ICoMST
49th International Congress of Meat Science and Technology
2nd Brazilian Congress of Meat Science and Technology

On-line Evaluation of Meat Quality: State of the Art

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SUMMARY

Carcass quality relates to the cut-out yield of a carcass as affected by subcutaneous and intermuscular fat, while meat quality relates to the sensory and processing properties of muscle and adipose tissues. This presentation is restricted to on-line evaluation of meat quality.

On-line evaluation of meat quality is applicable at two key points. Firstly, on-line evaluation may be used to sort or grade carcasses or their primal cuts by differences in meat quality. The information might be used to monitor genetic or production variables, to determine financial payments to producers, or to stream products for various applications. But this seldom happens. Grading or sorting for meat quality is usually done as it has been done for decades, by sorting on the basis of animal age, or the visual appearance of a major muscle. The main problems are lack of commercially available instrumentation, difficulty in obtaining access to interior muscles, and sampling error caused by variation within and between major muscles. At the second point of application, on-line evaluation may be used for continuous streams or batches of meat from heterogeneous sources for process control, emulsion formation, curing or cooking.

No contamination is allowable. This prevents the use of probes with unprotected glass electrodes or loose reagents. Measurements on carcasses or primal cuts are severely limited by allowable time and level of damage. These restraints are greatly reduced when measurements are made later on streams or batches of meat - where NIR methods are extremely useful.

Keywords

on line measurements, meat quality, electrical impedance, light scattering, connective tissue fluorescence

Figure 1. Dr. F.H. Banfield in 1971.

OBJECTIVES

Having been invited to consider the 'state of the art', this presentation deals only with the most recent progress in the field. But progress for carcasses and primal cuts is slow, despite the fact Banfield (1935) and Callow (1936) were using impedance and pH on-line to monitor pork quality in the 1930s. Seventy years later, quality-control data collected with essentially the same methodology cannot be regarded as 'state of the art'. Surely it must be possible to obtain rapid assessments of meat tenderness, water-holding capacity and marbling. How might this be done?

ELECTRICAL IMPEDANCE

Direct current (DC) measurements are difficult to use on meat because of galvanic effects on the surfaces of metal electrodes. Electrodes in contact with meat electrolytes discharge ions into meat fluids while ions in the fluid tend to combine with an electrode. This creates a charge gradient across an electrical double layer and the electrode-electrolyte interface behaves as a voltage source and as a capacitor in parallel with a resistor. Thus, if a DC current is passed through meat then disconnected, galvanic effects can light up a low-voltage light bulb connected to the terminals in the meat. This electrode polarization overwhelms measuring circuits applied to the electrodes. Thus, measurements are made using an alternating current (AC), sometimes with different pairs of electrodes for the stimulating current and the recording circuit. The AC current detects both resistance (the inverse of conductivity) and capacitance. Impedance is the product of resistance and capacitance.

Impedance measurements are affected by:

1. The metallic properties of the electrode
2. Electrode area
3. Electrode insulation by adipose cells
4. Current density and electrode separation
5. Postmortem changes in electrolyte composition
6. Temperature
7. Test current frequency

Resistance and capacitance in parallel are usually inversely proportional to the logarithm of frequency. Thus, measurements from one commercial system are difficult to compare with measurements from another system because of differences in electrodes and AC frequency.

Muscle has a much lower electrical resistance than fat because muscle contains electrolytes with a high conductivity. Although fat contains some electrolytes, it is dominated by globules of triglyceride filling adipose cells. Temperature has a strong effect on electrolyte conductivity. Thus, measurements must be made at a constant temperature, or temperature must be measured and used to adjust impedance. Impedance may be used to assess overall fatness, or the release of muscle electrolytes in PSE pork. But this immediately tells us fatness may detract from PSE detection and vice versa.

Most commercial systems are based on measuring resistance (conductivity) or impedance dominated by resistance. Capacitance is more difficult to measure with a simple system, but has much to commend it scientifically if not technically. Capacitance is determined mainly by the integrity of muscle fiber membranes, and is a sensitive indicator of PSE. Muscle fibers are extremely large cells, perhaps only about 0.1 mm in diameter but usually many centimetres in length. Muscle fiber membranes have strong dielectric properties and a capacitance of approximately 1 $\mu F\ cm^{-2}$. Pairs of probe electrodes may be inserted in three different planes relative to the longitudinal axes of muscle fibers. When electrodes and muscle fibers are coaxial, muscle fibers are usually compressed concentrically around the electrodes to increase the capacitance from membranes. But if the electrodes force open channels of extracellular fluid along muscle fibers, this shorts the test current and reduces the current density on membranes. Thus, on-line use of electrical probes requires standardisation of electrode placement relative to muscle fiber orientation. Likewise, depth of electrode penetration affects the area of electrode contact and the current density between the electrodes. There must be a method to control exposure and orientation of the meat to the electrodes, and it may be necessary to dry the carcass at points of measurement to avoid surface fluid shorting the electrodes.

There are no recent refereed publications making progress with these problems. Modern digital measuring circuits are superior to the analogue systems used by pioneers in the field, but there has been no great improvement in prediction accuracy. Impedance methods may give information on the state of muscle fiber membranes, and this might tell us a lot about meat quality. Before this can happen, however, we need basic research to improve the ways we connect our apparatus to the meat. We might hope that scanning through a series of AC frequencies (Morucci et al., 1996) may have a higher information content than measurements made at a single frequency, just as spectrophotometry is more powerful than monochromatic optical measurements.

Use of biolectrical impedance to detect PSE pork is well established (Swatland, 1995a). There has been recent interest in using the method to isolate genetic and environmental sources of the mild PSE condition affecting poultry meat, where fluid losses during secondary processing are paramount (Swatland et al., 2001a). PSE is seldom severe in Ontario turkey breast muscles, so the technology is pushed to its limit when working over a narrow range of meat quality.

