MEASUREMENT OF THE GRISTLE CONTENT IN BEEF BY MACROSCOPIC ULTRAVIOLET FLUORIMETRY

H. J. Swatland

University of Guelph, Guelph, Ontario, Canada

ABSTRACT

Ultraviolet light (365 nm) was directed at an angle of 45° onto meat samples in a circular aperture (3 cm diameter). Fluorescence emissions were measured with a monochromator and a photomultiplier tube. Intact tendons and elastic ligaments had a strong fluorescence emission peak around 440 to 450 nm and only weak fluorescence around 510 nm. Tissues such as lean meat and adipose tissue that contain a matrix of reticular fibers (Type III collagen) had very low fluorescence around 440 to 450 nm so that their peak emittance was the weak fluorescence peak at 510 nm. The 510/440 nm ratio of fluorescence emissions was measured in comminuted meat samples containing varying mixtures of lean meat and gristle and varying mixtures of muscles with a high and low gristle content. The 510/440 nm ratio was correlated with the ratio of lean meat/gristle (r = .96, P<.005). The 510/440 nm ratio was correlated with the ratio of longissimus/shank meat (two trials; r = .93, P<.01; and r = .94, P<.005). Results were only slightly changed when samples had dry surfaces or when samples were mixed with adipose particles. The relationship between the area of gristle in the samples and the 510/440 nm ratio was curvilinear with the greatest sensitivity to the fewest gristle fragments.

(Key Words: Meat Quality, Collagen, Elastin, Fluorescence.)

Introduction

Research using fiber-optics to study the fluorescence of different biochemical types of collagen (Swatland, 1987a) has shown that Types I and III collagen differ in their emission spectra. The emission spectrum of elastin is similar to that of Type I collagen. With excitation near the peak excitation wavelength of 370 nm, Type I collagen has a strong emission peak at 440 nm and weak emission around 510 nm. The fluorescence of Type III collagen is relatively weak at 440 nm, and peaks at around 510 nm. From these results it appeared that it might be possible to measure the gristle content of meat by macroscopic fluorimetry. The apparatus described in this report was constructed for this purpose.

The visible gristle in meat originates from a number of sources, including tendons (particularly from myotendon junctions), ligaments (such as the ligamentum nuchae in rib roasts), perimysium (particularly in the pennate extensors and flexors of the lower limbs), fasciae (such as the lumbodorsal fascia of the longissimus muscle) and intramuscular blood vessels (particularly arteries). All these sources are dominated by either Type I collagen or elastin, whereas the background of myofibers and adipose cells is dominated by Type III collagen. All the elastin-gristle and varying amounts of collagen-gristle retain their tensile strength after typical cooking procedures such as roasting and barbecuing (Bailey and Sims, 1977; Judge and Aberle, 1982; Pearson and Tauber, 1984; Purslow, 1985).

The overall objective of the research reported here was to develop a fluorimetric method to measure the gristle content (Type I collagen plus elastin) of macroscopic samples of ground beef. In Exp. 1 and 2, macroscopic emission spectra were compared with those obtained by fiber optics to check that comminution did not change the spectra. The characteristics of the method were studied in Exp. 3 to 5. The objective of Exp. 3 was to test whether the 510/440 nm fluorescence ratio could be used to estimate the amount of gristle when mixed with muscle. The objective of Exp. 4 was to determine whether the 510/440 nm fluorescence ratio was sufficiently sensitive to detect differences in the...
proportions of shank muscle (with a high gristle content) and longissimus muscle (with a very low gristle content). The objective of Exp. 5 was to examine the relationship between the number of gristle particles in the field measured and the 510/440 nm fluorescence ratio.

