CONFOCAL LASER SCANNING MICROSCOPY - A NON-DISRUPTIVE TECHNIQUE TO DETERMINE THE MICROSTRUCTURE IN MEAT AND MEAT PRODUCTS

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Background

Changes in the quality of meat and meat products are often related to changes in the meat microstructure. These changes can be followed and estimated by using different microscopic techniques. When conventional light- and electron microscopic techniques are used, only one horizontal section can be focused in each sample, and sample preparation and sectioning is needed before monitoring. Confocal scanning laser microscopy (CLSM) offers unique possibilities of showing important additional information about the microstructure of the product. One major advantage is that optical sectioning can be performed at different depths in the product, allowing disturbance-free observation of the 3-dimensional internal structure (Blonk and Aalst, 1993). No preparation except staining is needed. The CLSM technique has been developed and is mostly used in medical science, but interesting applications can be seen in the field of food science (Heertje *et al*, 1987).

SIK has possessed a CLSM since 1993, and it has been used for studies of meat systems as well as for other food products. A sample chamber in which atmosphere and/or temperature can be adjusted allows studies of different dynamic processes and transport phenomena during the processing and handling of meat such as freezing, heating or injection of salt- or phosphate solution. The microscope is connected to an image analysis system so that a quantitative estimate of the structure can be made.

Objectives

The objective of this paper is to show the possibility of using the Confocal Laser Scanning Microscopy (CLSM) as a fast, non-disruptive screening tool for detecting specific information about the microstructure of whole meat and meat products. After staining, and no further preparation, visual information is given on the 3-dimensional porosity of the meat product in combination with specific information about fat, protein and collagen structures in the meat.

Materials and methods

The confocal scanning laser microscope system used is a Leica TCS 4D, consisting of a Leitz DMR light microscope, an argon/krypton laser, two detectors for fluorescence and reflection and a CPU motorola control data system. The laser can give three different wavelengths, 488 nm, 568 nm and 647 nm, which can be used for individual lines or in combination. Other possibilities exist depending on what filter combinations of the detector are used, exploiting other emission lines (Engelhardt and Knebel, 1993). Numbers in brackets below indicate the emission wavelengths selected for the best separation of the fluorochromes. The defined laser wavelength scans an optical section or a focal plane in the sample. The scanning can be done both in XY- and XZ planes. The detector receives emission light only from the focal plane, resulting in very sharp focal images. The images from this optical sectioning can be put together to form a reconstructed three-dimensional picture.

Three types of products have been used:

1. Whole meat from shoulder of pork. The sample was stained with 0.05% Texas red (665 nm) specific for proteins, and the collagen in the perimysium and endomysium was labelled with polyclonal goat antibodies against collagen III and IV, and with FITC-labelled anti-goat IgG (488 nm) as a fluorochrome (Egelandsdahl *et al*, 1993)
2. Hamburger-like product; presalted minced meat from shoulder of pork, heat-treated to 70C. The myofibrillar proteins were stained with FITC (488 nm) and the collagen/gelatine with Sirius red (665 nm).
3. Meat emulsion from sausage. The myofibrillar proteins were stained with FITC (488 nm) and the fat with Nile red (590 nm).

Bulk samples were prepared by cutting out cubes (1x1x1 mm) from each of the products and staining them with fluorochromes specific for muscle protein, fat and collagen.

Result and discussion

CLSM offers a number of advantages over conventional techniques for studying the relation between composition, processing and final food properties. Sample preparation is easy, and optical sectioning has no impact on the structure.

Figure 1 gives an example of specific labelling of collagen. The staining of collagen III and IV in the whole meat sample clearly shows the thicker collagen threads in the perimysium between the unstained bundles of muscle fibres. Surrounding each fibre, like a stocking, the fine network of collagen IV also shows up clearly. The status and appearance of collagen in meat strongly affect the tenderness; any changes in the collagen structure are therefore of interest.