Fluid loss % by 24 hours post mortem	0.50 SD 0.35	N = 156
Prediction by resistance @ 120 Hz	-0.37	P < 0.001
1 kHz	-0.30	P < 0.001
10 kHz	-0.34	P < 0.001
Cooler drip % from 1 to 4 days	4.31 SD 1.91	N = 45
Prediction by resistance @ 120 Hz	-0.61	P < 0.001
1 kHz	-0.51	P < 0.001
10 kHz	-0.62	P < 0.001

None of the variation in fluid losses was attributable to genetic sources, thus implicating capture, transport and slaughter as the main determinants of fluid losses. The same samples were run against a battery of 'state of the art' optical methods involving spectrophotometry with polarized light (Swatland et al., 2001b), but with no improvement over the correlations detected with the much simpler and more robust impedance method.

LIGHT SCATTERING

Minolta colorimeter measurements of meat reflectance are used by many researchers to assess meat paleness. High reflectance is caused by high scattering, and scattering is related to pH, so there are also indirect correlations of reflectance with pH-dependent aspects of meat quality (Andersen et al., 1999). However, predictions of pH-dependent aspects of meat quality from optical measurements are not reliable enough for routine commercial use (unless there is a wide range in meat quality). Improvements in optical measurements may be the only hope of progress.

We have all seen low-pH meat with a higher reflectance than high-pH meat, but our textbooks give little explanation. Muscles are relatively dark immediately after exsanguination in an abattoir. Reflectance or paleness increases if post mortem glycolysis proceeds normally. Muscles thereby develop the relatively pale appearance of normal meat. But muscles may remain dark if postmortem glycolysis is truncated by absence of glycogen. Conversely, rapid or extended glycolysis may cause muscles to become abnormally pale (Bendall and Swatland, 1988). This inverse relationship between pH and reflectance is widely known and has great commercial importance, but we have no rigorous understanding of how it develops normally, why it sometimes fails, and why it sometimes proceeds too far.

The development of abnormally high reflectance, as in PSE pork, may be a different mechanism than the lesser increase in reflectance occuring in normal pork. Abnormal paleness may be caused by denaturation of sarcoplasmic proteins (Bendall, 1973). There is evidence this change is experimentally irreversible. Thus, if pH is artificially elevated by perfusion, the pork does not revert to a normal appearance (Swatland, 1995b). The normal development of paleness, on the other hand, is experimentally reversible. Rather than being caused by denaturation of sarcoplasmic proteins, there is evidence of a physical effect involving the myofilament lattice. Decreased electrostatic repulsion between myofilaments may cause shrinkage and increased refractive index of the myofilament lattice, thus increasing reflectance from myofibrillar surfaces (Hamm, 1960). We have no experimental proof, but the hypothesis seems reasonable. There is also another possibility to account for the experimentally reversible relationship between pH and reflectance. Low pH may increase refraction through myofibrils (as distinct from reflectance at the myofibrillar surface). Instead of penetrating deep into meat with minimal back-scattering at a high pH, light penetration at a low pH may be reduced by refraction through myofibrils. This may increase the intensity of light back-scattered to the meat surface to be perceived as paleness.

Bulk meat has extremely complex optical properties - hence the attraction of using single muscle fibers for experiments. Perhaps an understanding of the optical properties of single fibers will help unravel the mysteries of bulk meat?

Experiments on single muscle fibers showed myofibrillar refraction is involved in pH-related meat paleness. Refraction was detected by spectrophotometry at the Becke line, and by ellipsometry scanning across muscle fibers. Complications originated from the partly prismatic shape of muscle fibers and from differences in attenuation through red and white histochemical fiber types. In all configurations, transmittance through muscle fibers was decreased when pH was changed from 7 to 5.5, and vice versa.

Apparatus

Measurements were made with vintage 30-year old apparatus - a Zeiss Universal microscope and a side-window photomultiplier (Fig. 2, 1) above a filter changer (2) to remove high-order harmonics from a grating monochromator (3). Zeiss LD Epiplan objectives (7) were used to reach into a fluid filled chamber mounted on a scanning stage (8). The chamber was perfused with 0.2 M phosphate buffer with programmed changes in pH. Individual muscle fibers were isolated by microdissection and mounted on a metal frame orientable with a magnet from outside the sealed chamber. Solenoid valves stopped perfusion when optical measurements were made, otherwise bulging of the cover slip topping the sample chamber produced errors. Substage condensers (9) were changed to match the experiment, sometimes with a long working range and sometimes with quartz optics. A mirror was moved to select an appropriate illuminator, either a halogen source or a xenon arc (12). Illuminators were fitted with solenoid shutters (11) for measurement of the dark-field current of the photomultiplier.

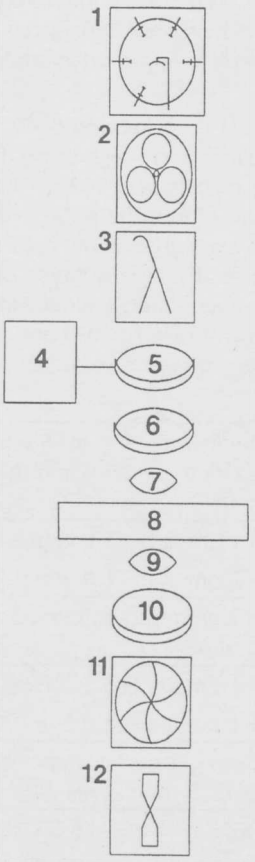

Figure 2. Microscope system.

