Rapid determination of connective tissue content in minced *longissimus dorsi* muscles by macroscopic fluorescence emission spectroscopy

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Introduction

Connective tissue (CT) is an important constituent of meat describing quality and relates to tenderness and texture. Today's methods for connective tissue extraction are tedious and demand use of several chemicals. Thus, a rapid measurement is desired. Swatland (1987) suggests that the autofluorescence spectrum can be used to monitor the connective tissue in meat slurries. In this preliminary study macroscopic fluorescence emission spectroscopy has been evaluated as a possible method for connective tissue determination in minced meat. The results from two different excitation wavelengths (335 nm and 380 nm) were compared.

Materials and methods

Non-stimulated longissimus dorsi muscles were collected at random from 20 animals at the slaughter house 2 days post mortem. Two adjacent cross section slices of about 200 g were cut from approximately the middle of the muscle. The slices were separately homogenised by first using a *Moulinex Masterchef 20* for 20 sec. and then a *Krups homogeniser* for 20 sec. to obtain a fine structured mince. One mince was used for CT extraction, the other for fluorescence measurements.

Fluorescence emission spectra were measured by an optical bench system (Figure 1). The two excitation wavelengths were generated by a xenon light source, a heat filter and 10 nm bandwidth excitation filters (335 nm and 380 nm). The excitation wavelength 335 nm was not chosen as an optimum for determination of CT, but seemed like a feasible wavelength from various previous work on meat (Egelandsdal et al., 1996) and was close to 340 nm which generate CT fluorescence (Munch, 1989). 380 nm was considered as appropriate although 370 nm has been found to be the excitation maximum for connective tissue (elastin, type I collagen) (Swatland, 1987). The light was guided onto the samples through a 50 mm diameter aluminium tube at an angle of 45°. The mince was crammed into sample cups exposing a flat circular surface area of 20 cm^2 for measurement. The detector was an *Acton SP-150* imaging spectrograph connected to a 512*512 *Princeton* CCD camera (Charge Coupled Device). The CCD was UV-coated to enable sensitivity in the spectral area 200-1100 nm. Further more it was back illuminated (reducing signal loss due to absorption) and cooled to -40°C resulting in a very low dark charge. The fluorescence spectra were obtained with an exposure time of 10 sec. and resulted from adding (binning) the signal from 300 lines of the CCD. The measurements were performed at room temperature in a laboratory with a minimum of disturbing straylight.

The amount of connective tissue determined as hydroxyproline was performed in duplicate and triplicate using *Technicon Auto Analyzer II* (NL 142-78 F). The mean standard deviation of the CT determinations was 0.060.

Multivariate calibration (partial least square regressions) were carried out using the *Unscrambler 5.0* software (Camo AS, Trondheim). The parameters root mean square error of prediction (RMSEP) and multivariate correlation were used to evaluate and compare the models.

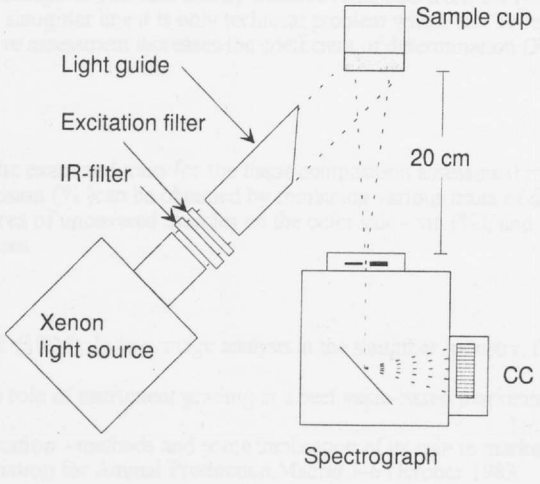

Figure 1 System for macroscopic fluorescence spectroscopy.

Results and Discussion

It is well known that connective tissue is one of the major contributors to the fluorescence emission signal in meat (Swatland, 1987). It can, however, be difficult to distinguish differences in emission spectra due to changes in connective tissue content because fluorescence emission from components in fat are dominating the spectra. Figure 2 shows emission spectra from minced beef for each of the excitation wavelengths. The curves marked "high fat" are signals from a sample with high fat (8 %) and high CT (0.8 %) content. The "low fat" lines are from a sample with low fat (0.5 %) but still high CT (0.9%). For the excitation 335 nm there are no clear changes in spectra due to differences in the amount of CT. An increase in fat content on the contrary gives rise to prominent increase in emission intensity in the range 420-540 nm. According to Swatland (1987) elastin and type I collagen have a strong emission peak at 440 nm. This peak is strongly overlapped by the emission from fat. The excitation 380 nm gives rise to emission spectra with more detailed shapes, but are more difficult to interpret with regard to fat and CT content. Figure 2 and prior knowledge emphasise the necessity of applying multivariate regression to be able to resolve information about variations in CT content and to obtain a meaningful regression.

