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An investigation of UV meat probe optics, comparing fluorescence, reflectance and colorimetry

Abstract

Fluorescent connective tissues make a provable contribution to the toughness of beef and venison. They may be quantified in a few seconds using a UV fiber optic probe – speed of measurement is essential to keep pace with the rate beef carcasses are processed in a commercial environment. With advancing technology in optoelectronics, might there be something better than simply measuring flashes of fluorescence as a probe penetrates and withdraws from the meat? Static apparatus was developed to answer this question, using a rotatable mirror to switch from visible to UV illumination of connective tissue samples, with illumination at 45° to minimize specular reflectance thus allowing colorimetry. Apart from some interesting findings on the surface optical properties of connective tissues from beef and venison, there was nothing to justify improving UV meat probes beyond simply measuring flashes of fluorescence.

Keywords: Connective tissue, Fluorescence, Reflectance, Colorimetry, Beef, Venison

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Introduction

Beef is a commodity of strategic importance in our global economy – like many other food commodities. But beef is highly variable because of numerous geographical sources, genetic biological variation between animals, time of year, and climatic factors affecting animal nutrition. Apart from local preferences in taste, color and method of preparation, the tenderness – toughness evaluation of beef is a major factor industrially. Connective tissue fibers are a notorious source of toughness in meat. Collagen and elastin fibers are fluorescent[1] and may be detected and quantified using a single optical fiber probe with a dichroic beam splitter to separate outgoing UV from incoming visible light.[2] As the fiber optic aperture on a probe passes into the meat, flashes of fluorescence are detected and their depth is found from a plate remaining on the meat surface.[3] Differences between way-in and way-out measurements enable tissue elasticity related to meat toughness to be monitored.[4] A strain gauge may be incorporated along the shaft of the probe to correlate resistance to penetration with fluorescence.[5] In addition to a single optical fiber detecting fluorescence, pairs of optical fibers may be used to detect reflectance – one fiber illuminating tissue structures and the other detecting their reflectance. This enables tough collagen fibers to be separated from soft adipose tissue[6] and raises some interesting questions as to how reflectance might affect fluorescence. Does high reflectance decrease UV penetration into tissues or reflect the long-pass edge of excitation illumination? Although the fluorescence of connective tissues in meat may appear almost white, much of colorimetry is directed at the measurement of white samples.[7] Think of the subtle colorimetric differences between white ceramics in a bathroom or sheets of white paper for different uses. In geological museums, colored minerals attract our attention if their color changes in UV. But what about the colorimetry of near white samples of connective tissue in meat when the illumination is changed?

The dominant experimental protocol in nearly all meat science research is to obtain small meat samples and to take them into a laboratory where it is clean and safe. This enables investigation with fragile equipment, nearly always purchased commercially and professionally engineered. It is far more difficult to build robust equipment to be used in an abattoir where it is wet, dangerous and subject to mechanical damage and electrical interference. Even with small meat samples analyzed in a laboratory, the problem of sampling error (anatomical and between animals) remains. How can small samples characterize a whole carcass or type of animal given seasonal variation? Rapid, non-destructive probe measurements may enable us to average sampling error, even to the point of detecting seasonal variation in a commercial environment.[8]