In Figure 2, one optical section taken from the centre of the minced meat product sample is shown. Both intact bundles of muscle fibres (MF) and unstained fat cell domains (F) can be seen. Remnants of collagen (arrowheads) are seen surrounding the muscle fibres, the fat cell domains, and covering the surface inside the pores (P). Optical sectioning in different directions through the structure revealed that the pores in this sample were connected by fine channels and were often filled with gelatine. The distribution of pores and channels in the product provides important information on the water-holding properties, texture and the sensory perception of the product. By combining signals of different wavelengths, we can see the continuous phase of the product (lower right of the picture) containing a protein matrix mixed with collagen/gelatine.

Figure 3 shows the distribution of fat droplets, pores and/or channels in a sausage emulsion. The large variation in the size of the fat droplets can be seen. The comminution of the raw material, the distribution of pores and fat droplets influence the fat- and liquid-holding capacities of the product. The fat droplet- and pore sizes can be quantified using the image analysis system. Fat structures are sensitive to conventional sample preparations involving dehydration for plastic sections or freezing for cryosections. Therefore the CLSM technique is superior when fat and emulsified fat structures are to be studied.

The micrographs shown here indicate that the CLSM technique will open up many opportunities for obtaining a comprehensive characterisation of the microstructure in meat and meat products, which is needed both to guarantee quality maintenance of a product and in product development. More material will be presented on the poster at Lillehammer.

Figures

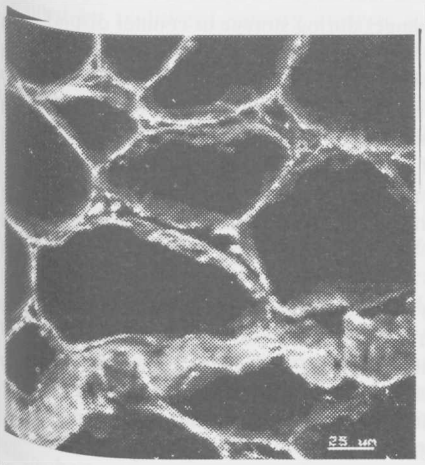

Figure 1.
Whole meat of pork shoulder. Collagen is seen in white surrounding and in-between the myofibrils.

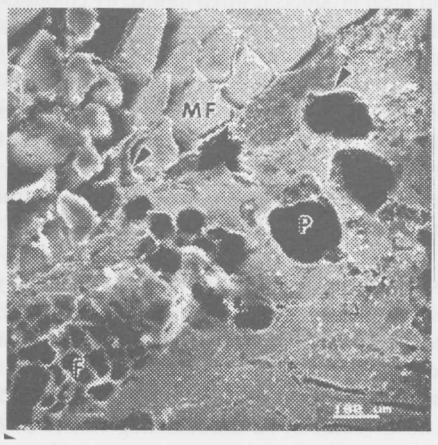

Figure 2.
Minced and heat treated meat from pork shoulder

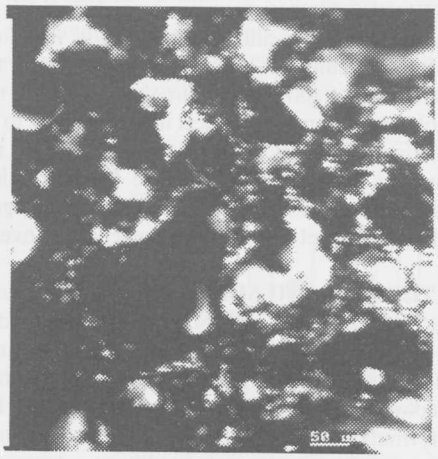

Figure 3.
Meat emulsion of sausage

For 3-dimensional appearance of the micrographs, colour reprinting is needed.

Conclusions

- CLSM is a most pertinent tool when screening bulk samples of meat and meat products.
- Information can be obtained about the 3-dimensional structure of the sample.
- Several structural components can be identified by a combination of wavelengths and staining techniques.
- CLSM makes it possible to follow the structural changes in a sample directly under the microscope.
- Images are generated in the computer and are suitable for image analysis.

Pertinent literature

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