Ellipsometry was used to measure muscle fiber birefringence. The original method of de Sénarmont (1840) was for light reflected from minerals, but was adapted for transmitted light by Goranson and Adams (1933). A de Sénarmont quarter-wave plate was used in conjunction with a rotary analyzer (Hartshorne and Stuart, 1970). The de Sénarmont compensator (Fig. 2, 6) was oriented diagonally in a NW-SE slot below the analyzer to give a N-S orientation of the slow axis. The polarizer (10) was oriented in N-S and analyzer (5) in E-W directions. The analyzer was turned positively under computer control (4) starting at 90° searching for an extinction position. Measurements were made with muscle fibers oriented longitudinally SW-NE. A depolarizer below the monochromator avoided interactions of polarized light with the grating. For ellipsometry, the wavelength was 589 nm with a 10 nm band pass. For spectrophotometry, with all polarizing optics removed, the monochromator was stepped at intervals of 10 nm with a 10 nm band pass. Operating principles and software protocols for ellipsometry with a motorized analyzer and for microscope spectrophotometry are detailed by Swatland (1998). Software was programmed in HP BASIC and instrumentation was controlled via an IEEE-488 bus.

Optical dispersion at the Becke line

Becke lines may be unfamiliar to meat scientists - but anyone who has measured sarcomere lengths has seen them. When muscle fibers are mounted in water they are delineated by bright or dark lines depending on the focus of the microscope.

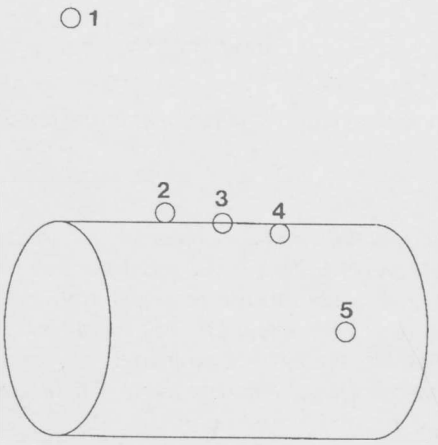

Figure 3. Measuring aperture positions relative to a muscle fiber.

Muscle fibers are packed with myofibrils. Myofibrils are formed from a dense myofilament lattice of thick and thin myofilaments. Individual muscle fibers exhibit strong Becke lines when mounted in 0.2 M phosphate buffer. The Becke line is caused by refraction (Kerr, 1977) and is the most intense fringe of a diffraction pattern caused by an asymmetrical wave spreading from the fiber edge (Faust, 1959). Rather than changing the focus above or below the fiber, which would have been difficult to quantify, the fiber was kept in focus and chromatic dispersion was measured as the pH was changed (Swatland, 2002a).

The photometer was standardized (transmittance = 1) away from the fiber (Fig. 3, position 1). Measuring aperture positions 2 to 4 were at, bisecting, or just past the edge of the fiber. Position 5 was half way across the fiber. After flushing out myoglobin, light at 300 nm was first to be attenuated stepping across the edge of the fiber from positions 2 to 4 (Fig. 4). One would expect light at short wavelengths to be refracted through a larger angle than light at long wavelengths. However, the wave shape of lines 3 and 4 of Fig. 3 indicates a secondary effect as well, perhaps from birefringence?

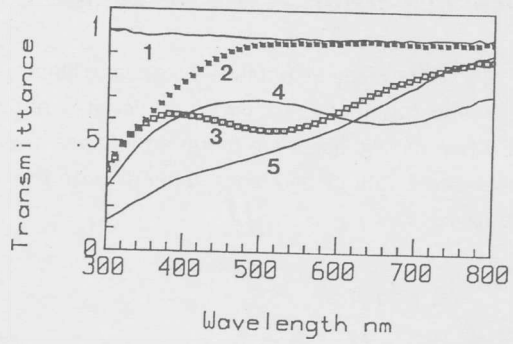

Figure 4. Spectra found at different aperture position across the fiber.

With a balanced experimental design starting 10 fibers at high pH (7.26 ± 0.15) and 10 at low pH (5.49 ± 0.06), then changing to the other pH, transmittance was higher at high than at low pH (300 to 740 nm, $P < 0.05$; 310 to 510 nm, $P < 0.01$) at position 5 half way across the fiber. It was concluded refractive index had increased as pH was decreased and vice versa.

Ellipsometry across the fiber

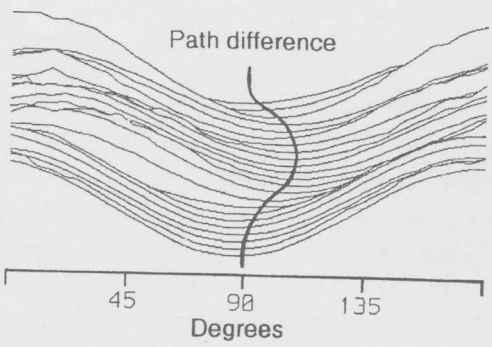

Figure 5. Birefringence path differences across a muscle fiber.

The longitudinal alignment of thick and thin myofilaments in myofibrils creates strong birefringence, well known to microscopists in the 1800s (Engelmann, 1878). Birefringence might have led to the early discovery of sliding filaments, but was strangely ignored until the much later revelations of electron microscopy (Huxley, 1980). Thus, we should keep birefringence in mind as we attempt to understand the optical properties of bulk meat, especially since we sometimes see interference colors on meat surfaces. With studies on individual muscle fibers, however, the length of the light path is short, divergence of ordinary and extraordinary rays is minimal, and interference creates first order shades of gray, as seen in the Michel-Levy charts of mineralogy texts (Kerr, 1977). Interference up to the first order is measurable by the de Sénarmont method, using a fixed quarter-wave plate and a rotary analyzer (Hartshorne and Stuart, 1970).

