FT-IR MICROSCOPY AS A TOOL TO INVESTIGATE PROTEIN SECONDARY STRUCTURAL CHANGES OF MUSCLE TISSUE IN INDUSTRIAL PROCESSES LIKE HEAT TREATMENT

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Introduction

FT-IR spectroscopy has traditionally been used within analytical chemistry for structural analysis of organic compounds. Since the 1990ies, its application has been extended to the characterisation of biological samples, amongst others intact biological cells such as microorganisms or single mammalian cells, and complex tissues. FT-IR spectra of biological samples display absorption bands due to vibrations of functional groups of proteins, polysaccharides, lipids, and nucleic acids. The use of FT-IR spectroscopy is thus providing information about the overall biochemical composition of the sample in one single measurement. FT-IR spectra of biological samples often contain a number of overlapping bands that may be difficult to interpret, but the spectral features of proteins, carbohydrates and lipids are generally separated well. As the position of spectral bands is governed by the molecular structure, intra- and intermolecular interactions and the amounts of the present molecules, FT-IR spectra can be used to evaluate the structure of the components in biological samples. By the invention of FT-IR micro-spectroscopy, a combination of spectroscopy and microscopy, it became possible to acquire spatially resolved FT-IR spectra, from slices of intact tissue (Wetzel *et al.*, 1999). FT-IR spectroscopy has become a very important technique in the investigation of meat and fish muscle tissue, since it offers a high specificity regarding the characterisation of protein secondary structure. Furthermore, FT-IR microspectroscopy allows the investigation of single muscle fibres and the spatial distribution of secondary structural modes within one muscle cell. In this paper we show the excellence of FT-IR for studying protein structural changes in meat tissue during heating.

Materials and Methods

Pork meat (*M. longissimus dorsi)* with high (6.5) and normal (5.4) pH, that was heated at 10 different temperatures (28.5, 34.0, 40.0, 46.1, 52.0, 57.6, 63.0, 69.3, 75.5 and 81 °C), was used for this experiment. The spectra were acquired from cryo-sections obtained from small meat tissue blocks (5x5x2 mm), that were immediately frozen in liquid nitrogen and stored at –80 °C prior to sectioning. The 8 µm thick cryo-sections were thaw-mounted on infrared transparent (2 mm thick) CaF_2 slides for the FT-IR microscopic measurements (IRscope II coupled to an Equinox 55 FT-IR spectrometer, Bruker Optics, Germany). The IR spectra were collected from single myofibres in transmission mode from 4000 to 700 cm^{-1} with a spectral resolution of 6 cm^{-1}. For each spectrum 256 interferograms were co-added and averaged. A background spectrum of the CaF_2 substrate was recorded before each sample measurement in order to account for variations in water vapor and CO_2. Spectra were pre-processed and normalised using extended multiplicative signal correction (EMSC) according to Martens *et al.*, (2003).

Results and Discussion

Figure 1a shows the amide I region (1700 cm^{-1}-1600 cm^{-1}) of the 2nd derivative of the FT-IR spectra from the pork meat with normal pH. Prior to taking the 2nd derivative the spectra were pre-processed by EMSC as described above. The minima in the 2nd derivatives refer to maxima (bands) in the original spectra. In Fig. 1a we are able to identify 9 bands at the wavenumbers 1695 cm^{-1}, 1682 cm^{-1}, 1668 cm^{-1}, 1660 cm^{-1}, 1652 cm^{-1}, 1639 cm^{-1}, 1628 cm^{-1}, 1619 cm^{-1}, and 1610 cm^{-1}. Table 1 presents a tentative assignment of these spectral bands.

In Fig. 1b shows a correlation loading plot obtained from an ANOVA-PLSR with the design variables (indicator variables) as *X* and the assigned FT-IR spectral bands as *Y*. The inner and outer circle in Fig. 1b refer to 50 and 100% explained variance, respectively. All bands have a very strong correlation to the temperature used for the heat-treatment. The correlation is also confirmed by considering plots of the single bands against temperature (not shown). The bands at 1695 cm^{-1}, 1668 cm^{-1}, 1628 cm^{-1} and 1619 cm^{-1} all have positive correlations towards temperature. The bands at 1682 cm^{-1}, 1660 cm^{-1}, 1652 cm^{-1}, 1639 cm^{-1} and 1610 cm^{-1} all have negative correlations towards temperature. PH showed no apparent effect on the protein structure. However, a potential effect of pH may be concealed by the influence of temperature.

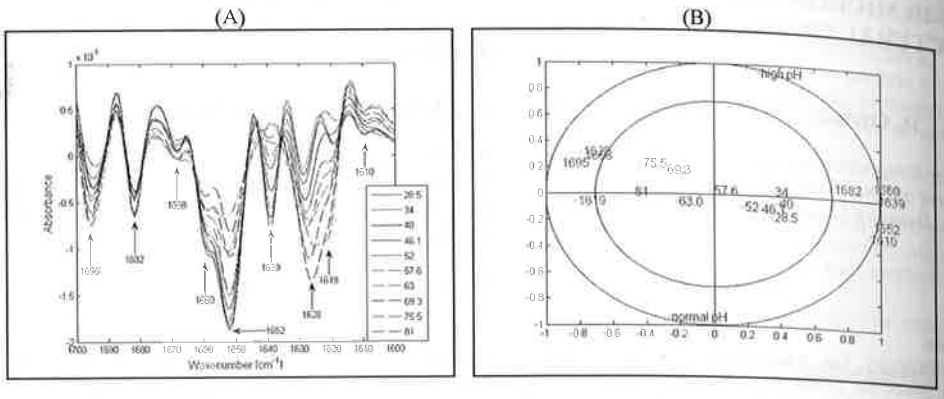

Figure 1: In (a) the 2nd derivative of the FT-IR spectra in the amide I region (1700 cm^{-1}-1600 cm^{-1}) of pork meat with normal pH (*M. longissimus dorsi)*at 10 different temperatures (28.5, 34.0, 40.0, 46.1, 52.0, 57.6, 63.0, 69.3, 75.5 and 81 °C). In (b) correlation of FT-IR spectral bands and design variables with the first and second PLS components of an ANOVA-PLSR (with design as X and selected FT-IR bands from amide I as Y) are shown.

Table 1: Band assignments (Jackson *et al.*, 1995, Fabian *et al.*, 2002).

1695 cm^{-1}	aggregated β-strands (intermolecular dipole coupling), high frequency
1682 cm^{-1}	native β-sheet structures (intramolecular), high frequency
1660 cm^{-1}	Possibly referring to native structures as loops in native structures
1652 cm^{-1}	α-helical structures
1639 cm^{-1}	native β-sheet structures (intramolecular), low frequency, O-H bending of water
1619/1628 cm^{-1}	aggregated β-strands (intermolecular dipole coupling), low frequency
1610 cm^{-1}	possibly the amino acid side chain tyrosine

The band positions shown above are very stable with respect to different animals and different treatments of the meat, though there are strong absorbance variations in the bands due to the different treatments that refer to protein structural changes. Our results show that FT-IR microspectroscopy can be used to measure structural changes in meat tissue proteins during heating. The type and extend of changes in secondary structure may have a potential as an indicator for product quality parameters of processed as well as aged meat.

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