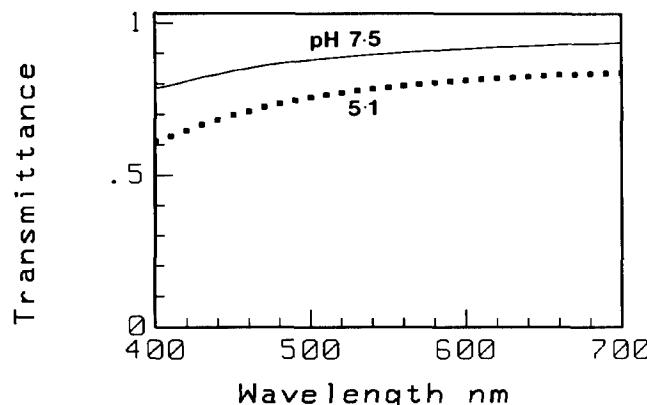
Scanning along a fibril from pork fillet, measuring the pattern of birefringence of sarcomeres (from Swatland, 1989a).

Figure 20



Effect of pH change ($+0.038 \text{ pH min}^{-1}$) on the optical path difference of a porcine psoas muscle fiber (Swatland, 1989a).

Figure 21



Effect of pH on the transmittance of light perpendicularly through an individual beef muscle fiber (from Swatland, 1990a).

Fibrils are strongly birefringent (with two refractive indices), as indicated by the naming of their A (anisotropic) and I (isotropic) bands, and this enables them to be investigated with polarized light (Engelmann, 1878). Fig. 19 shows the birefringence pattern of the sarcomere, scanning along a fibril under computer control. This sarcomere pattern may have been what Offer et al. (1989) observed at the fibrillar surface by laser microscopy, observed at the surface of the fibril, but originating from the optical properties of the filament lattice **within** the fibril.

If a whole muscle fiber is integrated in an aperture matched to its diameter, the overall birefringence can be measured. Where c = velocity of light in a vacuum and v = velocity of light through the fibril, refractive index (n) is given by

$$n = c/v$$

Only the frequency of light is constant as it passes through the fibril, so that wavelength decreases with n . Because fibrils are birefringent, transmitted light splits into two components that travel at different velocities, the ordinary ray (O) and the extraordinary ray (E), with $O \perp E$.

$$\text{Birefringence} = n_E - n_O$$

and may be positive or negative. Phase retardation occurs as light is transmitted through the fibrils, because the ordinary and extraordinary ray paths differ in length and interact when recombined. The path difference of a depth of muscle (Γ_m) may be measured by ellipsometry using a de Séarmont compensator

$$\Gamma_m [\text{nm}] = K\lambda [\text{nm/degree}] \cdot u^\circ$$

where u = angle in degrees required for compensation, and $K\lambda$ = the de Séarmont constant (path difference for 1° of rotation). Γ_m depends on pH (Figure 20), thus explaining at least some of the wide relationship between pH and light scattering.

I have been unable to find quantitative evidence of the other possible sources of light scattering in meat so, until that time, my **working hypothesis** is that birefringence is a major cause of pH-related differences in light scattering in meat. This working hypothesis allows some testable predictions to be made and, for that reason alone, is worthy of serious consideration. Thus, if pH-dependent changes in fibrillar refractive index contribute to meat paleness, one would expect changes in transmittance perpendicularly through muscle fibers also to change as a function of pH. Fig. 21 shows that they do.

However, there are still many unanswered questions concerning the effect of pH on fibrillar birefringence. In all samples I have measured, path difference has decreased as I have decreased the surrounding pH from 7 to near 5, and vice versa, since the effect is completely reversible. Often there has been a peak path difference around the isoelectric point, below which path difference has decreased, as would be predicted from theoretical grounds. But this effect is sometimes absent, and path difference may continue to increase at pH's below the isoelectric point. I do not know whether this is simply a methodological problem, or an indication of something more meaningful. Making these measurements is not exactly easy,

and I have not pursued this any further because the effect is beyond the normal pH range of meat and, I hope, does not impinge on my attempts to predict WHC from optical properties.