To scan across fibers mounted SW-NE, the scanning stage was stepped in x- and y-axis, using the hypotenuse as the scanning step (Swatland, 2002b). The path difference of birefringence was highest near the mid-point across the fiber (Fig. 5), and was higher at low pH than at high pH. A deception was caused by low resolution of path differences at the edge of the fiber - fibers looked wider at low than at high pH. Direct measurements of fiber diameter revealed the opposite - fibers shrank to a lesser diameter at a low pH.

1. Maximum path differences at pH 5.5 were higher than at pH 7.0, but variance was high and the difference was not significant

64.4 ± 15.1 nm at pH 5.5 versus

58.9 ± 15.2 nm at pH 7.0, $P > 0.05$, $n = 40$

2. However, the mean depth of fiber fragments at pH 5.5 was less than at pH 7.0

53.9 ± 15.1 μm at pH 5.5 versus

70.6 ± 16.1 μm at pH 7.0, $P < 0.005$, $n = 40$

3. Thus, path differences per micrometre of fiber depth were greater at pH 5.5 than at pH 7.0

1.25 ± 0.37 $nm^{-1\mu m}$ at pH 5.5 versus

0.87 ± 0.31 $nm^{-1\mu m}$ at pH 7.0, $P < 0.0005$, $n = 40$

In other words, a decrease in pH caused fibers to shrink and birefringence to increase.

Spectrophotometry across fibers

The Bouguer-Lambert-Beer law states the intensity of a beam of monochromatic radiation in an absorbing medium decreases exponentially with penetration distance (Parker, 1984). This predicts transmittance should decrease as path length is increased scanning across the width of a fiber to its maximum depth. But the photometric law assumes uniform distribution of chromophores. Under the microscope, distributional error may produce some interesting deviations (Swift and Rasch, 1956). Hence, a study was made of transmittance in relation to path length, using muscle fibers of different diameters to create different path lengths. Adapting microscope methods for textile fibers (Faust, 1959) unwittingly introduced the assumption muscle fibers would be cylindrical when isolated. This is how muscle fibers are shown in histology books. The results soon revealed otherwise.

Pork muscle fibers ($n = 50$) were mounted in 0.2 M phosphate buffer at pH 5.5 or 7.0. The scanning stage was used to measure transmittance in relation to fiber diameter, anticipating attenuation of transmittance with increasing

diameter. For some of the fibers, results were as expected, with transmittance 1 at each side of the fiber and marked attenuation at the thickest part of the fiber (Fig. 6). The serrations in Fig. 6 were caused by sarcomeres. Although scanning was nearly perpendicular to the longitudinal axes of fibers, and one might not expect to cross more than a couple of sarcomeres, myofibrils usually are skewed as fibers develop rigor mortis. Thus, scanning across a fiber perpendicularly also leads to scanning down the lengths of sarcomeres at an angle. Another complication was transmittance across fibers was often asymmetrical, apparently because fibers retained flattened sides after being released from bulk meat. Thus, fibers appearing cylindrical when isolated under the microscope may in fact be prismatic (Swatland, 2002c).

1. Lateral measurements of fiber diameters were compared with minimum transmittance at any point across a fiber

89.76 ± 17.07 μm at pH 5.5

97.43 ± 20.04 μm at pH 7.0, $P > 0.05$

2. No transmittance attenuation was detected at pH 7.0.

$r = 0.07$

3. At pH 5.5, instead of attenuation, the relationship of transmittance with diameter was positive.

$r = 0.44$, $P < 0.05$

This was the opposite to predictions and, at first sight, and appeared to contradict the photometric laws. However, other expectations from earlier experiments held true.

4. Attenuation at pH 5.5 was greater than at pH 7.0

2.76 ± 1.19 at pH 5.5 versus

1.42 ± 0.65 at pH 7.0 ΔT mm^{-1}, $P < 0.001$)

5. And was strongly related to wavelength

$r = 0.97$, $P < 0.001$

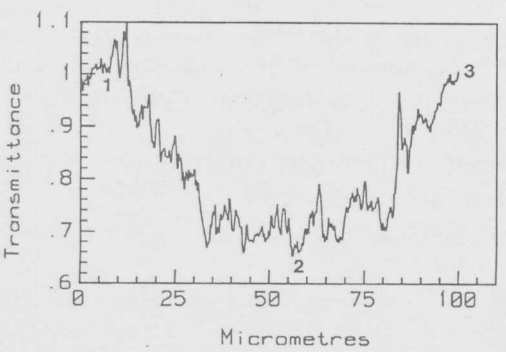

Figure 6. Transmittance across a fiber.

It is notoriously difficult to control the thickness of microscope sections through soft animal tissues. Even if a microtome cuts accurately, the sections may be variably flattened by dehydration or compression when mounted. Measuring isolated fibers and using a lateral measurement of diameter to give maximum path length through the center of the fiber looked like a solution to this problem. But even allowing for prismatic shaping of basically cylindrical fibers, however, something was wrong. Were anomalies caused by different radii of curvature in otherwise identical fibers, or did small and large diameter fibers differ in their intrinsic optical properties? To answer these questions, fibers were measured along their longitudinal axes.

Spectrophotometry along fibers

Relatively thick (1 mm) sections were cut across the grain of pork (Swatland, 2003a). The thickness of sections when mounted could not be controlled, but adjacent fibers differing in diameter could not have differed much in length. In bundles of pork muscle fibers, small-diameter red fibers (histochemical Type I) are located centrally. Red fibers have many mitochondria and a high myoglobin content. Large-diameter white fibers (histochemical Type II) are located peripherally. White fibers have few mitochondria and low myoglobin (Swatland, 1994). Sections were washed with phosphate buffer until highly soluble myoglobin was washed out and the Soret absorbance band was barely visible or absent. Polarized light was used initially to check there was no birefringence along the longitudinal axes of fibers. None was anticipated, but it seemed wise to check. Absence of birefringence also meant fibers were aligned with their longitudinal axes exactly in the optical axis of the microscope. As soon as fibers were tilted, they showed interference colors from birefringence.

