ESTIMATING THE CONNECTIVE TISSUE CONTENT OF COMMINUTED COMMERCIAL BEEF AND PORK GRADES USING TWO DIFFERENT AUTOFLUORESCENCE METHODS

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Background

The amount of connective tissue in a particular meat grading determines its commercial value as the functional properties of connective tissue proteins differ substantially from those of the myofibrillar proteins. In addition, some countries, like Norway, still regulates by law the amount of connective tissue being allowed into some meat products. Therefore the connective tissue content of meat grades is frequently measured by the meat industry. The technique of autofluorescence has been suggested as a possible method for rapid measurements of the connective tissue content of meat (Swatland, 1987a, Swatland and Barbut, 1991, Wold et al, 1997, Wold et al, 1999). The meat systems used in these previous reports were not commercial meat grades but carefully chosen, albeit highly relevant, meat model systems. In addition, the instruments used for autofluorescence measurements were not commercial.

Objective

The objective is to report on the accuracy of quantitative measurements of connective tissue content (measured as hydroxyproline) using two commercial grades of beef plus one commercial grade of pork. The instruments used were an optical bench system (Wold et al., 1999) as well as a commercial instrument, BioView, sold by DELTA Danish Electronics, Light and Acoustics. The latter instrumentation has proved useful in on-line monitoring of fermentation processes (Grønkjær Pedersen et al., 2000).

Methods

Meat samples : Samples were coarsely chopped or muscle pieces of two manufacturing meats of beef and one of pork (fat contents, 14, 21 and 23%, respectively). The samples were stored at –30 °C for 4-5 months before being measured. The samples were then thawed, homogenized (Grindo mix RD 2000 ; 5-8 sec) and conditioned to 16-18°C. Totally 50 samples were measured for each grading.

Connective tissue measurements: Connective tissue was determined as hydroxyproline (NT FOOD 127/NMKL no 127:1988). Practical root square error is 0.13% (Wold et al., 1999); a figure impaired by the heterogeinity of the grade.

Optical bench (OB) system: The instrumentation (Wold et al.,1999) consists of a 300 W Xenon arc lamp (Oriel 6258, Oriel Corporation , Stratford, CT), a spectrograph (Acton SP-150, Acton research Corp., Acton, MA) and as detection camera (a cooled charged coupled device, TEA/CCD-512-TKBMI, Princeton Instruments Inc., Trenton, NJ). Intererference filters: 332 nm and 380 nm (bandwith 10 nm); cut-off filters; 360 nm and 400 nm. Illumination was at an angle of 45°.Wavelength resolution of emission spectra was 5 nm. Iluminated area: approx 20 cm^2. Irradiance approx. 4 $\mu W/cm^2$. Exposure time per sample was 10 sec .

BioView(BV) On-line Spectrofluorometer: In this case the exciting light is transferred from a Xenon flash lamp on to the sample through an optical cable. The instrument is a filter instrument and different filters are used on excitation and emission range (20 nm steps; exciting range: 270 nm – 550 nm ; emission range 310 – 590 nm). If excitation is at 330 nm, then 12 emission measurements are available. Measuring area: approx 20 cm^2. Irradiance approx. 2 $\mu W/cm^2$ (average power). Illumination was at an angle of 45°. The cable was positioned in a lightsealed container. The detector was a photomultiplier. Measuring temp 20°C. 2-4 measurements were made on each sample.

Statistics : The emission spectra(X) from the samples were used to predict the chemical measurements of connective tissue (Y). The multivariate calibration methods partial least squares (PLS) was used (Martens and Næs, 1989) as contained in the software package Unscrambler (version 7.5) from Camo AS (Oslo, Norway). Full crossvalidation was used; i.e. one sample was left out at a time. Prediction error is given as root mean squar error of cross validation (RMSECV).

Results and discussion

Table 1. Shows the span in connective tissue content of the three grades. The pork grade had the last variation in connective tissue content.

Figure 1a shows the emission spectra (OB system) for three samples containing 3% connective tissue drawn from the different grades. It is known that connective tissue is autofluorescent in this wavelength range (Swatland 1987b; Egelandsdal et al. 1996). These spectra have typical shapes for meat samples (Wold et al., 1999). Note that the emission intensity is the lower for the beef samples since those samples are less red. The peak at about 380 nm is largely due to the characteristics of the cut–off filter. The valley at about 420 nm is believed to be due to myoglobin absorption. **Figure 1b** shows comparable emission spectra for the excitation wavelength 330 nm using Bioview plus OB (latter with 20 nm resolution simulated). The spectra are featureless compared to the spectra in Figure 1a. The different in absolute shapes can largely be explained as due to differences in the response characteristcs of filters and detectors used.

