STUDY ON SALT-INDUCED CHEMICAL CHANGES IN PORK MUSCLE BY FT-IR IMAGING

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Background

Processing methods like heating, freezing or salting are commonly used in muscle food preparation and all may lead to changes in texture. Texture is a complex property considered to be affected by muscle microstructure and composition, i.e. myofibrillar proteins, intramuscular connective tissue and fat distribution.

Therefore, there is a demand for techniques that enable us to gain more detailed knowledge about microstructural changes in these proteins.

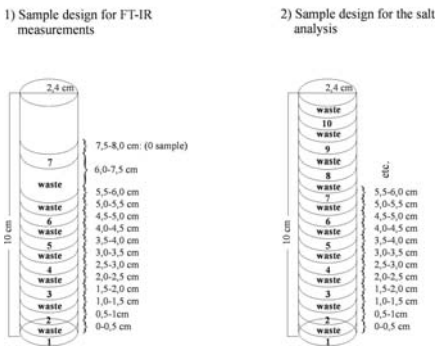
FT-IR-microspectroscopy and FT-IR imaging have been found to be a useful analytical tool so far mainly in biomedical science. Since it is a combination of spectroscopy and microscopy it allows to investigate those properties simultaneously and can provide information on chemical composition and morphology of complex tissues at the same time.
Most recently, a FT-IR imaging system was used to monitor denaturation induced by heating in beef muscle tissue (Kirschner et al. 2004).

Objective

The intention of this study was to investigate if FT-IR imaging can be used as a tool to monitor chemical changes such as denaturation processes in pork muscle tissue which occur during salting.

Materials and methods

Sample preparation



In this study samples from pork muscle (*m. semimembranosus)* with three different salt concentrations were investigated. The raw meat samples were placed in tubes (2.4 cm in diameter and 10 cm in height) that were kept in saturated brine (26% NaCl) so that the brine would diffuse into the samples resulting in salt gradient throughout the sample. After two weeks small blocks from regions of different salt concentrations were excised from the sample tube, frozen in liquid Nitrogen and stored at -80°C until use. For the FT-IR-imaging cryo-sections of 8 µm thickness (two sections each for low, medium and high salt concentration, i.e. from layer 1, layer 4 and layer 7 respectively) were prepared with a Leica cryostat CM3050 S (Leica microsystems, Nussloch, Germany) and mounted on infrared transparent CaF_2 slides.

Salt concentrations in the parallel sample (in the corresponding layers as shown above) determined by chemical Cl^--analysis were 1.6% for the low salt, 7.7% for the medium salt, and 15.4% for the high salt layer. In the following the samples will only be referred to as high, medium and low salt sample.

IR data collection and evaluation

An IR-microscope (IRscope II, BRUKER OPTICS, Germany) equipped with a 64 x 64 focal plane array detector (FPA) was used to collect 4096 spectra (a sample area of 270 µm x 270 µm which can be analysed simultaneously at a spatial resolution of 4 µm per pixel) on a pork muscle tissue sample. The analysis of the acquired IR images was done with in-house developed software in C++. The Microsoft C++ Developer

Studio was used to create Dynamic Link Libraries, that were linked to Dynamic Imager, an image analysis developer platform (Kirschner et al.2004). First, the image spectra where scatter-corrected using extended multiplicative signal correction (EMSC). EMSC is a pre-processing method that allows the separation of physical light-scattering effects (e.g. sample thickness) from chemical absorbance effects in spectra (Martens et al. 2003). Then, the raw spectra were submitted to a quality test, which among other things checks spectra for water vapour and signal to noise ratio. Spectra that did not pass the quality test were not used for further data analysis.

Results and discussion

The most prominent protein band in a FT-IR spectrum of muscle fibres is the Amide I band that appears in the frequency region of 1700-1600 cm^{-1}. It is mainly related to the carbonyl stretching vibration with minor contribution of C-N stretching and N-H bending vibrations. The Amide I vibration mainly depends on the secondary structure of the protein backbone and therefore is often used for secondary-structure analysis of proteins (Barth and Zscherp 2002).

