3:9 Sarcomere length measurement by laser diffraction and light microscopy

VANDENDRIESSCHE[*], F., BUTS, B., CLAEYS, E., DENDOOVEN, R. & DEMEYER, D.

Laboratorium voor voeding en hygiëne, University of Ghent, B-9230 Melle, Belgium
[*] Meat Proc. Plant Imperial, B-9920 Lovendegem, Belgium

Introduction.

With the discovery of the cold shortening phenomenon (Locker & Hagyard, 1963) and the associated toughening of the meat, measurement of the contractile state of the myofibril (sarcomere length) gained considerable interest. The traditional sarcomere length measurement, using a light microscope equiped with a calibrated eyepiece micrometer is accurate but rather time consuming. A more convenient method, first presented by Voyle (1971), consists of the illumination of the muscle fibre by coherent monochromatic light (Laser) to obtain a diffraction pattern. The muscle fibre acts as a diffraction lattice with the sarcomere lenght as the lattice constant. However as discussed by Rudel & Zite-Perenczy (1979), sarcomere length by means of laser diffraction is not as straightforward as believed. With a normal incidence of the laser beam not all of the illuminated myofibrillar clusters will contribute to the diffraction proces, but only a small proportion of them, corresponding to the Bragg condition : $2 d \sin \alpha = k \lambda$ (d = sarcomere length, α = glancing angle between incident light and lattice plane, k = diffraction order, λ = wave length). Possibly because of this reason, this method may give results differing from those obtained by light microscopy (Varcoe & Jones, 1983), especially after electrical stimulation (George et al., 1980).
We have compared both methods in routine analysis.

Material and Methods.

Animals and muscles:

One year old bulls were slaughtered in the slaughterhouse of our laboratory and the carcasses were treated to obtain differences in contractile state due to rapid cooling (cold shortening) or electrical stimulation (Demeyer & Vandendriessche, 1980).
Muscle samples (n=73, 1-2 g, 3x2x2 cm^3) were taken at various times (up to one week at 2°C post mortem) from Longissimus dorsi 1st-3rd thoracic rib (12 samples, LD1) and 6th-7th rib (8 samples, LD2), Infraspinatus (8 samples, TF), Gastrocnemius (9 samples, G) and occasional other muscles (16 samples). Samples were fixed in glutaraldehyde (see below) and sarcomere length (SL) determined by light microscopy and laser diffraction. For 41 samples (LD1, n=7; LD2, n=8; ST, n=8; ST, n=8; IF, n=8; G, n=6) the SL was also measured on fresh samples with the light microscope.

Sarcomere lenght measurement:

a. Preparation of samples:

- For measurement on fresh muscle (microscope) 1 or 2 g of fresh muscle was minced with a knife and gently homogenized with an Ultra-Turax (type TP 18/10 Janke and Kenkel, KG Staufen, BRD) in circa 20 ml of a buffer solution of pH 7.6 (0.25 M sucrose, 0.05 M Tris, 1 mM EDTA and HCl for pH adjusting) Davey & Gilbert (1974).
- For measurement after fixation (microscope and laser) 1 or 2 g of fresh muscle was fixed during 2 hours in a solution containing 0.1 M KCl, 0.039 Boric acid and 5 mM EDTA in 2.5% glutaraldehyde and then transferred to a solution of 0.025 M KCl, 0.039 M Boric acid and 5 mM EDTA in 2.5% glutaraldehyde (Laser manual).

b. Measurement:

- light microscopy: SL of the suspended myofibrils were measured using a Reichert-Biovar (Austria) light microscope equiped with a calbrated eyepiece micrometer (Magn. x 1250). For each sample 5 to 15 sarcomereswere observed (depending on their length).
- laser diffraction : Within 48 h from sampling SL was determined, from the measuring distance (2T in mm) between first order diffraction lines obtained after illumination of a muscle fiber with a Spectra physics n.145-02, 1.5 mW He-Ne Laser 632.8 nm (Spectra physics, Mountain View, USA) fixed in vertical position. From the Bragg condition it can be calculated that the sarcomere length (µm) is given by :

$$SL\ (\mu m) = \frac{0.6328 \times D \times \sqrt{(T/D)^2 + 1)}}{T}$$

D : Distance in mm between the muscle fibre and the screen.

