EVALUATION OF PROBE DESIGNS TO MEASURE CONNECTIVE TISSUE FLUORESCENCE IN CARCASSES

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ABSTRACT

Three probes were evaluated for their effectiveness in measuring connective tissue fluorescence within carcasses or primal cuts: 1) a quartz-glass rod, 2) a light guide formed from bundles of optical fibers, and 3) a single optical fiber. The shape of the fluorescence emission spectrum of tendon was altered by the method of measurement, probably because of differences in the intensity of excitation. The single optical fiber design provided the best solution to the problem caused by the irregular distribution of connective tissue in meat, and a modified fat-depth probe was tested as a prototype. Beef shank had more fluorescence peaks per millimeter ($P < .01$), a greater area above minimum fluorescence ($P < .01$), and a greater mean peak intensity ($P < .005$) than did psoas major.

Key Words: Grading, Carcass Composition, Connective Tissue, Toughness


Introduction

The development of sensors, control systems, and robotics is important for continued technological progress in the meat industry (Krol et al., 1988). Tenderness is the primary factor that determines the commercial value of meat from a healthy beef animal. Tenderness strongly influences how we grade, sell and cut the carcass and is a major factor in the economics of beef consumption for the average consumer. A method for the rapid measurement of connective tissue in meat could improve the reliability of beef grading systems and help to maintain the consumer demand for beef, provided that efforts are made to avoid the cold-shortening of beef.

The autofluorescence of connective tissue is well known (Pearse, 1968), and fluorimetry may be used to measure the amount of connective tissue appearing on cut surfaces of meat (Swatland, 1987). Unfortunately, flat-cut surfaces of meat are not readily available when carcasses might be sorted on the basis of their potential connective tissue toughness. The major technical problem is that the penetration of UV light through meat is very low relative to that of visible light at long wavelengths (because scattering tends to be inversely proportional to the power of wavelength). The major biological problem is that connective tissues are not uniformly distributed through the carcass musculature but are concentrated toward tendons, aponeuroses, and fascias. Therefore, sampling location is critical.

The objective of this study was to evaluate three potential methods of measuring the fluorescence of connective tissue inside primal cuts or carcasses and to test a prototype based on the most promising method.

Materials and Methods

Apparatus for the Evaluation of Probe Designs. Figure 1 shows the construction of the three probes (not to scale). The principle of both the quartz-rod and light-guide probes is that the area of meat in contact with the probe is relatively large and any fluorescence in that area is integrated with a single measurement. The area of the single-fiber probe in contact with the meat is much smaller than that of the other two probes. The single-fiber probe is designed to detect fluorescent boundaries as it is pushed through the meat.

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The quartz-rod probe was constructed as follows. A quartz rod (12.8 mm diameter, 20 cm length) was coated with aluminum on its sides to give it an internally reflecting surface (A in Figure 1). One end was cut at a 45° angle and polished to give an area in contact with the meat of approximately 300 mm². A dichroic mirror (B in Figure 1; part 360-30-2D) was used to separate incoming (excitation) and outgoing (emission) light. Incoming light was from a UV fluorescent pencil lamp (shown from top view as C in Figure 1; model 36-380). The diameter of the pencil lamp was 9.5 mm and the distance of the lamp surface mirror axis was 35 mm. Closer spacing gave a higher intensity of fluorescence, but the emission spectrum was contaminated by stray light from the lamp. Outgoing light (D in Figure 1) passed through a grating monochromator (Zeiss 474345 with 477216), a stray light filter (Zeiss 477215), and onto a side-window photomultiplier (HTVR928) in a housing (Zeiss 474230).

The light-guide probe was made from a pyrex test tube (E in Figure 1) containing a central core of approximately 320 UV-conducting fibers (1) surrounded by approximately 250 Crofon (2) collecting fibers. In the side view of the light guide probe in Figure 1, three diagrammatic fibers (solid black) represent the several hundred fibers present in the tube. The curved area of glass in contact with the meat was approximately 260 mm², but only the center of this area was intensely illuminated. For the incoming light path (G in Figure 1), excitation was from a high-pressure short-arc mercury lamp (HBO 100W) operated from a stabilized power supply (Zeiss 910235), and the band-pass was controlled with a prism monochromator (Zeiss M4Q III). The outgoing light path (F in Figure 1) was similar to that used for the quartz rod probe.

The single-fiber probe (H in Figure 1) was composed of .5 m of optical fiber (type HFBR10) with an optical area of approximately .78 mm² interfacing with the meat (there was a narrow air space of approximately .5 mm between the optical fiber and the meat when this design was evaluated by surface scanning of the meat). The beam splitter was the dichroic mirror and exciter filter of a fluorescence microscope (I in Figure 1; Zeiss III RS with G365 and FT 395). A microscope objective (J in Figure 1; Zeiss Neofluar × 6.3, NA .2) was used to couple the fiber to the optical axis of the microscope. The outgoing light path (K in Figure 1) went through the Zeiss grating monochromator mounted on the microscope and onto the side-window photomultipler. The light path in the monochromator could be switched to bypass the grating. The incoming light (L in Figure 1) was from the short-arc mercury lamp mounted on the microscope.