1. From 460 to 700 nm, red fibers always had lower ($P < 0.01$) transmittance than white fibers.
2. Differences persisted after two hours perfusion with 0.2 M phosphate buffer and were unlikely to have been caused by myoglobin in red fibers.
3. Elevating pH from 5.5 to 7.0 increased transmittance in both red and white fibers.

$P < 0.001$ at 400 nm and from 430 to 700 nm, $n = 20$

Conclusions

Lateral spacing between myofilaments is maintained by negative electrostatic repulsion. X-ray diffraction shows interfilament spacing decreases from 46 to 42 nm when pH drops from 7.0 to 5.5 (Irving et al., 1990). Thus, acidification by post-mortem glycolysis causes the myofilament lattice to shrink. This increase in density probably accounts for the increases in myofibrillar refractive index reported here. Does myofibrillar refraction contribute to paleness in the same way refractive particles contribute to the whiteness

or opacity of paint? White paint with strongly refractive particles appears whiter then inexpensive paint with weakly refractive particles because strong refraction shortens the mean light path in expensive paint, scattering incident illumination back to the surface to be seen as whiteness. Weak refraction allows light to penetrate deeply, and less is scattered back to the surface (Judd and Wyszecki, 1975). For meat, we need to know how much light is scattered by reflection from myofibrillar surfaces, as proposed by Hamm (1960), and how much is refracted through the myofibrils. Both may depend on myofibrillar refractive index relative to the refractive index of surrounding fluids. Perhaps understanding the optical properties of bulk meat may help us improve optical sensors for the measurement of scattering.

CONNECTIVE TISSUE FLUORESCENCE

Both elastin and collagen are strongly autofluorescent when excited with UV light. Although this is a nuisance in detection of microbial spoilage by fluorescence spectroscopy, it enables enables a fiber-optic probe to detect the relative amounts of connective tissues in beef (Fig. 7). Pyridinoline cross linking causing increased cooking resistance also increase fluorescence. Research to validate the technology was all based on intramuscular measurements within major muscles of primal cuts. But, of course, this was totally ignored in commercial attempts to exploit the technology, and measurements were made on intact carcasses with little regard for anatomy. Also following from the triumph of commercial opportunism over scientific rigor, other well known causes of beef toughness were completely ignored - short sarcomeres and inadequate post mortem autolysis. It was no surprise commercial exploitation failed.

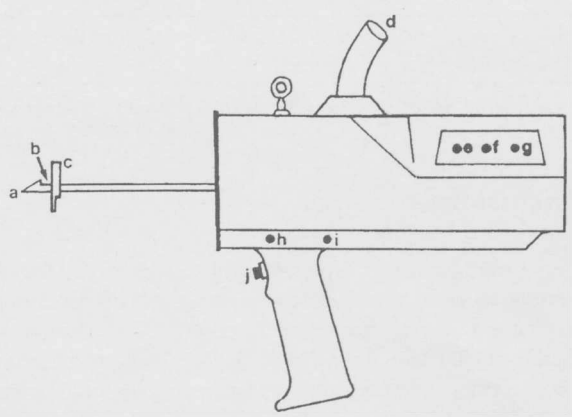

Figure 7. CT Probe.

Anecdotal results

The 'state of the art' is now very modest. I am using the technology on beef from my local butcher, enabling us to find superior sources of beef with low connective tissue levels. Our primary objective was to identify local abattoirs producing cold-shortened beef. This happens very easily in the Canadian winter because refrigeration systems are too effective when the outside air temperature is very cold. Attempts at using 'state of the art' polarized light probes for sarcomere length failed, so we had to use polarized light microscopy instead. Over one hundred years and no progress! The same measurements could have been made by Engelmann in 1878. However, an interesting discovery was made. In conjunction with a seasonal effect on tenderness caused by cold-shortening, there was also a hitherto unsuspected seasonal effect on connective tissue. I quite appreciate the research now to be described does not reach the standard required in a refereed journal (because sensory evaluation was uncontrolled and copious amounts of red wine were used to cleanse palates). However, being able to use technology to improve the tenderness of my regular Sunday roast beef gives me great personal satisfaction.

1. Thirty well-marbled (Canada AAA), well-aged (30.3 ± 4.5 days) rib roasts were evaluated from January to August 2002.
2. The meat was never frozen.
3. The sensory scale ranged from -4 (exceptionally tough) to 4 (exceptionally tender) and averaged 1.93 ± 1.43.
4. Tenderness and sarcomere length increased with time (respectively, $r = 0.45$, $P < 0.01$, and $r = 0.35$, $P < 0.05$), thus proving cold-shortening as a cause of winter toughness.
5. Sarcomere length averaged 1.70 ± 0.19 µm and was correlated with tenderness ($r = 0.66$, $P < 0.0005$).
6. But tenderness adjusted for sarcomere length remained correlated with time ($r = 0.33$, $P < 0.05$), thus implicating an accessory seasonal effect.
7. The accessory effect was probably from connective tissue because fluorescence peaks cm^{-1} were correlated negatively with tenderness, maximum $r = -0.36$, $P < 0.05$.
8. Fluorescence decreased with time.
   maximum $r = -0.73$, $P < 0.0005$
9. Fat depth was correlated positively with tenderness $r = 0.43$, $P < 0.01$.
10. Whereas longissimus thoracis depth was correlated negatively with tenderness ($r = -0.52$, $P < 0.005$).