Materials and Methods

The apparatus for macroscopic fluorimetry is shown in figure 1. The primary illuminator (component A in figure 1) was a Zeiss fib-type HBO 100W/2, which can tolerate a 65° orientation. Readers are warned to check manufacturers' data before using other types of burners at this angle. Light at 365 nm is near enough to the peak excitation wavelength (370 nm) to produce the desired results, and this wavelength allows the very strong peak emittance of a mercury source to be used to full advantage. The power supply was stabilized (Zeiss 910235). Light from the primary illuminator passed through a 365-nm filter (component B in figure 1) and was directed down a tube with a silvered internal coating (component C in figure 1) to strike the sample (component D in figure 1) at 45°. The sample filled a petri dish to the depth of the side walls so that it could be held against the sample aperture by a spring-loaded platform (component E in figure 1) without bulging into the aperture. The circular sample aperture was 3 cm in diameter. Fluorescence emissions from the sample passed through an oblique aperture in the silvered tube and were reflected from a mirror (component F in figure 1) and directed into a collimator (component G in figure 1). The parallel rays of light passed through a rectangular aperture and a lens (component H in figure 1), through a motorized continuous interference-filter monochromator (component I in figure 1), through a solenoid-operated shutter (component J in figure 1) and were focused on a side-window photomultiplier (component K in figure 1). The monochromator, the shutter and the photomultiplier were operated from a microcomputer (Zeiss Zonax).

To enable the system to be standardized, the secondary illuminator (component L in figure 1) was a 100-W halogen source connected through a fiber-optic light guide (component M in figure 1) to a small internally-reflecting prism (component N in figure 1) that enabled a plate of barium sulfate powder in the sample position to be evenly illuminated. The intensity of illumination was varied by a series of pin-hole stops between the light guide and the secondary illuminator so that the illuminator could be kept at a constant voltage to generate a known emission spectrum. The light path of the apparatus was shielded from ambient illumination by a metal housing with a trapdoor for viewing the specimen in the sample aperture. Ultraviolet safety glasses were worn by the operator when checking the samples.

The protocol to standardize the system was as follows: 1) shut off the primary illuminator by replacing the excitation filter with a blackened block of metal; 2) place a plate of compressed barium sulfate powder in the sample aperture; 3) illuminate evenly with light from the secondary illuminator at an intensity slightly higher than that anticipated from the fluorescence of a real sample; 4) set the high voltage of the photomultiplier and the gain of the amplifier at each wavelength from 440 to 600 nm in increments of 10 nm, so as to use most of the dynamic range of the analog to digital converter; 5) use the solenoid-operated shutter to find the appropriate photomultiplier output when in darkness; 6) turn off the secondary illuminator and obtain incident ultraviolet light by replacing the metal block with the 365-nm excitation filter; 7) replace the barium sulfate

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Figure 1. Apparatus for macroscopic fluorimetry. Mercury illuminator, A; 365-nm excitation filter, B; tube with silvered internal surface, C; 3-cm diameter sample aperture, D; spring-loaded platform to hold sample in petri dish against aperture, E; mirror, F; collimator, G; aperture and focusing lens, H; motor-driven continuous interference-filter monochromator, I; solenoid-operated shutter, J; side-window photomultiplier, K; secondary halogen illuminator, L; fiber-optic light guide, M; and internally-reflecting prism, N.

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3 Carl Zeiss, 7082 Oberkochen, W. Germany.
4 Osram, Berlin, W. Germany
plate with a plate of activated charcoal previously cleaned of any fluorescent particles; 8) measure this fluorescence blank at each wavelength to obtain data with which to make corrections for imperfections in the separation of incident excitation and radiated fluorescence and 9) replace the charcoal blank with a real sample to measure its fluorescence.

The data were corrected to allow for the fact that there was a low output from the photomultiplier even in darkness and for imperfections in the optical system (from steps 5 and 8 of the protocol). Fluorescence emission spectra were adjusted to allow for the fact that the halogen secondary illuminator did not emit all wavelengths at equal intensity (from steps 3 and 4 of the protocol). It is very difficult to measure fluorescence in absolute units of luminance, and all the data presented in this report are in relative units. When several spectra are shown together in a figure this indicates that they were all measured together with the same standardization of the photomultiplier. Thus, a standardization for when the sample aperture was filled with strongly fluorescence elastic ligament, for example, was not suited for the measurement of the much weaker fluorescence of intact muscle (see step 3 of the protocol).