Figure 1 displays five spectra across a randomly chosen fibre (spectrum 1 located at the periphery, with increasing numbers moving towards the centre of the fibre and 5 representing a spectrum located in the centre), which were extracted from the FT-IR image obtained from a cryo-section with high salt content. Spectra from an area around the edge of the fibre cell (Spectrum 1 and 2) exhibit a decrease in absorbance at 1654 cm^{-1} compared to those taken from inner parts of the fibre.
In order to gain a better resolution of the Amide I components derivation was applied, which is illustrated in Figure 2. The second derivatives of the five spectra shown in Figure 1 reveal the mentioned effect at 1654 cm^{-1} more clearly. The frequency region of 1648-1658 cm^{-1} is considered to correlate to α-helical protein structures (Barth and Zscherp 2002). The observed decrease at 1654 cm^{-1} might therefore indicate either an increase in denaturation with a shift from α-helix to β-sheet structure as observed by Kirschner et al. (2004) in heat-treated beef myofibre samples in the range of 45°C to 70°C, or it might be due to a salt-induced spatial diffusion of α-helix or β-sheet compounds within the fibre.

Figure 3 shows chemical images for three different salt concentrations. The chemical images were reconstructed from the spectra obtained from FDA-detection by employing the I_{1630}/I_{1654} band ratio, which gives a measure of the level of change: blue corresponds to a low while red corresponds to a high band ratio. Black domains in these images refer to spectra that did not pass the quality test, i.e. they mostly correspond to extracellular areas which can be seen when compared to photomicrographs. Figure 3c exhibits a fairly low band ratio indicated by predominantly dark blue colouration in the chemical image. Figure 3b reveals a lighter blue colour, which is caused by an increase in protein I_{1630}/I_{1654} band ratio. This effect is further increased in Figure 3a. Here, the higher degree of denaturation at the outer parts of the fibres is even more distinct than in Figure 3b. This supports the results presented in Figures 1 and 2, i.e. that the outer regions of the muscle fibre cells reveal a higher degree of either denaturation or an increase in β-sheet or decrease in α-helix structure respectively compared to the fibre centre. As seen from the photomicrograph at high salt content the fibre cell appear shrunken to high degree and thus the increase of extracellular space (Knight and Parsons 1988) is the reason for the significant increase in black pixel in the corresponding chemical image.

The possible reasons for the increase in the I_{1630}/I_{1654} band ratio at the fibre periphery may be as already mentioned a change in secondary structure for individual protein types or a possible diffusion effect that causes migration of protein structures. The latter may lead either to an increase in β-sheet-components or a decrease in α-helix which causes the higher I_{1630}/I_{1654} ratio in the outer parts of the fibre cells.

Conclusions

- The FT-IR imaging technique employing FPA-detectors might provide a useful tool for the detection of chemical changes like protein denaturation or diffusion effects. These changes can be followed in a spatially resolved manner.
- It was suggested that high salt contents may induce a higher degree of denaturation on the periphery compared to the centre of a muscle fibre cell.
- This method could possibly be used also to gain a better understanding in the mechanisms in salt diffusion in muscle tissue

References

Barth, A., and Zscherp, C. (2002). What vibrations tell us about proteins. *Quarterly Reviews of Biophysiscs*, **35**, 369-430

Kirschner, C.; Ofstad, R.; Skarpeid, H.-J.; Høst, V.; and Kohler, A. (2004). Monitoring of denaturation processes in aged beef loin by FT-IR microspectroscopy. *Journal of Agricultural and Food Chemistry* (submitted).

Knight, P., and Parsons, N. (1988). Action of NaCl and polyphosphates in meat processing: Responses of myofibrils to concentrated salt solutions. *Meat Science*, **24**, 275-300

Martens, H.; Nielsen, J.P.; and Engelsen, S.B. (2003). Light scattering and light absorbance by extended multiplicative signal correction. Application to near-infrared transmission analysis of powder mixtures. *Analytical Chemistry,* **75***,* 394-404.

Figures

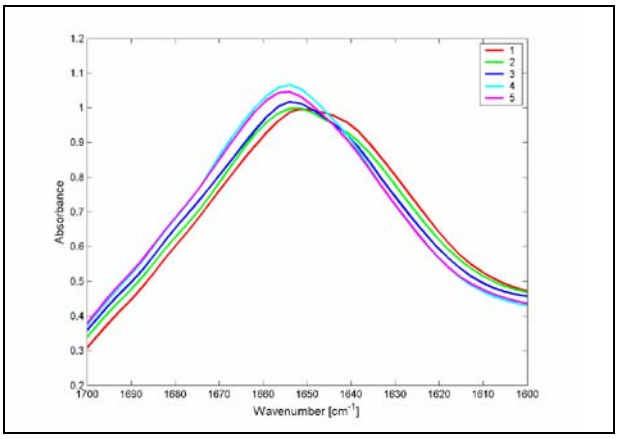


Fig.1: IR-spectra across a single myofibre from the high salt content sample shown in the frequency range 1700-1600 cm^{-1}; numbers 1-5 represent spectra from the edge towards the centre of the fibre cell