Thus, no surprise to me or my butcher, roasts with compact muscles surrounded by abundant fat were more likely to be tender than lean roasts with bulging muscles. Fully controlled experiments usually provide the most reliable scientific advances. But, despite countless controlled

experiments, beef toughness still occurs in Canada and causes great annoyance to myself and other consumers. A major difficulty with controlled experiments on beef toughness is all the meat to be tested arrives in a short period of time. Thus, sensory panels can only cope if the meat is frozen. Freezing may change meat texture (Shanks et al., 2002). Sampling error within the target muscle is another potential problem avoided here by integration of responses for whole roasts.

These results are anecdotal - meaning only I and my butcher take them seriously. It would be a mistake to apply the findings globally, especially to the mainstream beef industry. Few consumers have their meat fully aged in a traditional aerobic manner. The effects detected here may only become evident after such treatment, and traditional beliefs about seasonal effects and the subjective appearance of a roast may only hold true if the meat is aged and cooked in a traditional manner.

It is always important to distinguish between correlation and causality, but particularly in anecdotal research. The correlations reported here are probably based on a complex network of underlying causal relationships. For example, there was no evidence of longissimus thoracis depth changing from winter to summer ($r = -0.29$, $P > 0.05$), but there was evidence of fat depth increasing during this time ($r = 0.40$, $P < 0.025$). As well as insulating against cold-shortening, it is quite likely fatness ameliorates connective tissue toughness, although it is difficult to cite supporting evidence in relation to gourmet cooking. Underlying relationships also may involve seasonal effects on animal physiology, nutrition, growth attainment, and market sources. Thus, no claim is made to understand fully what has been shown to exist.

There is little kudos to be gained in demonstrating traditional beliefs. Butchers and chefs who already believe require no proof, while sceptical scientists eschew uncontrolled experiments. Thus, the implications of this research are for those concerned with niche marketing and gourmet cooking, rather than with mainstream supermarket beef. The challenge is to develop a scientific understanding of superior traditional practices, combining new objective sensors with gourmet cooking.

As for the bottom line, was the anecdotal research successful?

The answer is yes. Using the fluorescence probe as a guide, we found a new source of beef with less connective tissue fluorescence and more fat to lessen the risk of winter cold-shortening.

Technical progress - a reflectance correction for fluorescence

While improving the Swatland family beef supply, an attempt was made to improve the technology for connective tissue detection (Swatland, 2003b). The key component of a fluorescence probe is a dichroic mirror separating outgoing fluorescence excitation from incoming fluorescence emission. The separation is imperfect because excitation and emission maxima are quite close. With double-monochromator laboratory apparatus giving an effective separation of fluorescence excitation and emission without a dichroic mirror, fat exhibits only weak fluorescence from reticular fibers around adipose cells. But with a carcass probe, where connective tissue fluorescence is detected through a single optical fiber using a dichroic mirror as a beam splitter to separate excitation and emission wavelengths, the fluorescence signal passing through the dichroic mirror may contain traces of reflectance from the upper edge of the excitation band-pass (pseudofluorescence), depending on the reflectance of tissues at the probe window (Swatland, 1995a). Thus, without correction, bulk fat reflectance may resemble connective tissue fluorescence.

The plan of components is shown in Fig. 8. The reflectance window on the probe shaft (1) is the common opening of a bifurcated light guide. The illumination branch contains a green filter (2), a solenoid shutter (3) and a tungsten source (4). The receiving branch contains another green filter (5) and a photomultiplier (6). The fluorescence window (7) is connected to a dichroic mirror as a beam splitter (8). The separates the excitation pathway containing a low-pass filter (9), a heat filter (10), a solenoid shutter (11) and a mercury arc (12) from the emission pathway containing a long-pass filter (13) and a photomultiplier (14). At standardization there are internal checks to ensure minimal cross-talk between channels and components, and these low levels are cancelled. Testing against bar codes was used to find and correct the functional offset between the two windows (1 and 7).

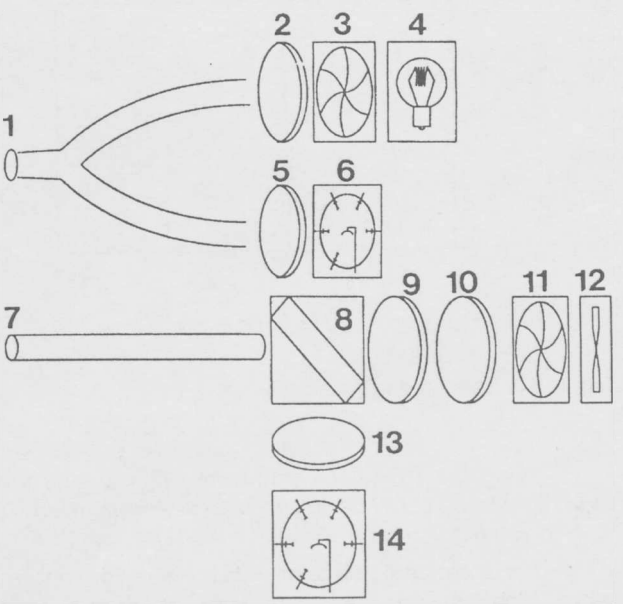

Figure 8 . Dual channel CT Probe

The dual channel probe with two optical windows was enabled the fluorescence signal to be corrected using a ratio, F/R. Thus, fat with weak fluorescence and strong reflectance was expected to give a lower ratio than collagen with strong fluorescence and strong reflectance. Fig. 9 shows a typical probe measurement through subcutaneous fat and into the longissimus thoracis using only the fluorescence channel. Fluorescence peaks detected by the counting algorithm are marked with vertical lines. The high fluorescence intensity for the first 20 mm is not true fluorescence from connective tissue, but pseudofluorescence (reflectance from fat at the cross-over of the dichroic beam splitter).