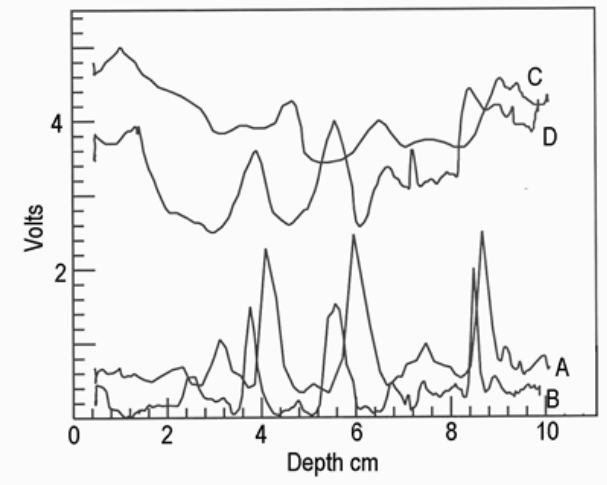

Figure 1 A typical result of a dynamic probe measurement in a beef carcass through the *Longissimus thoracis*. Line A shows the fluorescence signal on the way in. Line B shows the fluorescence signal on the way out, it is displaced negatively for distance because of tissue elasticity. Line C shows white light reflectance on the way in. Line D shows white light reflectance on the way out, it is displaced negatively for distance because of tissue elasticity and has sharper peaks because of tissue damage on the way in. On many depths might it be possible to measure the color of skeletal muscle between connective tissues?

In all the meat probe measurements made so far, the x-axis has been an anatomical position (depth) against which fluorescence (peak emission wavelength) and monochromatic reflectance measurements have been displayed on the y-axis (Figure 1). This loses spectral information and colorimetry that might also be used to predict meat quality, but now the apparatus is getting rather complex. There is no time to use a mechanical monochromator for spectrophotometry if a

probe measurement must be made in a few seconds - a photodiode array is fast enough[9] but not easy with low cost apparatus in a very difficult working environment. There are other possibilities for making simultaneous fluorescence and reflectance measurements varying the illumination with photodiodes,[10] but again, can this be done with a probe in a few seconds in a hostile environment? Meat color is commercially important, especially with defects such as dark-cutting beef caused by glycogen depletion in the live animal and a subsequent failure of light scattering responsible for a normal bright color.[11] Between the peaks of connective tissue fluorescence can the background reflectance of skeletal muscle detect the color of skeletal muscle? There is no knowing what new optoelectronic components might solve these problems in the future, but to get started, the research reported here reverted to taking small samples into a laboratory and measuring them slowly with a mechanical monochromator.

Materials and methods

Beef samples were from Canada Grade A carcasses. Venison samples were from farmed deer from Quebec. They were tested with the apparatus shown in Figure 2. The photometer was a side window photomultiplier (Figure 2, 1; Hamamatsu HTV R928, Hamamatsu City, Japan) below a grating monochromator (Figure 2, 3; Zeiss 474345, Oberkochen, Germany) with filters to remove high order harmonics (Figure 2, 2; Zeiss 477215). Light to the photometer came through a Zeiss Universal microscope from a vertical optical axis orthogonal to the sample which was illuminated at 45° by a quartz light guide thus avoiding specular reflectance as in a conventional colorimeter (Figure 2, 8). There were two illuminators that could be switched into the illuminating fiber by a rotating mirror (Figure 2, 7). One illuminator was a halogen source (Figure 2, 5) operated at 12 v from a stabilized transformer to calibrate the system for reflectance measurements using white Teflon tape as a standard.[12] The other illuminator was a UV diode to create fluorescence in the samples (Figure 2, 6; Nichia NVSU233B, Tokushima, Japan). The protocol was to set up the system for reflectance colorimetry using the weighted ordinate method,[8] then to switch illuminators for the colorimetry of fluorescence in the samples.

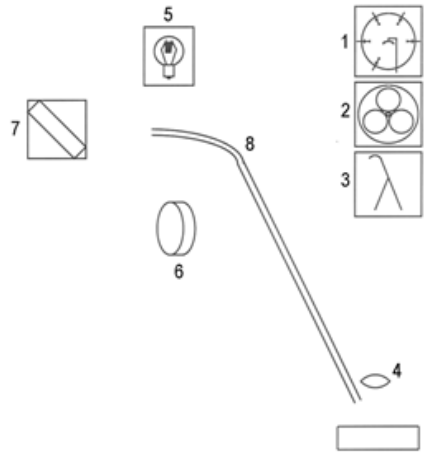

Figure 2 Apparatus for reflectance colorimetry and fluorescence. Components are a photomultiplier (1), filter wheel to remove high order harmonics (2) from a grating monochromator (3), quartz microscope objective (4), tungsten filament bulb (5), UV LED (6), rotatable mirror (7) and light guide (8).