**Meat Samples**

Meat samples were obtained from Agriculture Canada grade A beef carcasses in the University of Guelph abattoir. The animals were crossbred beef animals with live weights ranging from 400 to 500 kg. There were no obvious differences in fluorescence related to the source of the sample nor to the time lapse from slaughter to sampling.

Exp. 1. Samples of the lamellar part of the ligamentum nuchae, tendinous collagen from the gastrocnemius bursa of the hock, sternal adipose tissue, psoas major and deep digital flexor of the forelimb shank plus surrounding muscles were obtained intact from a beef carcass and arranged so as to fill the sample aperture. The two muscle samples were sectioned transversely.

Exp. 2. Tissues from similar sources to those in Exp. 1 were obtained from five other beef carcasses. Tissues of the same type from each animal were pooled together. The tissues were comminuted through a plate with 3-mm diameter apertures and thoroughly mixed. The auger and plate were cleaned between different types of tissue and the tissues were first cut into small cubes to prevent excessive heating and pressure inside the grinder.

Exp. 3. From an experiment in which a whole beef carcass was being comminuted for proximate analysis through a plate with 12.5-mm diameter apertures, samples of lean skeletal muscle and collagen from tendons and ligaments were isolated with forceps. Tissue fragments were sorted into five petri dishes by weight to give samples with 0, 25, 50, 75 and 100% tendon with the balance being lean skeletal muscle.

Exp. 4. Experiment 4 was replicated (n = 2). Samples were taken from the longissimus muscle over the posterior ribs and from the distal parts of the extensor and flexor muscles of the forelimb shank (tendons were trimmed off level with the distal end of the muscle). The samples were comminuted through a plate with 3-mm diameter apertures and the fragments were searched with a binocular dissection microscope to remove all visible traces of adipose tissue. Meat fragments from these two sources were sorted by weight into petri dishes to give mixtures with 0, 25, 50, 75 and 100% longissimus muscle with the balance being shank muscle.

Exp. 5. Samples were taken from the thoracic part of the longissimus muscle and from the tendons of foreshank extensor and flexor muscles. Samples were comminuted through a plate with 3-mm diameter apertures and the tendon fragments were sorted and trimmed under a binocular microscope to produce small cylinders of tendon with a diameter of 3 mm and a length of 3 mm (approximately). The comminuted muscle from which all traces of adipose tissue had been removed was pressed into eight petri dishes to the depth of the side wall. The small cylinders of tendon were pressed vertically into the centers of the petri dishes to give a range of samples with from zero to seven cylindrical tendon fragments per dish. The dishes were mounted under the sample apertures so that the tendon fragments were in the center of the field.

**Results**

The results obtained in Exp. 1 are shown in figure 2. The elastic ligamentum nuchae had the strongest fluorescence, and is not shown in figure 2 because it would have been off the scale. The shape of emission spectrum for elastic ligament was very similar to that of the tendinous collagen. The tendinous collagen had the next
strongest fluorescence with peak emission around 450 nm (figure 2). Adipose tissue had a weaker fluorescence with a peak at 510 nm. Both psoas and shank muscles had much weaker fluorescence than the connective tissues. The 510/440 nm ratios, previously proposed as a means to measure the ratios of Types III to I collagen (Swatland, 1987a), were .65, .58, 1.24, 1.98 and 3.15 for elastic ligamentum nuchae, tendinous collagen, adipose, shank muscles and psoas, respectively.

Figure 3 shows the emission spectra for psoas and shank muscles on an expanded scale after restandardizing the apparatus (with a lower luminance to set the dynamic range of the photomultiplier). In the emission spectrum of the shank muscle with its high gristle content could be seen higher emissions from 430 to 500 nm relative to the essentially gristle-free psoas muscle.

