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Sarcomere Length and Meat Quality

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Since the report by Locker (1960) that tenderness in beef is influenced by the degree of muscular contraction a number of workers have investigated the correlation between sarcomere length, as a measure of the contractile state of a muscle, and tenderness. The latter may be determined subjectively by taste panel assessment, or objectively by texture measuring instruments. The influence of contractile state on texture is seen most forcibly in the phenomenon of 'cold-shortening' (Locker and Hagyard, 1963; Marsh and Leet, 1966), and in the effect on a number of muscles of the posture of the carcass during rigor mortis (Herring, Cassens and Briskey, 1965; Hostetler et al, 1970).

Although there are several contributing factors to the textural state of a muscle as meat, a dominant one is the connective tissue content. This may override the effect of variation in other factors, as has been shown in attempts to correlate sarcomere length with tenderness in muscles having different connective tissue content, (Locker, 1960; Cooper, et al, 1969; Hostetler, et al, 1970). In the longissimus dorsi muscle, for instance, where the connective tissue content is relatively low, a significant correlation between sarcomere length and tenderness has been reported, but in the semitendinosus muscle, with a higher connective tissue content, change in sarcomere length did not produce a significant change in texture.

If the contractile state of a muscle is to assume any importance in determining the quality of muscle as meat then it is desirable that a simple, yet reliable method of measuring sarcomere lengths be employed. Sarcomere lengths have been measured in a variety of ways. The preparation of thin sections of embedded tissue followed by the application of a suitable staining technique makes it possible to observe and measure sarcomeres, using a light microscope at an appropriate level of magnification. The spacing between adjacent Z-discs, defining the sarcomere, can be measured against a calibrated eyepiece graticule. Phase-contrast microscopy of unstained preparations also reveals the detailed striation pattern of the muscle fibre. A more sophisticated method of measuring the spacings between Z-discs is to record the image photographically and then to obtain a densitometric trace from the negative. The distance between peaks representing the Z-discs in the photographic image represents the individual sarcomere length. These methods are accurate but time consuming.

An alternative method is to prepare a suspension of myofibrils from the muscle sample. A drop of the suspension may be examined by phase-contrast microscopy and measurements of sarcomere lengths obtained. This method is more convenient than those requiring preparation of tissue sections, but it has been shown that mechanical disruption of the fibres produces a shortening of the sarcomere, probably in the I-band, (Rome, 1967).

A method currently in use at the Meat Research Institute derives, initially, from the observations of Ranvier (1874) that a striated muscle acts as a transmission grating when placed in the path of a beam of light. Diffraction patterns are formed on a screen, the separation of the lateral orders of the diffraction pattern being determined by the contractile state of the muscle. This concept has been applied by several workers who reached a variety of conclusions about the structure of muscle and in particular, the nature of the striations observed under the microscope. However, in the light of more recent knowledge of the fine structure of muscle fibres the significance of the diffraction patterns is more clearly understood. A recent development in this concept is the use of a gas laser as a source of coherent, monochromatic light. This innovation was first applied to optical diffractometry by Klug and De Rosier (1966) in their work on the analysis of electron microscope images. The use of a laser in determining sarcomere spacings in muscle fibres has been reported by Cleworth and Edman (1969) in observing changes in sarcomere length during isometric tetanic contractions, and by Rome (1967) in her work on the filament lattice of rabbit psoas muscle.

The apparatus consists, very simply, of a He-Ne laser of low power (1 mW) which emits radiation of wavelength λ = 632.8 nm. This is mounted on an optical bench along with a specimen-holding device and a screen. For additional convenience, a lens system is inserted between the laser and the specimen. This enables the beam to be directed precisely on to the fibre bundle in the specimen holder. The screen, which consists of a vertically mounted white card bearing a central millimetre scale, may be replaced by a piece of photographic film or paper. A permanent record may then be made of the diffraction pattern given by the specimen being examined. It is useful to incorporate a shutter on the optical bench if such photography is to be carried out and, of course, it will be necessary to set up the apparatus in a darkroom.

In order to determine the spacing of the grating elements, i.e. sarcomeres, giving rise to the lateral bands in the diffraction pattern the following parameters must be determined :

θ_n = angle subtended at the screen by n^{th} order diffraction band

λ = wavelength of radiation

It can be shown that

$$d \text{ (sarcomere spacing)} = \frac{n\lambda}{\sin \theta_n}$$

Since for small angles the sine approximately equals the tangent of the angle, tan θ can be substituted into the above expression. Then

$$\tan \theta = \frac{\text{Separation between } 0^{th} \text{ and } n^{th} \text{ order } (S)}{\text{Distance between specimen and screen } (D)}$$

$$d = \frac{n\lambda}{\tan \theta} = \frac{n\lambda D}{S}$$

n can conveniently be unity, D and S are expressed in mm., and λ is known, so that

$$d = \frac{632.8 \times 10^{-3} \times D}{S} \quad \mu m$$

It is also advantageous to make D constant. d is then proportional to $\frac{1}{S}$. Once the apparatus is set up under standard conditions, values of d (i.e. sarcomere length) may be read off from a table or a straight line graph for the relevant value of S.

Specimen Preparation

A thin slice of tissue from the muscle sample to be examined is cut with known orientation of the fibres. This is fixed for about 30 minutes in a buffered glutaraldehyde solution in order to facilitate the teasing of a fine bundle of 2-3 fibres. Samples which have been subject to cooking do not require this preliminary fixation. Comparison of sarcomere lengths in parallel samples of fixed and unfixed material shows that the fibres are not adversely affected by the action of the fixative. The fine bundle of fibres is mounted in a drop of buffered sucrose between two glass coverslips which are held in the perspex specimen block. If pre-rigor muscle is to be examined the usual precautions should be taken to avoid contraction of the fibres on excision, e.g. fixing of muscle length by tying a strip of tissue to a perspex splint.

With the specimen block mounted in its holder on the optical bench, the specimen is aligned in the laser beam to give a horizontal array of diffraction bands on the screen. This is achieved when the bundle of fibres is perpendicular and normal to the beam.

The resulting pattern represents an average of all the unit spacings illuminated by the beam, which has a diameter of about 1 mm. If there is a high degree of uniformity in these spacings the pattern will be one of well-defined, fairly thin bands. If there is a wide range of unit spacings the bands will be more diffuse. It will not be possible to get an ordered diffraction pattern if the fibres in the teased bundle are in disarray.

This method is of particular value when sarcomere length measurements are required on a large number of samples. It provides only limited information, however, about the morphology of the fibres. For this an examination of stained sections by conventional methods of microscopy is necessary.

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