Results

Initial tests on bovine tendon and fluorescent minerals such as selenite ($CaSO_4.2H2O$) revealed an anomalously high reflectance ≥ 400 nm that would have prejudiced colorimetry calculations. The first suspect was a narrow separation between the excitation wavelengths and the emission wavelengths. Although reflectance at 400 nm has a minimal effect on calculating chromaticity coordinates, this problem had to be solved. In other words, the higher wavelengths of the excitation spectrum were reflected to overlap the lower wavelengths of emission spectrum. This was seen by examining the output of the UV LED (Figure 3).

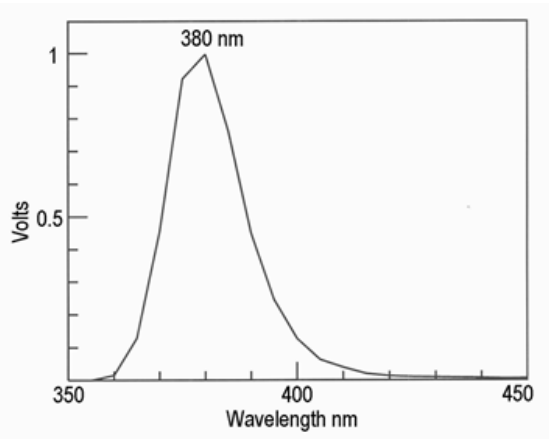

Figure 3 Normalized photomultiplier output (volts) for the excitation spectrum (nm).

Before finding a solution to this problem, it was thought best to check the colorimetry optics and software. There was no point in solving the crossover problem if no reasonable colorimetry data were obtainable. The system was tested on blue, yellow and red fluorescence reference standards (Ted Pella Inc. 2273, Redding California). Although excitation wavelengths crossing over from excitation to emission at 400 nm might prejudice colorimetry of white fluorescence, this was considered to be negligible with a wide separation between excitation and emission for colored fluorescence standards. Figure 4 shows the chromaticity coordinates of blue (Figure 4, A; peak 410 nm), yellow (Figure 4, B; peak 520 nm) and red (Figure 4, C; peak 600 nm). The slight deviation of the yellow slide towards green was not considered to be a problem because this test slide was approximated to acridine orange which has a green tinge like uranyl glass. Since the colorimetry optics and software seemed subjectively reasonable, the next problem was to find a solution to the crossover from excitation to emission that might have affected colorimetry of white fluorescence.

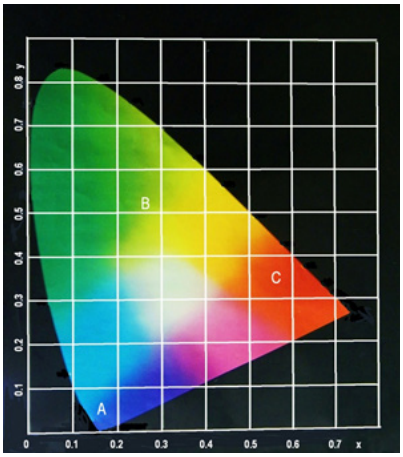

Figure 4 Chromaticity coordinates of blue (A), yellow (B) and red (C) fluorescence standards.

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One solution attempted was to incorporate a correction for the crossover in the standardization protocol for setting up the reflectance measurements. Here a diffuse reflectance standard was scanned across the visible spectrum after setting the photometer to near its peak sensitivity (560 nm). The reflectance at each wavelength from 400 to 700 nm at 10 nm intervals was found together with a dark field measurement at each wavelength (the result with a shutter blocking the photometer). This corrected for some of the ambient illumination which was minimal working in a dark room, but computer screens and keyboard illumination are always a risk. Reflectance at each wavelength was then found from the photometer response minus the dark field measurement. So it was possible to make another correction, like the dark field correction, by rescanning a dark but reflective standard illuminated by the UV LED. Thus, reflectance was taken as the reflectance measurement minus the dark standard illuminated by UV. For a dark standard with reflectance but no fluorescence the choice was granules of activated charcoal briefly flamed to burn off any dust, which in a laboratory is normally derived from human keratin which has strong white fluorescence.