Figure 3 shows how the 510/440 nm ratio is sensitive to the presence of either Type I collagen or elastin on a background of Type III collagen fibers around myofibers or adipose cells. Collagen and elastin have a very strong fluorescence around 440 to 450 nm so that even minute traces are detectable when the photomultiplier is set at a high voltage and high amplifier gain to measure the much weaker fluorescence of dispersed Type III collagen fibers. From the results shown in figures 2 and 3, it might be argued that 450 nm or even 460 nm would be a better denominator for the ratio. However, an arbitrary decision was made to stay with 440 nm because this wavelength gives the optimum sensitivity with fiber optics. Thus, a very small degree of sensitivity was sacrificed in order to obtain standardization between different optical methods. The 10-nm positive bias in emission peaks at 440 or 450 nm found with the optical system developed for this study relative to those obtained by fiber-optics might have been caused by chromatic aberration.

The results of Exp. 1 and 2 showed that comminution did not produce any obvious changes in fluorescence emission spectra of connective tissues or muscle. The fluorescence emission spectra that were obtained from the different tissues originating from five animals were essentially the same as those shown in figures 2 and 3. Hence, no further attention was given to the possibility of variation in the shape of emission spectra between different animals or caused by comminution.

In Exp. 3, with five samples ranging from 100% gristle to 100% muscle, the 510/440 nm fluorescence ratio was correlated with the proportion of muscle (r = .96, P<.005; figure 4). For 100% gristle, the minimum value of the 510/440 nm fluorescence ratio was close to the value measured on intact tendon and elastic ligament. For 100% muscle, the ratio was between the value found for psoas and shank muscles.

In Exp. 4, with five samples ranging from 0 to 100% longissimus muscle with the balance being shank muscle, the 510/440 nm fluorescence ratio was correlated with percent longissimus muscle (r = .93, P<.01; figure 5). When the experiment was repeated a second time with meat from a different beef carcass, the result was similar (r = .94, P<.005). To each of the samples was added 25% by weight of comminuted adipose tissue and each sample was thoroughly remixed. With the samples now diluted by adipose tissue, the 510/440 nm fluorescence ratio was correlated with percent...
longissimus muscle, $r = .90$, $P < .025$. Samples from the second replicate of the experiment (without added adipose tissue) were left uncovered in a refrigerator overnight at 4°C so that the meat surface became dry and metmyoglobin coloration started to develop. When re-measured, the correlation of the 510/440 nm fluorescence ratio with percent longissimus muscle was similar to that obtained before storage ($r = .95$, $P < .005$).

In Exp. 5, each gristle particle occupied about 1% of the area of the sample aperture. The number of particles was correlated with the 510/440 nm fluorescence ratio ($r = -.84$, $P < .005$, figure 6). However, the relationship was not linear and it showed that the 510/440 nm ratio was more sensitive in the detection of one or two gristle particles rather than in resolving larger numbers of particles. The change in ratio from zero to one or two particles was as great as the change from two to seven particles. In retrospect, evidence of non-linearity was also obvious in the results of Exp. 4 (figure 5).

Discussion

Despite the great amount of research that has been undertaken on meat toughness, it is still a characteristic that is difficult to measure and even more difficult to predict. The contributions of connective tissues and shortened sarcomeres to meat toughness are well known (Lawrie, 1985), and the traditional focus of attention on postmortem enzyme biochemistry (Schwimmer, 1981) has been augmented by ultrastructural studies, most recently on the cytoskeleton (Locker and Wild, 1982). Rheological methods for the measurement of meat toughness, however, have not attempted to isolate toughness caused by gristle from toughness originating from the state of myofibrillar and cytoskeletal components. Nevertheless, most consumers are sensitive to the presence of gristle in meat because it can be both seen by eye and detected by mouth. Even in ground beef, gristle fragments may be detected by mouth and create what might be called a gritty texture after dry-cooking. Apart from laboratory analyses such as hydroxyproline content and histological sectioning, there is at present no routine method to measure specifically the gristle content of meat. Existing laboratory methods require samples to be taken and they are too costly and time-consuming to be used commercially in the meat industry. Macroscopic ultraviolet fluorimetry might be devel-

Figure 4. Relationship between 510/440 nm fluorescence ratio and percent muscle in mixtures of comminuted gristle and muscle.