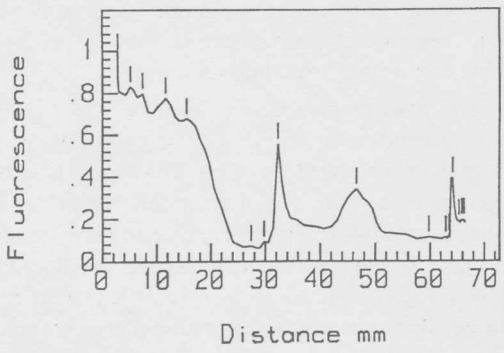

Figure 9. Uncorrected fluorescence probe transect.

Corrected transects were quite different. Pseudofluorescence through the subcutaneous fat was lost to emphasize the true fluorescence of the conspicuous epimysium over the longissimus thoracis, as in Fig. 10.

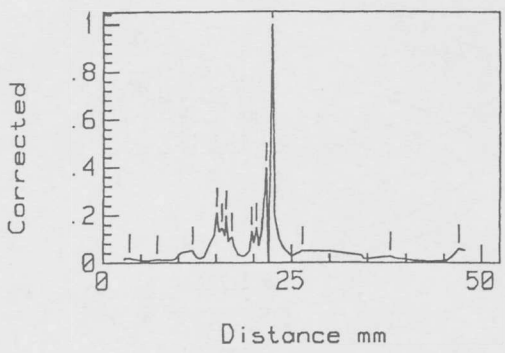

Figure 10. Corrected transect.

Using the F/R ratio strengthened ($P < 0.001$) the mean correlation of F/R peaks cm^{-1} with time from mean $r = -0.47$ ($P < 0.01$) to mean $r = -.59$ ($P < 0.001$).

The anatomical source of signals is a major concern when probe measurements are made through various types of tissue in an intact side of beef. Fluorescence signals from muscles and fat overlying longissimus thoracis in beef rib roasts were compared with intramuscular signals from longissimus thoracis, using anatomical data from cut surfaces. The scientific question was whether or not extramuscular and intramuscular connective tissues develop independently. Anatomical measurements were used to differentiate between signals exterior to the longissimus thoracis (EXLT) and intramuscular signals (INLT). Each roast was probed in eight locations, giving 32 sets of signals (position, WI or WO, EXLT or INLT). F/R peaks cm^{-1} in EXLT and INLT were correlated, $r = 0.24$ for WI and $r = 0.57$ for WO, showing development of extramuscular and intramuscular connective tissues was linked ($P < 0.0005$, $n = 480$).

This is what one might expect but, to my knowledge, it has never before been checked before. The implication is limited sampling can assess the general level of connective tissue development in a beef carcass - but, of course, great care is needed to make sure the sampling site is constant. Thus, the best engineered and most reliable probe possible is quite likely to be commercially useless in the hands of a careless or ignorant operator.

NIR FOR PROCESS CONTROL

NIR methods are especially suitable for the fat content of mince or ground beef (Tøgersen et al., 1999) and are ideal for plates and continuous streams of comminuted meat products (Isaksson et al., 1996). NIR also has been used for carcasses (Chen, 1992; Chen and Massie, 1993) and may be used to predict functional properties of meat in processed products and to detect previously frozen meat (Downey and Beauchene, 1997). Species identification is possible with homogenized meat (McElhinney and Downey, 1999; Ding and Xu, 1999) as well as detection of microbial spoilage (Ellis et al., 2002).

The typical components in a system are a tungsten-halogen source, a grating monochromator from 800 to 1100 nm, an optical system to direct the light through or onto a plate of comminuted meat, and a photometer. Multiple scanning and rotation of the sample may be used to cancel sample heterogeneity, and smoothed absorbance measurements at about 100 wavelengths may be used to make predictions from a partial least squares chemometric calibration. Typical problems involve drying of the sample surface, pH-related changes in light scattering, metmyoglobin formation, and sample temperature. These may limit the applicability of a prediction equation to a situation identical to that at calibration. NIR spectrophotometry is very sensitive to fat content and fat content has many effects on product quality. Thus, it may be difficult to isolate the signal relating directly to protein functionality. Chemometric methods are widely used to calibrate NIR systems against specific quality requirements. We may not know why it works, but if it works - it works. On that happy note we may conclude this review of the 'state of the art' in on-line evaluation of meat quality.

REFERENCES

Andersen, J. R., Borggaard, C., Rasmussen, A. J. and Houmøller, L. P. (1999). Optical measurements of pH in meat. **Meat Sci. 53**, 135-141.

Banfield, F. H. (1935). The electrical resistance of pork and bacon. Part 1. Method of measurement. **J. Soc. Chem. Ind. Trans. Comm. 54**, 411T.

Bendall, J.R. (1973). Postmortem changes in muscle. In, **The Structure and Function of Muscle**, Bourne, G.H. (Ed.), Vol II, Part 2. New York, Academic Press.

Bendall, J.R. and Swatland, H.J. (1988). A review of the relationships between pH and physical aspects of pork quality. **Meat Sci. 24**, 85-126.

Callow, E. H. (1936). The electrical resistance of muscular tissue and its relation to curing. **Special Report 75, Dep. Sci. Indust. Res. Food Invest. Board, London.** pp 75-81.

Chen, Y. R. (1992). Nondestructive technique for detecting diseased poultry carcasses. **Soc. Photo-Optical Instrument. Engineers 1796**, 310-321.

Chen, Y. R. and Massie, D.R. (1993). Visible / near-infrared reflectance and interactance spectroscopy for detection of abnormal poultry carcasses. **Trans. Amer. Soc. Agric. Engineers 36**, 863-869.