From each muscle 20 different measurements of 2T were obtained from one fixed sample.

Results and discussion :

For the fixed samples SL, from laser diffraction for LD1 (1.21-3.24 µm), LD2 (1.69 - 2.09 µm), ST (1.83 - 2.91 µm), TF (2.22 - 3.63 µm) and G (1.98 - 3.57 µm) were not significantly different from values derived from light microscopy. With IF however laser diffraction gave significantly (paired t-test $p < 0.05$) higher values (Mean value : 2.11 µm) compared to light microscopy (Mean value : 1.97 µm).
Linear regression analysis of the microscopic (Y) versus the laser values (X) for all data (n=73) gives the following ewuation :

$$Y = 0.36 + 0.80\ X \quad (r=0.91,\ rsd=0.19)$$ (figure 1)

The regression slope and intercept differ significantly ($p < 0.05$) from 1 and 0 respectively. The net result of this regression is an overestimation (or overprediction) of sarcomere length by the laser method. These results are similar to those found by Varcoe & Jones (1983) who compared laser on fixed an microscopy on fresh muscle. An explanation for the deviation of the slope from unity and the intercept from zero may be found in the following : As is mentioned by e.g. Savell et al (1978) sarcomeres may appear in some particular cases (electrical stimulation, supercontraction) as periodical contraction nodes or zones which, using the laser method, could be erroneously seen and measured as sarcomere lengths. However if the periodicity of those contraction zones differs sufficiently from the periodicity of the sarcomeres, little or no problem will arise in sarcomere length measurement. Also the diffraction pattern obtained may appear in some cases as diffuse, indicating that a large range of sarcomere lengths were illuminated by the laser beam. Such a diffuse pattern would cause some difficulties in measuring the 2T value, as a mean value for all the illuminated sarcomeres. With a laser beam of 1.5 mm diameter more then 1000 sarcomeres are illuminated. So the sarcomere length, calculated as a mean of 20 different fibres of the same muscle, is based on illumination of about 2.10^6 sarcomeres, whereas using the microscopic method the data are based on the observation of maximum 300 sarcomeres. While the laser method is easier and more simple to operate it is not possible to provide as many details about the morphology as a microscopical examination will do.
For individual muscle fibers however Varcoe & Jones (1983) found an almost perfect agreement between the two methods of estimating sarcomere length. They therefore concluded that the laser method to determine sarcomere length was only accurate when individual fibres were compared.
To obtain information on how laser sarcomere length and microscopic SL measurements (on fixed muscle) are related when changes in sarcomere length were considered, changes in sarcomere length were calculated as the ratio between the values obtained for carcass sides treated to induce various degrees of shortening (e.g. hot-boning, electrical stimulation etc...) as described by Demeyer & Vandendriessche (1980) and the value obtained for the same muscle of the opposite control side.

Such ratios were calculated using results obtained by laser diffraction and by ligth microscopy on the same fixed samples (35 ratios obtained from 35 paired measurements). Linear regression of microscopic ratios (Y) on laser ratios (X) gives the following equation :

$$Y = 0.14 + 0.87\ X \quad (r=0.83,\ rsd=010,\ n=35)$$

with intercept and slope not significantly ($p < 0.05$) different from 0 and 1 respectively. So it is allowed to fit the regression through the origin (Snedecor & Cochran, 1978). This results in the following equation : $Y = 1.02\ X$ ($r=0.82$, $rsd=0.10$, $n=35$) (figure 2).

From this equation it can be concluded that the laser method does not differ from the microscopic method in measuring changes in SL induced by various treatments, although laser diffraction may give erroneous absolute values. For measuring SL changes the laser diffraction method does save time compared to oil immersion microscopy.

As mentioned in the material and methods section the sarcomere length determination was also performed with the microscope on fresh samples for 41 of the 73 samples. This makes it possible to compare the accuracy of the three methods (table 1).

Table 1 : Mean variation coefficients (VC) of the different methods used to determine SL (on 41 different samples).