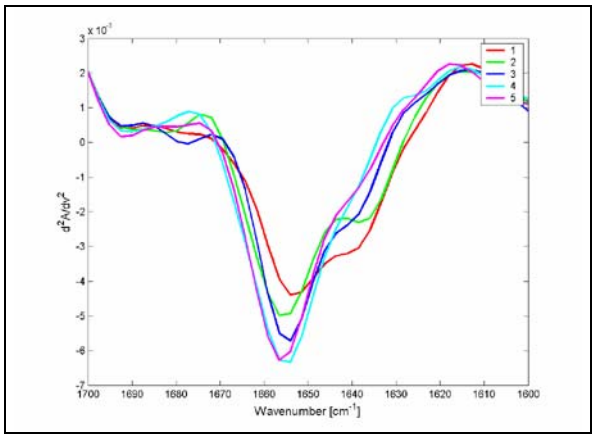


Fig.2: 2.Derivatives of IR-spectra from the high salt content sample displayed in the frequency range 1700-1600 cm^{-}; numbers 1-5 represent spectra from the edge towards the centre of the fibre cell

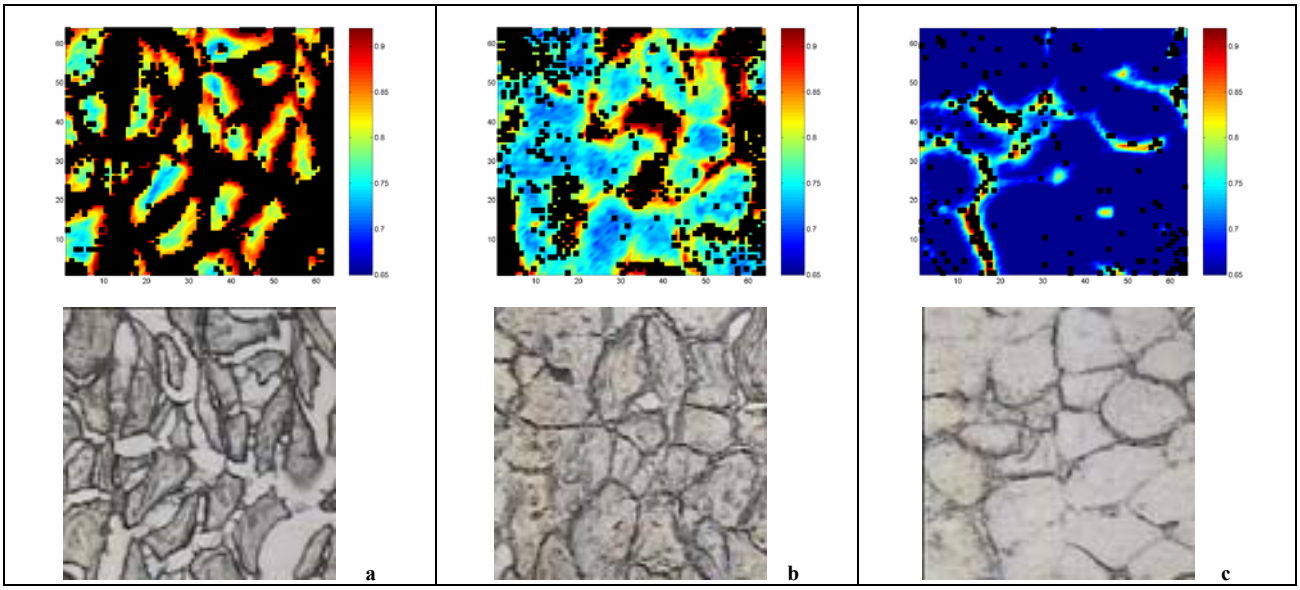


Fig.3: Chemical images (showing the I_{1630}/I_{1654} band ratio) obtained for 3 salt concentrations: high (a), medium (b), low (c) (from left to right). Corresponding photomicrographs are shown below the respective IR-image.