Investigating muscle fibre properties by FTIR microspectroscopy and micro tensile tests.

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Abstract

Muscle fiber properties of *m. Semimembranosus* of Charolais animals have been investigated as a function of cooking temperature and time. Protein secondary structural properties have been studied by FTIR spectroscopy and mechanical properties by micro tensile tests. Small muscle samples have been cooked in water bath for 2, 4 and 8 minutes at the temperatures 50, 60, 65, 70 and 80°C in a factorial design. For all the conditions mechanical measurements and FTIR microspectroscopic measurements of single myofibers have been performed. It is shown that both cooking time and temperature has an effect on the protein secondary structure and different parameters obtained through mechanical measurements. The mechanical parameters could be related directly to the shares of aggregated protein structures and native protein structures.

Materials and Methods

Heat treatment: The samples were excised from the *m. Semimembranosus.* Each sample was 6 x 6 cm and 2 mm thick. They were stored at – 80 ºC. Before heating they were thawed for 15 minutes in an 18 ºC water bath. Then they were heated at 5 different temperatures, 50, 60, 65, 70 and 80 ºC at 3 different times, 2, 4 and 8 minutes. Two replicates (2 samples) were used for each time-temperature treatment, in total 30 samples.

FTIR Microscopy: After heat-treatment the samples were taken out of the water bath, kept 1 min in room temperature, and then cooled to 18 ºC in water bath. Then the samples were cut into small pieces along the fibre direction and covered with O.C.T. compound (Tissue-Tek, Sakura Finetek, USA) and frozen immediately in liquid nitrogen. Until sectioning the samples were stored at - 80 ºC. Sectioning were carried out on a cryosat (Leica CM 3050 S, Nussloch, Germany) at – 22 ºC transversely to the fibres direction. 10 µm thick sections were prepared on infrared transparent 2 mm thick CaF2 slides for FT-IR microscopic measurements. An IR microscope (IRscope II) coupled to an Equinox 55 FT-IR spectrometer (both Bruker Optics, Germany) was used to measure the tissue sections. The microscope was equipped with a computer-controlled x,y stage. The Bruker system was controlled with an IBM- compatible PC running OPUS-NT software, version 6.5.

IR spectra were collected from single and multi myofibers in transmission mode from 4000 to 700cm^{-1} with a spectral resolution of 4 cm^{-1}. A mercury-cadmium-tellurium (MCT) detector was used. For each spectrum 128 interferograms were coadded and averages. A background spectrum of the CaF_2 substrate was recorded before each sample measurements in order to account for variation in water vapour and CO_2 level. In addition the instrument was sealed using a specially designed box.

Preprocessing of FT-IR Spectra: The spectra were pre-processed by using extended multiplicative signal correction (EMSC) and 2nd derivative. For the spectra analysis only the amide I region was used 1700 to 1600cm^{-1}.

Micro tensile tests: Tensile tests were carried out on a micro-tensile device developed at INRA. Muscle fibres where isolated mechanically under a stereo microscope and glued on aluminium frames. The fibres on the aluminium frames were then fixed on the micro-tensile device and the frame cut. During dissection and during the test the fibres were immerged in the liquid of cooking loss. The diameter of fibres is determined under a microscope at x 50 magnification.

The fibres were extended to fracture at a rate of 130 µm/s. The software allows the determination of initial modulus, threshold of elasticity, slope above the threshold, breaking stress, breaking energy and breaking strain

Data analysis

The FT-IR spectra and measurements from Micro tensile tests were averaged resulting in just one spectrum per experimental condition. Every data block, FT-IR data and Micro tensile tests, contained the sample spectra as rows and the variables as columns. The sample spectra were ordered in order to obtain a row to row correspondence between the two data blocks, i.e. the first sample spectrum in the FT-IR data is corresponding to the first sample spectrum in the Raman data and so on. The data were then analyzed by multiblock principal component analysis (MPCA)[1]. MPCA belongs to the group of multiblock methods that maximize a common variation pattern, while another group (e.g. canonical correlation analysis CCA) maximizes the correlation between block scores, and third group of methods - used for predictive purposes - which establishes causal models between blocks. The aim of MPCA for the analysis of spectroscopic multiblock data is to visualize variation that is common for the two data blocks, variation that is specific for every data block and to find co-variant interpretative structures between the different data blocks, i.e. to analyze, if certain interpretable bands in the FT-IR spectrum are correlated to certain interpretable bands in the Raman spectrum. The major advantage of using MPCA with respect to PCA, is that it is focused on the consensus in between two (or more) data blocks. The same components are referring to the same type of information. This gives the possibility to investigate how strong a common information is reflected by both data blocks and to visualize the interpretative bands in both data blocks that are contributing to this common variation. Correlations between the interpretative bands in FT-IR and Raman are visualized by correlation loading plots[1]. Data analysis was carried out using an in-house program written in MATLAB version 7.3