Figure 5. Relationship between 510/440 nm fluorescence ratio and percent longissimus muscle in mixtures of comminuted longissimus and shank muscles.

Figure 6. Relationship between 510/440 nm fluorescence ratio and number of gristle particles in the sample aperture.
opened as a method for measuring the gristle content of meat under industrial conditions. Some of the salient features of this proposed method are discussed below.

Firstly it is necessary to attempt to identify the most useful application of macroscopic fluorimetry. The great sensitivity of the method to small particles of gristle (figure 6) suggests that a conventional optical system in the incident-light mode (such as that shown in figure 1) would be more effective than a fiber-optic system (as used in the initial research; Swatland, 1987a). With optical fibers directly in contact with the meat sample, it might be difficult to estimate the overall gristle content from the chance occurrence of gristle in the small area of tissue examined. The system shown in figure 1 enabled the fluorescence of a relatively large area to be integrated. Thus, the most useful application of the method might be to measure the gristle content of ground beef or comminuted tissue mixtures used for further processing. In the first case this could be for the purposes of quality control, while in the later case information on gristle content might be used to control the connective tissue content of certain products. The ratio of myosin to collagen is a critical factor in emulsion formation in many types of meat products (Pearson and Tauber, 1984), and a continuous measurement of Type I collagen content might be used for least-cost formulations.

For industrial use, the system shown in figure 1 could be reconfigured to have three short and roughly parallel light paths—one for incident excitation and two for measuring fluorescence emissions at around 440 and 510 nm. Exactly how the system should be used and how the results should be interpreted call for further research and some improvement in our understanding of exactly what is being measured. However, further studies with microscope fluorimetry confirm that the fluorescence originates from connective tissue fibers (Swatland, 1987b).

The fluorescence of tryptophan, tyrosine and phenylalanine (Konev, 1967) and pyridinoline (Nakano et al., 1985) occurs at lower wavelengths than those used to excite fluorescence in the research reported here. Thus, it is likely that most of the fluorescence observed here originated from hydroxyproline. Although it is possible that differences in the amino acid composition of the alpha chains that compose Types I and III collagen might be responsible for differences in the fluorescence emission spectra of these two types of collagen, a more simple and, hence, a more likely explanation is that fluorescence emission spectra are influenced by collagen fiber diameter.

Fibers formed from Type I collagen typically have much larger diameters than those formed from Type III collagen. Furthermore, a major technical difficulty in measuring fluorescence is that fluorescence tends to fade, often very rapidly. Reference to figure 1 will reveal that there was no high-speed shutter between the primary illuminator and the specimen. Putting these facts together can give rise to the following speculation. When strong ultraviolet illumination is used to excite fluorescence in meat, perhaps what happens is that the fluorescence of small-diameter fibers is rapidly quenched, whereas the quenching of fluorescence in large-diameter fibers proceeds more slowly as successive outer layers fluoresce and then fade. Thus, after a certain degree of exposure to ultraviolet light, relatively large fragments of gristle may continue to fluoresce with a peak around 440 nm, while small-diameter collagen fibers may lose their emissions around 440 nm and only produce the much weaker post-fading spectrum that peaks at around 510 nm. Experimental evidence in support of this hypothesis is shown in figure 7. Figure 7 shows the drift in the values of the 510/440 nm fluorescence ratio on repeated remeasurements (r = .95, P<.005) and gives some idea of the error of measurement of the apparatus. A tentative explanation of this increase in the value of the ratio is that the demoninator is decreasing in magnitude (in other words, the 440-nm peak of large-diameter collagen fibers is slowly fading).
Whatever the cause of the phenomenon shown in Figure 7, it is of obvious importance in any future industrial use of the method because the degree of exposure to ultraviolet light before measurement would have to be tightly controlled. At the other extreme, the data in figure 7 suggest that a high-speed electronic flash illuminator might initially induce similar pre-fading spectra from both Types I and III collagen. Although further research along these lines is needed, the results already available show that macroscopic fluorimetry might be useful for measuring gristle in meat.

Literature Cited