Ding, H.B. and Xu, R.J. (1999). Differentiation of beef and kangaroo meat by visible / near-infrared reflectance spectroscopy. **J. Food Sci. 64**, 814-817.

Downey, G. and Beauchene, D. (1997). Discrimination between fresh and frozen-then-thawed beef M. longissimus dorsi by combined visible-near infrared reflectance spectroscopy. **Meat Sci. 45**, 353-356.

Ellis, D.I., Broadhurst, D., Kell, D.B., Rowland, J.J. and Goodacre, R. (2002). Rapid and quantitative detection of the microbial spoilage of meat by Fourier transform infrared spectroscopy and machine learning. **Appl. Environ. Microbiol. 68**, 2822-2828.

Engelmann, T.W. (1878). Neue Untersuchungen über die mikroskopischen Vorgänge bei der Muskelcontraktion. **Pflügers Arch. 18,** 1-26.

Faust, R.C. (1959). Optical properties. In, **Physical Methods of Investigating Textiles**, Meredith, R. and Hearle, J.W.S. pp. 320-345. New York: Textile Book Publishers.

Goranson, R.W. and Adams, L.H. (1933). A method for the precise measurement of optical path-difference, especially in stressed glass. **J. Franklin Inst. 216,** 475-504.

Hamm, R. (1960). Biochemistry of meat hydration. **Adv. Food Res. 10**, 355-463.

Hartshorne, N.H. and Stuart, A. (1970). **Crystals and the Polarising Microscope.** 4th edit. London: Edward Arnold.

Huxley, A. (1980). **Reflections on Muscle.** Princeton: Princeton University Press.

Irving, T.C., Swatland, H.J. and Millman, B.M. (1990). Effect of pH on myofilament spacing in pork measured by x-ray diffraction. **Can. Inst. Food Sci. Technol. J. 23,** 79-81.

Isaksson, T., Nilsen, B. N., Tøgersen, G., Hammond, P. R. and Hildrum, K. I. (1996). On-line, proximate analysis of ground beef directly at a meat grinder outlet. **Meat Sci. 43**, 245-253.

Judd, D.B. and Wysecki, G. (1975). **Color in Business, Science and Industry**. New York: John Wiley.

Kerr, P.F. (1977). **Optical Mineralogy**. 4th edit. New York: McGraw-Hill.

McElhinney, J. and Downey, G. (1999). Chemometric processing of visible and near infrared reflectance spectra for species identification in selected raw homogenised meats. **J. Near Infrared Spectrosc. 7**, 145-154.

Morucci, J-P., M.E. Valentinuzzi, B. Rigaud, C.J. Felice, N. Chauveau and P-M. Marsili. (1996). Bioelectrical impedance techniques in medicine. **Critical Rev. Biomed. Engineer. 24**: 223-677.

Parker, S.P. (1984). McGraw-Hill Dictionary of Scientific and Technical Terms. 3rd edit. New York: McGraw-Hill.

De Sénarmont, H. (1840). Sur les modifications que la réflexion spéculaire à la surface des corps métalliques imprime à un rayon de lumière polarisée. **Annal. Chimie Phys. 73,** 337-362.

Shanks, B.C., Wulf, D.M. and Maddock, R.J. (2002). Technical note: the effect of freezing on Warner-Bratzler shear force values of beef longissimus steaks across several postmortem aging periods. **J. Anim. Sci. 80**, 2122-2125.

Swatland, H.J. (1994). **Structure and Development of Meat Animals and Poultry**. Lancaster, Technomic Publishing.

Swatland, H.J. (1995a). **On-line Evaluation of Meat.** Lancaster, Technomic Publishing.

Swatland, H.J. (1995b). Reversible pH effect on pork paleness in a model system. **J. Food Sci. 60**, 988-995.

Swatland, H.J. (1998). **Computer Operation for Microscope Photometry**. Boca Raton, CRC Press.

Swatland, H.J., Uttaro, B., Mohr, J. and Buddiger, N. (2001a). Relationships between electrical impedance and fluid losses from turkey breast meat. **Arch. Tier. 44**, 109-113.

Swatland, H.J., Uttaro, B., Buddiger, N. and Mohr, J. (2001b). Spectrophotometry of polarised light transmitted through and reflected from slices of turkey breast meat in relation to pH and fluid losses. **Food Res. Internat. 34**, 117-121.

Swatland, H.J. (2002a). Optical dispersion through muscle fibers isolated from pork. **Food Res. Internat. 35**, 559-564.

Swatland, H.J. (2002b). Ellipsometry across isolated muscle fibres indicates a refractive contribution to paleness in pork. **Meat Sci. 63**, 463-467.

Swatland, H.J. (2002c). Relationship between pork muscle fiber diameter and optical transmittance measured by scanning microphotometry. **Can. J. Anim. Sci. 82**, 321-325.

Swatland, H.J. (2003a). Photometry of light passing longitudinally along red and white pork muscle fibers at pH 5.5 and 7.0. **Food Res. Internat.** In press.

Swatland, H.J. (2003b). Seasonal changes of connective tissue fluorescence in well-aged beef roasts, correcting for fat reflectance and signal source. **Can. J. Anim. Sci.** In press.

Swift, H. and Rasch, E. (1956). Microphotometry with visible light. In, Oster, G. and Pollister, A.W. (Eds) **Physical Techniques in Biological Research**. Vol. 3. pp. 353-400. New York, Academic Press.

Tøgersen, G., Isaksson, T., Nilsen, B. N., Bakker, E. A. and Hildrum, K. I. (1999). On-line NIR analysis of fat, water and protein in industrial scale ground meat batches. **Meat Sci. 51**, 97-102.