FLUORIMETRY AS A POTENTIAL METHOD FOR MEASURING THE TOUGHNESS OF CONNECTIVE TISSUE

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SUMMARY

Perimysial sheets from raw masseter were dissected and their fluorescence emission spectra (345-510nm) determined. The same sheets were afterwards stretched to breakage and three tensile parameters were calculated. The fluorescence intensity, single readings at particular wavelengths or the complete spectrum, was related to the three different tensile parameters. The major source of variation, up to 87%, in two of the tensile parameters could be explained by the fluorescence spectra. A better model seems to need more accurate tensile measurements as well as inclusion of data on connective tissue thickness and light penetration depth.

Introduction

Tenderness is the most important parameter that determines the commercial value of beef. Methods that non-destructively determine beef tenderness are therefore searched for. Such methods need to be sensitive to several phenomena; myofibrillar contraction state, fiber thickness, connective tissue content and physical properties, and so further. It may prove difficult to find one single method that can give the needed information sufficiently accurate. Fluorimetry is a very sensitive, rapid and non-destructive technique which can provide information about connective tissue and fat, but will probably not be able to include all the complex phenomena needed to determine beef tenderness. Thus its potential, provided its information content technique is unique, may be as a supplement to other methods. Fluorimetry has previously been shown to determine the gristle content in comminuted beef systems (Swatland, 1987). In the present paper we present a first approach to determine the tensile properties of dissected *perimysial* connective tissues.

Materials and methods

Materials: The *masseter* muscle was excised 30 min *post mortem*, and then kept at 15°C for 24 hrs. Samples (5 x 2 x 1 cm, pH 6.15) were then vacuum packed in small bags, frozen in liquid nitrogen and stored at -80°C, later thawed at 4°C a few hrs before analysis.
Dissection of perimysium: 33 different *perimysium* sheets were dissected from the above samples under a stereomicroscope.
Fluorescence analysis: The slices (Figure 1) were positioned between 2 quartz plates and installed in the Front surface Accessory of a Perkin Elmer LS-50 Luminescence Spectrometer. The reflectance emission spectra was recorded using excitation at 335nm and at 26±0.5°C. A small drop in signal intensity over time was estimated from measurements on standardized blocks, and linearly corrected for.
Tensile measurements: The samples were dumbbell shaped (Figure 1) by a bent razor blade knife. The procedure used was a modification of that used by Lewis and Purslow (1989). The sample was fixed to arterial grips fitted into a Texture Analyzer (Model TAXT2, Stable Micro Systems, Haslemere, Surrey).
Statistics: Principal component regression(PCR) was performed on the Unscrambler Software (Camo, Trondheim). To validate the models, full cross validation was used.

Results and discussion

Figure 2 shows the two most extreme fluorescence emission spectra. The increase in fluorescence intensity (I_f) towards 340nm is due to excitation light tailing. The other spectra, of intermediate intensities (not shown), have shapes in agreement with Figure 2 although the relative intensity ratio $I_{f,380nm}/I_{f,470nm}$ might vary. Figure 2a

indicates that the presence of connective tissue enhances the fluorescence intensity at about 380nm, and in particular at about 470nm.

Figure 3 gives the two most extreme tension curves. In panel 3a the parameters breaking load, slope and 'toe' are defined. The 'toe' and slope decreases and increases, respectively, with increasing breaking load. The range observed for the breaking loads can be seen in Figure 4.

The two maxima in fluorescence intensity, between 350-510nm, were correlated to the three tensile parameters by univariate analysis. The experimental parameters were also transformed into logarithmic values before the regressions were performed. The correlation coefficients are given in Table 1. All correlations were significant on a 1% level. However, strong correlation ($R > 0.9$, explained variance > 81%) was only observed between breaking load and the fluorescence intensity determined at about 470nm. When the whole spectra between 345-510nm was used, and a multivariate analysis (PCR) performed, the correlation coefficients increased for breaking load and slope but not for 'toe'.

A simple approach to a theoretical understanding of the relationship between tensile force (F) and fluorescence intensity (I_f) can be given as follows. The tensile force (F), here best related to the slope, is a function of the sample's crossectional area ($A = l_t \times$ width, l_t is the thickness of the connective tissue) as well as the concentration of loadbearing compounds (c_t) by the equation: $F \propto l_t (c_t)^n$ where $n \geq 2$ (Ross-Murphy, 1984). The fluorescence intensity, in the linear range, is related to the concentration of fluorescent compounds (c_f) and optical path length (l_f) by the equation: $I_f \propto l_f c_f$ (Guilbaut, 1989).
The condition of interest here is obviously were $c_t \propto c_f$, where we have: $F \propto l_t (I_f/l_f)^n$. This equation suggest a nonlinear relationship between tensile force and the fluorescence intensity, also after a log transformation of F and I_f. Only for the case where measurements of l_t are included in the model, and l_f is a constant, should a linear relationship between logarithmically transformed values of (F/l_t) and I_f be expected. Neither l_t nor l_f was measured in this first approach to look at the relationship between tensile parameters and fluorescence intensity. Such measurements will be of interest to future experiments. Non-linearity is not evident upon visual inspection (see Figure 4), ignoring $l_t l_f^{-n}$ might therefore, for these data, not induce non-linearity. However, the range spanned here with respect to the mechanical properties of connective tissue are quite large, and thus non-linearity was expected.

High accuracy is needed for detecting the exact shape of the relationship between the tensile parameters and the fluorescence intensity. A final issue to be raised therefore is about the accuracy of the parameters used for calculation, in particular for the tensile parameters which are the most difficult ones to determine. For weak *perimysial* sheets our standard error of the mean is estimated, by comparing sheets with practically equal I_f, to be at least 15% and for the stronger sheets around 10%. These values compare with error estimates given by Lewis and Purslow (1989) and Oxlund and Andreassen (1980) who have performed similar measurements. This magnitude for the error suggests that the type of relationship between tensile parameters and fluorescence intensity should not be overinterpreted, and that the maximum variance explained in these preliminary results in fact satisfactory.

Conclusion

The major source of variation (up to 87%) in the tensile parameters breaking strength and slope can be modelled by fluorescence analysis. Thus fluorimetry should be further investigated with respect to predicting the mechanical properties of connective tissues. Theoretically a better model should be obtained by including measurements of the thickness of the connective tissues as well as the light penetration depth. However, it presently seems that a better model also depends on more accurately determined tensile parameters.

References

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Legends for figures

Figure 1. The shape of the sample used for fluorescence and tensile measurements. The surface subjected to fluorescence measurements, dotted area, was released of muscle fibre fragments on one side only (sample thickness about 2mm). The sample's backside, in the narrow region, was released of muscle fibers before tensile testing leaving only connective tissue to become load bearing.

Figure 2. The emission spectra obtained after excitation at 335 nm. Panel a, lower curve, shows a system not containing visible connective tissue and the upper curve shows the connective tissue having the lowest fluorescence intensity of the population used. Panel b shows the emission spectra of the connective tissue with the population's highest fluorescence intensity.

Figure 3. Panel a and b show tension curves for the population's weakest and strongest connective tissue, respectively.

Figure 4. The figure shows the range spanned for the breaking loads, as well as the relationship between measured and predicted breaking loads.