Results and discussions

The second derivative spectra are shown in the amide I region in Fig 1a for the 60°C samples and the 3 different heating times. Negative peaks refer to different spectral bands, since the second derivatives are shown. We can see that an increase in heating time leads to an increase of aggregated protein structures and a decrease of native structures. The assignment of the different bands is shown in Table 1.

Table 1. Assignment of spectral bands in the amide I region according to Böcker et al. (2007)[2]

1694		Aggregated β-sheet structures
1682		antiparallel β-sheet structures
1668	*Amide I* (80%	non- hydrogenated C=O groups
1659	C=O stretch,	loop structures
1653	10% C-N	α-helical structures
1639	stretch, 10% N-	antiparallel β-sheet structures
1628	H bend)	aggregated β-sheet structures
1619		aggregated β-sheet structures
1610		n.a.

In Figure 2 the correlation loading plot for the amide I region of FT-IR spectra (black), the measurements from Micro tensile tests (blue) and the design (green) is shown (For the correlation loading plots the global scores, obtained by MPCA of FT-IR and micro-tensile tests were used, the design was not included in the MPCA analysis and has therefore no influence on the global pattern). In the correlation loading plot, the outer circle is corresponding to 100% explained variance, while the inner circle is corresponding to 50% explained variance. Variables that have high explained variance and that are near each other are likely to be highly correlated. From the correlation loading ploit we see that denaturated protein structures are related to longer treatments and higher temperatures, while native protein structures are correlated to shorter heating times and low temperatures. We can see clearly that the mechanical measurements maximum stress, breaking strain and breaking energy are highly correlated to denaturated structures while modulus 2 is strongly correlated to native protein structures.

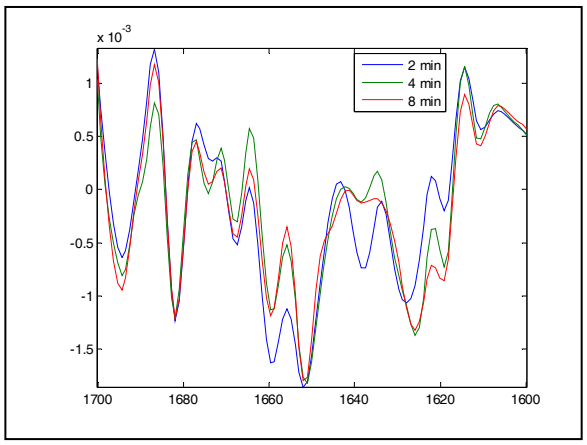

Figure 1a: FTIR Amide I for 60°C and three times.

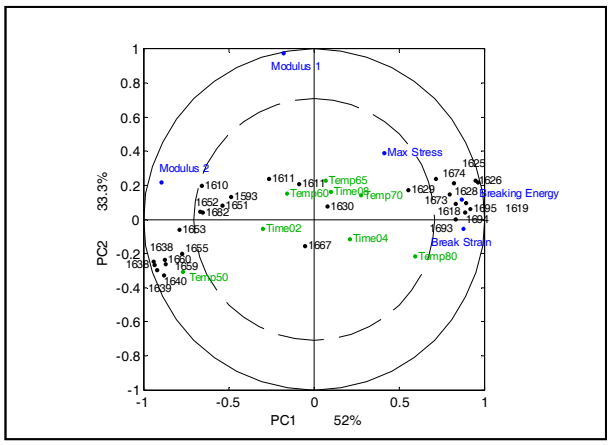

Figure 1b: Correlation loading plot

Conclusions

We have shown that FT-IR microspectroscopy and micro tensile tests can be used in combination to study how micro-mechanical properties of single myofibre and protein secondary structure are related. Micro tensile properties, associated with 'toughness' of single myofibers, are strongly correlated with denaturated protein structures. Modulus 2, the second slope of stress strain curves after the elastic limit, is related to native structures. This shows that stress-strain properties of single isolated musclefibres are related to their protein secondary structure.

Acknowledgements

Funding of this work by the grant ProSafeBeef of the European Commission under the Sixth Framework Programme and the Fund for the Research Levy on Agricultural products in Norway is greatly acknowledged.

References

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