To utilize birefringence as a method for predicting WHC, some changes are necessary because light scattering in meat is so strong that polarization is rapidly lost as the light passes through the meat. Exploiting the fact that scattering is inversely proportional to a power of wavelength, near infrared (NIR) light may be used so that the birefringence measurements can be scaled up from the microscopic (one muscle fiber) to the macroscopic level (1 mm of meat). Rotating one polarizer (the analyzer) relative to another, the extinction coefficient is given by

$$k = \log_{10} (T_0 / T_{90})$$

where T_0 is with the analyzer parallel to polarizer, and T_{90} is with the analyzer perpendicular to the polarizer. My apparatus has $k = 1.56$ at 800 nm and follows the Malus Law for crossed polarizers where T for the azimuth angle (α) between the axes of the polarizers is given by

$$T_\alpha = T_{90} + (T_0 - T_{90}) \cos^2 \alpha$$

A slice of meat, carefully controlled for thickness and fiber orientation, is placed between the analyzer and polarizer. I originally intended to use a compensator ($\lambda/4$) but found that the optical field did not have a homogeneous path difference, so that ellipsometry was not of much value. However, transmittance at certain analyzer angles was strongly correlated with the nature of the samples.

From Fig. 19, it may be reasoned that if pH-related differences in fibrillar refractive index make a major contribution to pH-related meat paleness, the transmittance of polarized NIR through pork should be affected by sarcomere length. In optical conditions where birefringence is the primary determinant of transmittance (muscle fibers at 45° between polarizers crossed at 90°), transmittance should increase as sarcomere length is decreased to the length of a thick filament, so that the whole field is filled with strongly birefringent A bands with little or no appearance of weakly birefringent I bands. As seen in Fig. 22, transmittance increased as sarcomere length decreased from $3.5 \mu\text{m}$ down to $1.5 \mu\text{m}$ but, below this length (which is close to the length of the thick filament), transmittance was decreased. The decrease in birefringence of highly shortened sarcomeres ($< 1.5 \mu\text{m}$) might be caused by disruption of the regular alignment of filaments (which is necessary for birefringence) caused by thin filaments overlapping at the midlength of the sarcomere. Separating the data in Fig. 22 at about the length of a thick filament, transmittance was correlated with sarcomere length, $r = 0.85$, slope $2.92 \text{ T } \mu\text{m}^{-1}$, $P < 0.025$ at sarcomere length $< 1.5 \mu\text{m}$. At sarcomere length $> 1.5 \mu\text{m}$, transmittance was correlated with sarcomere length, $r = -0.59$, slope $-0.39 \text{ T } \mu\text{m}^{-1}$, $P < 0.025$. I realize that those who do not agree with me will simply put a regression line through the whole lot and say that nothing happened, but argument is the catalyst of progress and helps keep us all awake!

At certain degrees of rotation, this system is strongly affected by pH ($R = 1$) while at other angles the effect of sarcomere length is dominant ($R = 0.98$), all of which is of general interest, but can it predict WHC? Using data kindly provided

by my colleague, Prof. Shai Barbut, we can find quite useful relationships with WHC ($r = 0.85$, $P < 0.0005$) and cooking losses ($r = -0.82$, $P < 0.005$) in turkey meat. These compare favorably with the correlation of pork WHC with laser scattering ($r = 0.84$; Irie and Swatland, 1992b) and with non-polarized NIR ($r = 0.74$; Swatland and Barbut, 1990). Being based on simple correlations, there is some hope that birefringence measurements are robust relationships that are not overly sensitive to instrumental conditions, as are multivariate predictors.

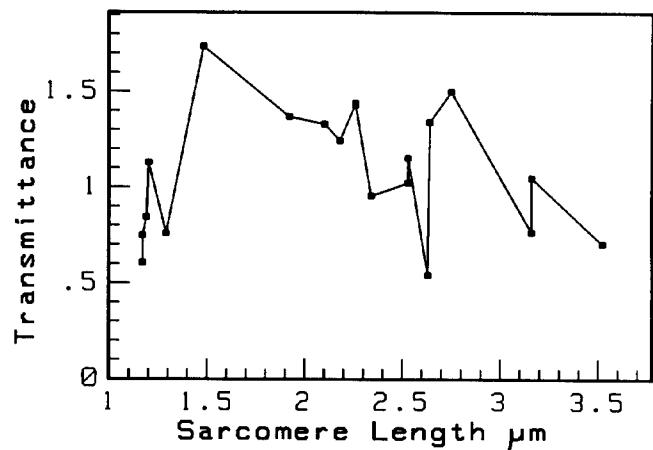
In summary, a third possible for the optical prediction of WHC (in addition to spectrophotometry and goniophotometry) is to use polarized light at long wavelengths to minimize scattering. Scattering is still a strong effect and can be used to detect pH-related aspects of WHC, but now it is also possible to obtain information on sarcomere length.