Method	Laser Diffract.	Microscope	
	fixed samples	fixed samples	fresh samples
Mean VC (%)	5.18	7.16	8.91
SE on Mean	0.23	0.42	0.52
Range	1.6 - 10.2	4.0 - 17.1	4.7 - 18.1

Variation coefficients obtained using both microscopical methods differ very significantly ($p < 0.001$, paired t-test) from those derived from the laser method, and they differ significantly ($p < 0.05$, paired t-test) from each other. From this table it can be concluded that the laser method is more accurate (significantly lower variation coefficients). The above variation coefficients are comparable to those found by Cross et al (1981), who concluded that using the laser method less measurements (34) are required to assure a 99 % precision than with the microscopical method (45).

This fact in combination with the above information may lead to the conclusion that laser diffraction can be recommended for measuring changes in SL induced by different treatments, although the method is not suitable to measure the correct SL value itself.

Aknowledgements :

The authors are grateful to Mrs A. Beets and D. Baeyens for carrying out the measurements. This work was sponsered by the IWONL, Brussels.

References :

CROSS, H.R., WEST, R.C. & DUTSON, T.R. (1981). Meat Sci., 5, 261.
DAVEY, C.L. & Gilbert, K.V. (1974). J. Food Technol., 9, 51.
DEMEYER, D. & VANDENDRIESSCHE, F. (1980). Ann. Technol. agric., 29,635.
GEORGE, A.R., BENDALL, J.R. & JONES, R.C.D. (1980). Meat Sci., 4, 51.
LOCKER, R.H. & HAGYARD, C.J. (1963). J. Sci. Food Agr., 14, 787.
RUDEL, R. & ZITE-FERENCZY, F. (1979). Nature, 278, 573.
SAVELL, J.W., DUTSON, T.R., SMITH, G.C. & CARPENTER, Z.L. (1978). J. Food Sci., 43, 1606.
SNEDECOR, G.W. & COCHRAN, W.G. (1978). Statistical Methods 6th ed. Iowa State Univ. Press. Ames Iowa, USA.
VARCOE, G. & JONES, S.D.M. (1983). Can. Inst. Food Sci. Technol. J., 16, 82.
VOYLE, C.A. (1971). Proc. 17th Europ. Meet. Meat Res. Workers, Bristol, p. 95.

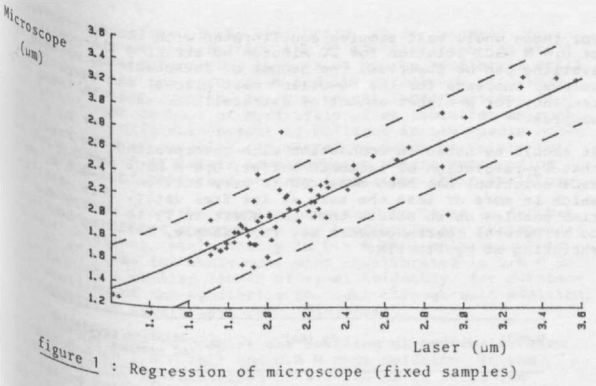

figure 1 : Regression of microscope (fixed samples) on Laser diffraction

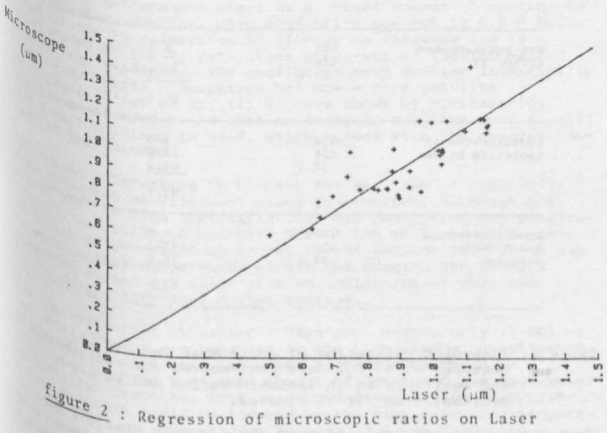

figure 2 : Regression of microscopic ratios on Laser ratios