This did not work well. Some of the carbon granules had surfaces with high reflectance of the UV excitation, while others did not. So the apparatus in Figure 2 was rebuilt to incorporate a dark field shutter (Ilex No. 1, Synchro, Rochester, New York) in front of the light guide (Figure 2, 8,) instead of before the photomultiplier (Figure 1&2), thus enabling a superior correction for ambient illumination. With the excitation spectrum rather close to the emission, to remove any doubt that the excitation was prejudicing emission colorimetry, an interference filter (Linos, Königsallee 23, 37081 Göttingen, peak 366 nm, zero at 400 nm) was added in front of the UV diode. Thus, as shown in Figure 5, the peak emission at 400 nm that might have been prejudiced by a crossover from excitation to emission in the apparatus survived after removal off any possibility of crossover in the apparatus.

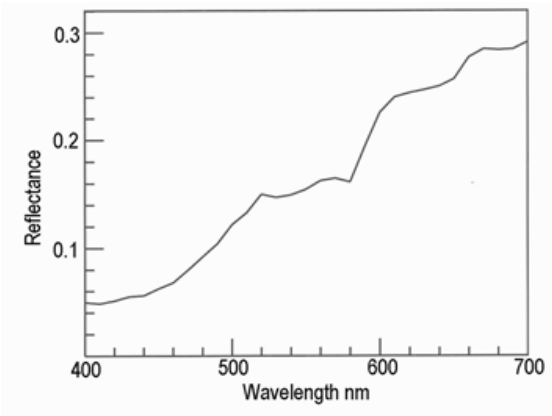

Figure 5 Reflectance of elastin (bovine *ligamentum nuchae*) relative to white Teflon with illumination at 45°.

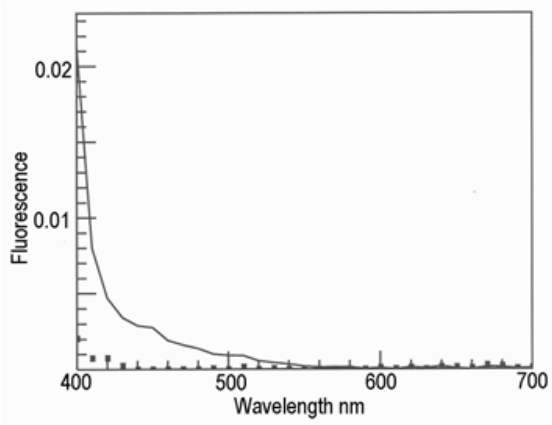

Figure 6 Fluorescence of elastin (bovine *ligamentum nuchae*) relative to the reflectance of white Teflon with illumination at 45° at two excitation peaks, solid line at 380 nm and boxes (■) at 366 nm.

The reflectance of elastin fibers of the bovine *ligamentum nuchae* (Figure 5) gave a typical result for biological fibers acting as a light trap with ramp from 400 to 700 nm. The dimple around 555 nm matches that known for hemoglobin, most likely from the few blood capillaries in the *ligamentum nuchae*. The hue was a pale yellow (x = 0.432, y = 0.402, Y% = 17). The fluorescence with 380 nm excitation was a dull blue (x = 0.165, y = 0.093, Y% = 0.33), with a shift to a darker blue with 366 nm excitation (x = 0.29, y = 0.15, Y% 0.03). In other words, UV excitation of elastin fluorescence produced an emission plus reflectance in the visible spectrum sufficient to affect colorimetry measurements. Thus, peak fluorescence emission at 400 nm from elastin was a real phenomenon not completely an artifact from the apparatus. These samples of bovine *ligamentum nuchae* were examined to test the apparatus in Figure 2. A commercial application is most unlikely because the *ligamentum nuchae* is removed from beef carcasses during meat cutting operations and is too strong to be penetrated with a UV probe. However, all the connective tissues in an animal are part of a continuous system and, until proven otherwise, a surface measurement of the *ligamentum nuchae* might be related to connective tissue toughness in prime meat cuts.