Refractive Index

The refractive index of the filament lattice and fluids within meat have an obvious importance in any attempt to predict WHC from optical properties. One would expect the water in the filament lattice to contain fewer solutes than the sarcoplasm, so that the release of water from the filament lattice as pH declines might be accompanied by a flushing effect from fluid with a low refractive index passing through downstream compartments (filament lattice \rightarrow sarcoplasm \rightarrow interfiber \rightarrow interfascicular \rightarrow surface). Thus, the refractive index of fluid that drips from pork slices may be inversely related to paleness ($r = -0.95$; Irie and Swatland, 1992b). PSE meat releases more fluid than DFD meat, and the extra fluid contains fewer solutes and lowers the refractive index of the drip fluid.

Obviously this has an important bearing on the light scattering mechanism proposed by Hamm (1960), that shrinkage of fibrils at a low pH increases the refractive index difference between fibrils and the sarcoplasm, thus increasing scattering from the fibrillar surface. This mechanism might explain the transient decrease in reflectance seen shortly after slaughter (Fig. 13), which one would expect from this system when the filament lattice and the sarcoplasm reached the cross-

Figure 22



Effect of sarcomere length on transmittance of polarized NIR light through slices of pork (Swatland, research in progress).

over point for refractive index (lattice ascending, sarcoplasm descending). Whether or not we can develop this into an online method remains to be seen. The distal window of an optical fiber may be used as a refractometer (Swatland, 1991), but making it sensitive to the small changes we are looking for in meat could be a serious technical challenge ($n = 1.353$ to 1.365).

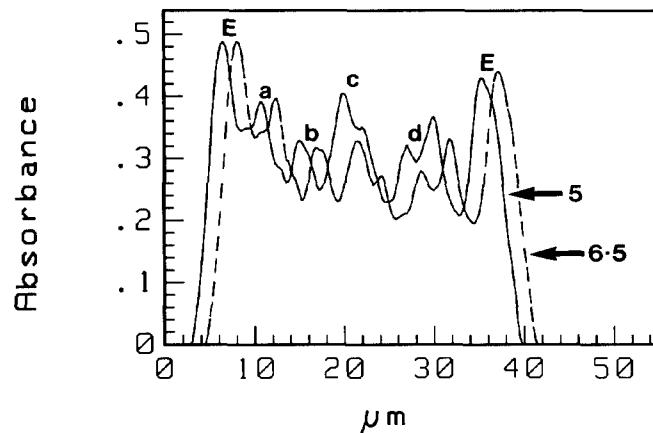
I have not yet attempted optical measurements down at the level of the fibril surface in meat, but am working on the easier problem presented by the fiber surface (Swatland, 1994). If Hamm's (1960) suggestion of scattering at the fibril surface is correct, there could be a similar effect at the fiber surface, although complicated by plasma membranes and the endomysial tube.

Sarcomeres seldom preserve their perpendicularity in meat, and usually are skewed so that several may appear when scanning perpendicularly across an individual muscle fiber (Fig. 23; a, b, c and d). One trace is offset by 2 μm relative to the other, caused by movement of the fiber progressively throughout the experiment (from pH 5 to pH 8, in steps of 0.5) with loosening of the knots by which the fiber was stretched across the metal specimen frame.

The edges (Fig. 23, E) of the fiber are indicated by high absorbance peaks that do not change with pH. In scanning across a cylindrical structure, the edge of the specimen has the minimum depth, while the maximum depth is half way across. But the muscle fiber has a higher refractive index than the surrounding buffer, which creates the refractive effect of a bright halo outside a dark rim (depending on the plane of focus), and creates the sharp edges seen in Fig. 23 at E. This effect is often called the Becke line. If structures have a higher refractive index than their surrounding medium, the Becke line moves inwards towards the structure when the objective is raised, and outwards when the objective is lowered. The muscle fiber is almost round in cross section, so that it func-

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Figure 23



Scans across an individual beef psoas muscle fiber at pH 5 and 6.5, showing absorbance peaks at the edge (E) caused by refraction, and absorbance peaks, sometimes dented, caused by A bands (a, b, c, d).

tions as a convex lens creating a strong Becke line similar to that used in Heyn's method for measuring the refractive index of textile fibers. Changing the pH caused only a small change in refractive index of the buffer, and little or no change in the Becke line. But scanning across the fiber, four A bands with a high refractive index were detected (Fig. 23; a, b, c and d), and their absorbance was inversely proportional to pH: as pH went up, absorbance went down, as predicted from what we know about the effect of pH on the birefringence of meat (Swatland, 1989a). But this just about brings us up to date (June 1994), and more research is needed before we can be sure of these effects and proceed to the surface of the fibril.

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