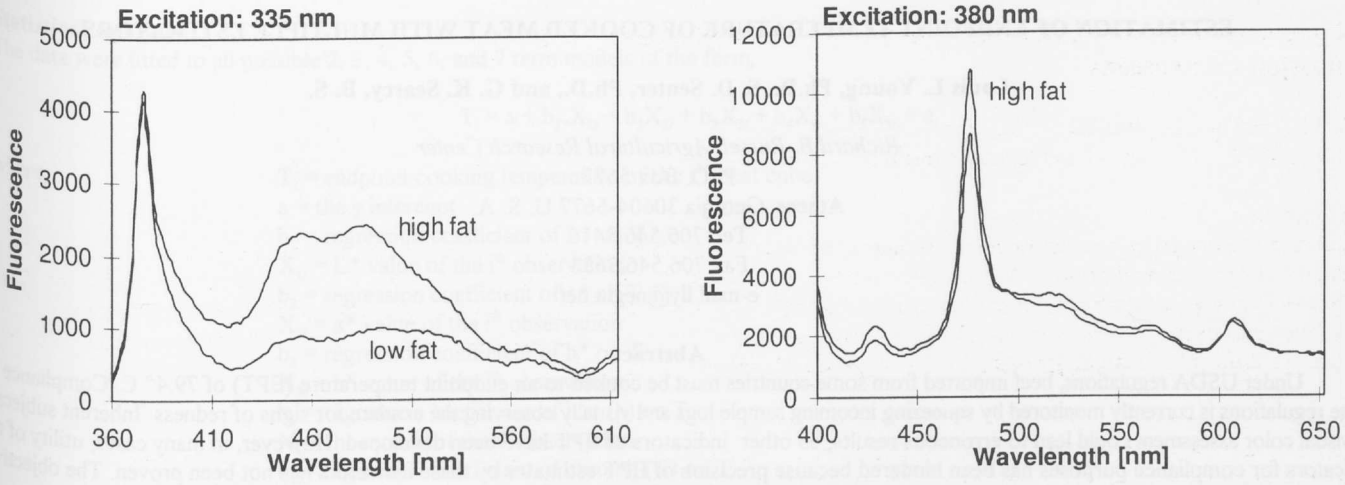

Figure 2 Fluorescence emission spectra from meat originating from excitation wavelengths 335 nm and 380 nm.

Figure 3 shows the relationship between measured and predicted amount of CT for the two excitations. For 335 nm a correlation of 0.81 and a RMSEP of 0.065 % were obtained. For 380 nm the relationship was poorer with a correlation of 0.66 and a RMSEP of 0.087 %. Both models were validated using full cross validation.

We would have expected a better result for 380 nm relative to 335 nm since it is close to the CT excitation maximum 370 nm. The great difference between the emission spectra arising from the two excitations (Figure 2) indicates that different excitations can be more or less selective to CT and/or that they generate fluorescence in different chemical components. That is, even if 370 nm is the peak excitation wavelength for CT it may not be the optimal excitation for this method, taking into account that fluorescence from fat and other constituents overlap. The method can probably be improved by optimising the excitation wavelength. A screening of different excitations would be appropriate.

A drawback with the method is that it is very sensitive to lamp intensity and homogeneity and structure of the samples. It will probably perform best on products in which the fat content is quite stable. A greater range in CT content would probably give a more stable model than we have achieved in this study. A suitable application would then for instance be to control the amount of CT in sausages which is not allowed to exceed 2 % (in Norway).

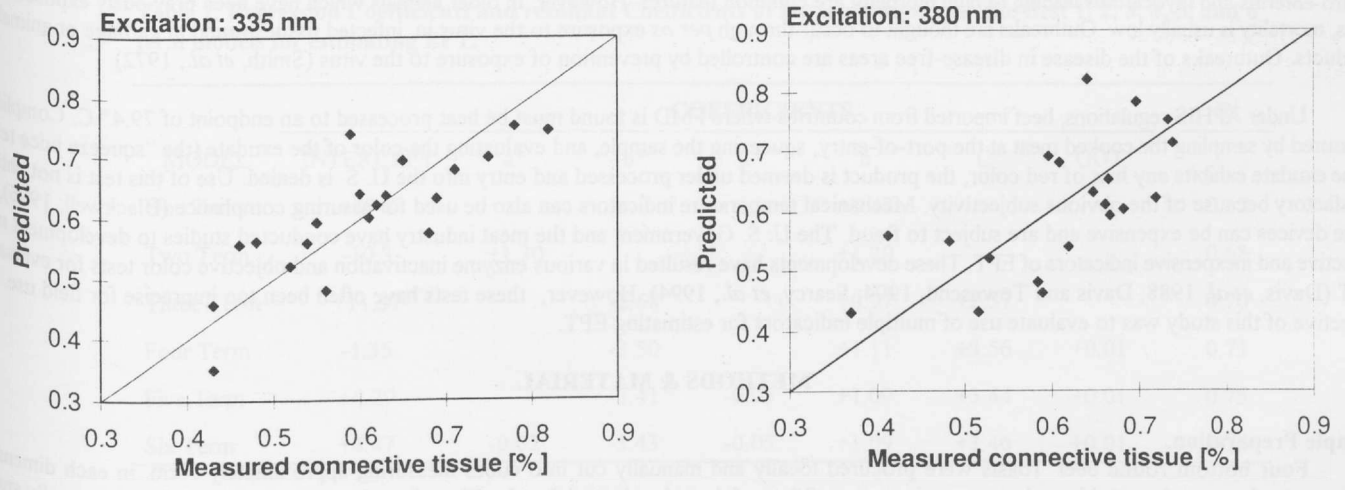

Figure 3 The relationship between measured and predicted amount of connective tissue using fluorescence emission spectroscopy.

Conclusion

The preliminary work performed here suggests that the technique of macroscopic fluorescence emission spectroscopy has potential as a rapid method for determination of connective tissue in minces of meat.

Acknowledgements

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References

Egelandsdal, B., Neegård, S., Sponnich, A., Enersen, G. and Kvaal K. 1996. Determination of the amount of intramuscular fat in beef - a comparison of two spectroscopic techniques. Poster proc. 42nd International Congress of Meat Science and Technology. 1-6 Sept.

Industrial method NL 142-78 F, Technicon Instruments BV, Rotterdam, Nederland.

Munck, L (ed.). 1989. Fluorescence Analysis in Foods, Longman, NY, USA.

Swatland, H. 1997. Measurement of the gristle content in beef by macroscopic ultraviolet fluorimetry. J. Anim. Sci. 65, 158-164.