To evaluate the probe designs, it was essential for the observer to see the area being measured. Thus, in the initial evaluation phase of the study, many of the measurements were made on the cut surfaces of meat samples. For the initial evaluation of the single-fiber probe, instead of being pushed through the meat, the fiber was scanned across the surface of the meat sample so that the tissues under the fiber tip could be observed.

No attempt was made to undertake radiometry of connective tissue fluorescence, and all

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measurements were relative. The three probes were standardized for the measurement of relative spectral fluorescence intensities following an accepted procedure (Zeiss, 1980). Spectra were measured from 400 to 600 nm at intervals of 10 nm. Except when stated otherwise, the band pass was 10 nm. Data were normalized for each type of probe (i.e., data were adjusted to give a maximum value of 1 for the strongest spectrum). The standardization protocol was tested by measuring the fluorescence emission spectrum of uranyl glass (Zeiss GG17).

A fluorescence blank was measured at each wavelength and subtracted from the fluorescence of the sample. The blank was either a black-velvet light trap (for light-guide and single-fiber probes) or wrinkled aluminum foil (for the quartz-rod probe). Factors that were included in the fluorescence blank were the following: 1) tail of the excitation band-pass, 2) stray light passing through the excitation monochromator and/or dichroic mirrors, and 3) intrinsic fluorescence in the optical system. However, because the refractive index of the blank (air) was different from that of samples, the fluorescence blank did not exactly simulate sample conditions. The most notable defect of this protocol was pseudo-fluorescence from 400 to 430 nm caused by reflectance of the tail of the excitation band-pass. This was tolerated because a narrower excitation band-pass would have weakened the fluorescence and made it more difficult to measure.

**Samples for the Evaluation of Probe Designs.** Meat samples were removed from the carcases of 16 cattle of various types. Considerable differences were found among carcass samples but no attempt was made to study them. All the data reported here for the evaluation of probe designs are typical of mature animals (maturity group III in the Agriculture Canada beef grading system). Extensor carpi radialis muscles were dissected to obtain sections of extramuscular tendon, epimysium backed by muscle, and muscle belly without any grossly visible connective tissue septa. Slices from across the extensor and flexor muscles of the forelimb shank and slices of psoas major were comminuted in a meat grinder (hole diameter 3.3 mm). The comminuted meat was mixed in various ratios (100 to 0% shank in increments of 10%, n = 11) and packed tightly into beakers for measurement. Mixtures were measured by inserting the light-guide probe halfway down the depth of the beaker. Gelatin solutions were prepared from technical grade gelatin granules.\(^\text{11}\)

**Test of Prototype.** The single fiber probe (Figure 1) was adapted for use as a prototype by incorporating it into a Danish MQM fat-depth probe.\(^\text{12}\) The original infrared diodes and associated circuitry were removed from the probe, and the single optical fiber was inserted through the probe to form an elliptical window (approximately 1 mm\(^2\)) just behind the cutting head. The photomultiplier used in the evaluation of probe designs was replaced with a flat-response silicon cell (SEE015/F\(^\text{13}\)) to give a faster response, and there was no monochromator in the outgoing light path. The probe was operated from a Hewlett-Packard microcomputer (model 9826 with Multiprogrammer 6942A). Thus, instead of giving infrared reflectance from fat vs depth in the carcass, the modified probe gave UV fluorescence of connective tissue fibers vs depth in the carcass.

The probe was tested by multiple (n = 10) penetrations of a forelimb shank and a psoas major taken from a beef carcass (Canada Grade A). Each penetration was at a different position. Thus, the variance in each set of measurements was the product of variation in connective tissue distribution and instrument response. Standard deviations of observations (\(\pm\)) are given after the reported means. Differences between means were evaluated with a \(t\)-test.

**Results**

**Evaluation of the Quartz-Rod Probe.** Fluorescence emission spectra of tendon, epimysium, and muscle belly obtained with the quartz-rod probe are shown in Figure 2. The fluorescence intensity of tendon was stronger than that of muscle from 460 to 600 nm, and the epimysium was intermediate in intensity.

**Evaluation of the Light-Guide Probe.** Fluorescence emission spectra of tendon, epimysium, and muscle belly obtained with the light-guide probe are shown in Figure 3. The peak for fluorescence of tendon was at 460 nm rather than at 480 nm, as was found with the

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11 Matheson, Coleman and Bell, Norwood, OH.
12 Danish Meat Research Institute, Roskilde, Denmark.
13 International Light, Newburyport, MA.
quartz-rod probe (Figure 3). Relative to spectra made with the quartz-rod probe, epimysium was stronger and muscle was weaker.

The light-guide probe was used to study the effect of fluorescence intensity on the shape of emission spectra using the following three techniques: 1) increasing the intensity of excitation by increasing the band-pass at the 365 nm mercury peak, 2) increasing the concentration of the fluorescent material, and 3) increasing the incidence of fluorescent material.