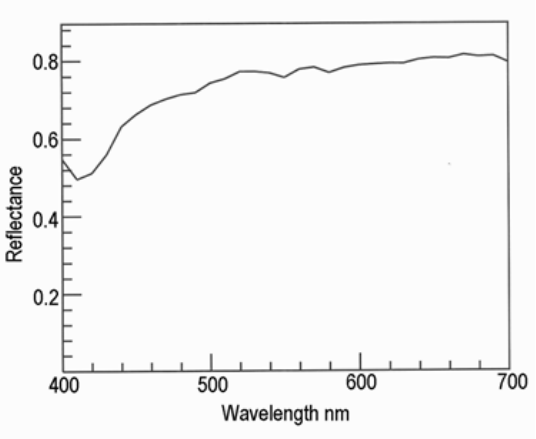

Figure 7 Reflectance of epimysium (collagen on the surface of the bovine *longissimus thoracis* muscle) relative to white Teflon with illumination at 45°.

Elastin fibers in beef have an effect on tenderness because they are heat stable and unaffected by cooking. But elastin fibers are microscopic and unlikely to be resolved in a UV meat probe. Far more important for understanding the operation of a UV meat probe are the larger structures composed of Type I collagen – epimysium on the surface of a muscle and perimysium around bundles of muscle fibers within the muscle. These structures are almost white, as can be seen from the reflectance spectrum in Figure 7, which was x = 0.34, y = 0.35, %Y = 77.1. The hue was almost the same when visible light illumination was replaced by UV at 366nm, x = 0.38, y = 0.41, %Y = 0.66. This is a fortunate coincidence. It shows that reflectance and fluorescence of collagen are similar in hue and unlikely to prejudice the detection of epimysium and perimysium.

In Figure 1, it may be seen there are many places between fluorescence peaks where reflectance would allow colorimetry of the

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striated muscle, as in the example shown in Figure 8 (x = 0.46, y = 0.36, %Y = 10.5). Might this be possible with a fast photodiode array for a meat probe? The spectrum shows myoglobin with its signature of a dimple around 560 nm.[13]

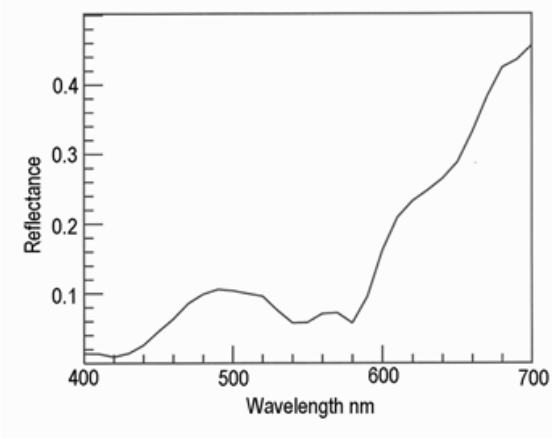

Figure 8 Reflectance of striated muscle between major fluorescence peaks of epimysium in bovine *Longissimus lumborum* relative to white Teflon with illumination at 45°.

Conclusion

This work was undertaken in ideal laboratory conditions to investigate how meat probes might be working in the extremely difficult conditions of a commercial abattoir – only seconds to make measurements on meat carcasses moving on an overhead rail. Spray water everywhere, not to mention the unexpected effects of static electricity on digital electronics and the risk of high voltage start-up circuits for UV arc lamps. There is little to be gained by colorimetry differences of connective tissue fluorescence and reflectance, but colorimetry of skeletal muscle between connective tissues may be possible.

Acknowledgments

Author declares no acknowledgement.

Conflicts of interest

None – unfunded research prompted by scientific curiosity.

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