Table 2. shows that the OB method generally gave the best prediction results. If the resolution using the OB method was reduced to 20 nm, i.e. to the resolution of BV, then the predictability of OB method was reduced but was still better than that obtained by the BV method. This is presumably due to the reduced irradiance obtained for the BV method.

For the beef systems (OB method) the predictability following excitation at 332 nm is the same or better than the predictability given by Wold et al. (1999). Excitation at 380 nm gave, however, poorer prediction results. The beef 21% and pork 23% systems gave lower correlation coefficients (not shown) than did the beef 14% system, presumed due to the more narrow ranges in connective tissue.

An unexpected high number of PLS components (up to 14) was needed to predict the connective tissue content of the samples compared to the number of components (1-4) used by Wold et al. (1999). The increased number of components is most likely due to the greater complexity of the systems studied here compared to the pure muscle system studied earlier. Work is in progress in our laboratory where we study the causes of the greater complexity for industrial relevant systems using designed samples.

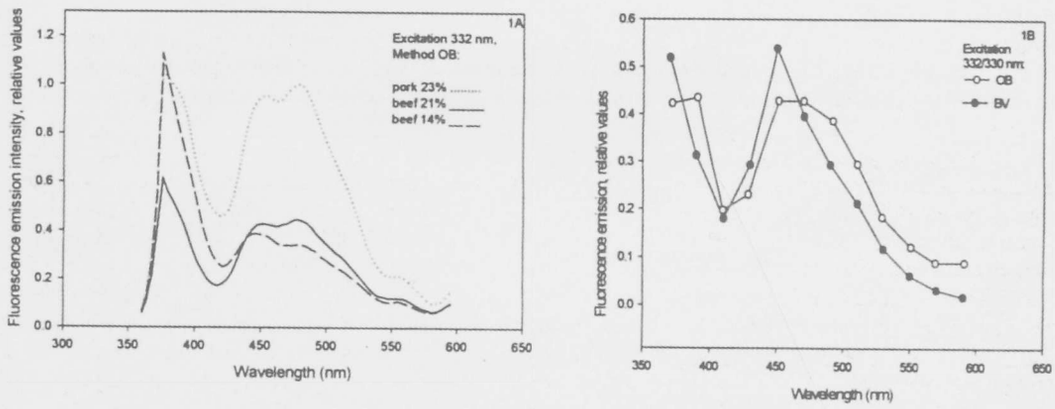
Table 1. The connective tissue contents of the three grades used.

Grades	Span(%)	Mean(%)
Beef 14%	**1.4-5.3**	**2.9**
Beef 21%	**1.8-5.2**	**3.7**
Pork 23%	**1.4-3.3**	**2.1**

Table 2. The results of the regression analysis using the two instrumental methods. In parenthesis: number of components equal to the BV method.

Grad-es	Instru ment	Resolu-Tion (nm)	Excita-tion (nm)	Opt. comps.	Corrrelation coeff., R	Pred. error (%), RMSECV
Beef 14%	OB	5	332	6	0.80	0.49
	OB	20	332	8	0.67	0.60
	OB	5	382	7 (4)	0.75 (0.48)	0.54 (0.72)
	OB	20	382	8 (3)	0.69 (0.42)	0.59 (0.74)
	BV	20	330	4	0.32	0.79
	BV	20	370	3	0.31	0.79
All	OB	5	332	12	0.82	0.56
	OB	20	332	9, (2)	0.80 (0.53)	0.60 (0.82)
	OB	5	382	14	0.81	0.58
	OB	20	382	6, (3)	0.67 (0.62)	0.72 (0.76)
	BV	20	330	2	0.56	0.80
	BV	20	370	3	0.58	0.79

Figure 1 (A and B). Emission spectra as a function og wavelengths.



Conclusion

The best prediction error for connective tisssue was 0.49-0.56%. The commercial instrument performed less well than the optical bench system most likely due to reduced resolution and reduced irradiance.

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