With uranyl glass (Zeiss, 1980) as a standard, the correct shape of the emission spectrum and the correct peak emission of 530 nm were obtained with an excitation band-pass of 2.5 nm (.1 mm slit width on a Zeiss M4Q III monochromator). When the band-pass was increased to 3 nm (.2 mm slit), the peak fluorescence intensity or the uranyl glass standard was approximately doubled to give an incorrect peak emission of 560 nm.

Using a 2% gelatin solution as a standard, peak fluorescence emission was at 450 nm. In a 4% solution, fluorescence intensity was increased (× 1.5), and the peak was at 500 nm.

With an increase in the ratio of shank muscle to psoas major, fluorescence intensity and the peak emission were increased, as shown in Figure 4. Fluorescence at 540 nm was correlated with the percentage of shank meat ($r = .68; P < .025; n = 11$).

**Evaluation of the Single-Fiber Probe.** The fluorescence emission spectrum of a septum of
in intramuscular tendon along a scanning transect is shown in Figure 5. The vertical offset of the spectra indicates an increment of 2 mm along the scanning transect. Peak fluorescence for intramuscular tendon was at 450 nm.

Figures 6 and 7 show typical results obtained by scanning across the surfaces of slices of forelimb shank and T-bone psoas major, respectively. The data have been simplified by combining the fluorescence at different wavelengths to give a single total at each point along the scanning transect (i.e., there was no monochromator in the outgoing light path). In Figure 6, the transect was along the medial to lateral axis (posterior to the radius) and passed across the ulnaris lateralis, deep digital flexor, and superficial digital flexor muscles. The edges of the slice were at 10 and 120 nm. In Figure 7, the transect was along the ventral to dorsal axis in the midlumbar region.

With this method, fluorescence peaks occurred whenever there were visible signs of connective tissue (fascia, epimysium, aponeurosis, or intramuscular tendon). For shank slices, background fluorescence over the muscle belly was relatively low. Muscle belly fluorescence was higher for psoas major than for muscles of the shank.

Test of Prototype. Examples of measurements made from within the meat (rather than scanning on the surface) are shown in Figure 8. Measurements made in the shank (top line in Figure 8) had more conspicuous fluorescence peaks than those made in the psoas major (bottom line in Figure 8). In a comparison of 10 measurements on a shank and 10 measurements on a psoas major, shank had more peaks per millimeter than psoas (0.11 ± 0.05 vs 0.06 ± 0.03, respectively; P < .01), had a greater area per millimeter above the minimum fluorescence intensity (0.57 ± 0.32 vs 0.24 ± 0.17; respectively, P < .01), and had a greater mean peak intensity (0.95 ± 0.38 vs 0.5 ± 0, respectively; P < .005). The minimum fluorescence intensity did not differ significantly between shank and psoas major (1.20 ± 0.48 vs 1.25 ± 0.49, respectively). The output of the silicon photometer was in steps of 0.5 (to obtain a fast response), which accounts for the standard deviation's equaling 0 for mean peak intensity of fluorescence in the shank and for the stepwise shape of the data shown in Figure 8.
Discussion

The results showed that the intensity of excitation may change the fluorescence emission spectrum of a sample. Apparatus was not available to measure the amount of energy delivered by the three probes. However, by subjective assessment of the intensity and area of fluorescence excited in uranyl glass, the quartz-rod probe seemed to be the strongest, the light-guide probe was intermediate, and the single-fiber probe was the weakest. Respectively, the fluorescence emission peaks for tendon were 480, 460, and 450 nm. Thus, in an industrial application, it may be difficult to use a ratiometric method for the measurement of pre- and post-quenching spectra associated with different ratios of Types I to III collagen because the results may be affected by the intensity of excitation. This factor may explain why the precise shape of individual fluorescence emission spectra reflects the apparatus with which they are recorded as much as the nature of the sample (Bashford, 1987).

Each of the probe designs had specific features that may be of some future use. For example, further investigation (Swatland and Barbut, 1991) has proved that the quartz-rod probe (which integrates fluorescence over a single, relatively large site) may be used to measure the fluorescence of macerated poultry skin used as a filler in poultry products. This enables predictions of gel strength \( (r = .99) \), cooking losses \( (r = .99) \), and fluid-holding capacity of the finished product \( (r = .92) \).

Relative to the objective of the present study (choosing a probe design to measure connective tissue distribution in carcasses), the single-fiber probe is the most favorable configuration because it offers the best solution to the problem caused by the irregular distribution of connective tissue in meat. With the single optical fiber mounted in the shaft of a conventional fat-depth probe, it is possible to sample the connective tissue in a transect of 8 to 10 cm through the tissue. Fat depth probes cause very little damage to the carcass and are fast enough to be used at typical line speeds. Therefore, building on this existing technology has many advantages.

Implications

If further development is successful, there is a possibility that connective tissue fluorimetry via a single optical fiber added to a fat depth probe could be used as a rapid method to sort meat or carcasses for connective tissue toughness. This might be used to select carcasses with a low connective tissue content for premium treatment (extended conditioning and superior packaging).

Literature Cited


