CONFOCAL SCANNING LASER MICROSCOPY OF MEAT PRODUCTS

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ABSTRACT: Confocal Scanning Laser Microscopy (CSLM) is a new optical microscopic technique which is evaluated as a renaissance in light microscopy. It promises to play a role of a bridge between light and electron microscopy and offers significant advantages over conventional microscopy. Confocal Scanning Laser Microscopy possesses a unique optical sectioning which permits the disturbance free observation of the three-dimensional internal structure. We have used Confocal Scanning Laser Microscopy to study the internal structure of the frankfurter and the localization of the bacteria (lactobacilli) in the summer sausage. The internal structure of the frankfurters was visualized and revealed the size and arrangements of the fat globules by optical sectioning at 0, 5, 10 and 15 μm. The localization of the bacteria (lactobacilli) in the summer sausage was studied by optical sectioning at 0, 6, 12 and 18 μm. Confocal Scanning Laser Microscopy marks a new era in the study of meat products.

INTRODUCTION: Over the past 5 years, the field of microscopy has undergone a technical revolution that is now having a major impact on the biological science (Agard, 1989). Confocal scanning laser microscopy (CSLM) is rapidly becoming a major technique for study of the three-dimensional morphology of biological objects (Brakenhoff et al., 1988, 1989; Van der Voort HTM et al., 1989). Confocal scanning optical microscopy, arguably the most significant advance in biological light microscopy in this decade, enables one to obtain quantitative non-invasive optical sections through labelled biological specimens, virtually free from out-of-focus blur (Shotton and White, 1989). Perhaps the most useful property of the confocal scanning optical microscopy is its unique optical sectioning or depth discrimination property (Wilson and Sheppard, 1984) cited by Wilson and Carlini (1989). An overview of Confocal Microscopy is made by Shuman et al., (1989). Confocal microscopy promises to form a bridge between light and electron microscopy. With it, it may be possible to see the interiors of cells within living tissue, without the artefacts introduced by preparing specimens for EM observation. In addition, dinamic events and structures that once were unobservable may now be visible. In a review on confocal scanning optical microscopy and its applications for biological specimens Shotton (1989) explaines the principles of scanning optical microscopy and blur-free confocal imaging, discusses the various imaging modes of confocal microscopy and illustrates some of its early applications. Confocal scanning optical microscopy (CSOM) has the ability to reduce out-of-focus blur, and thus permit accurate non-invasive optical sectioning, that makes confocal scanning microscopy so well suited for the imaging and three-

dimensional tomography of stained biological specimens. Heertje et al., (1987) have used confocal scanning laser microscopy to observe fat spreads, cheese and rising dough. They have concluded CSLM appears to be a very useful tool in the study of food microstructure and it may mark a new era in the study of food products. The aim of this paper is to observe the internal structures within the actual depth of thick sections of processed meat by confocal scanning laser microscopy (CSLM).

MATERIALS AND METHODS: Commercial frankfurters and commercial summer sausage were used in this investigation. Samples of about 0,5 cm per side were fixed for 24 h. The fixed samples were frozen in isopentane cooled with liquid nitrogen and then sectioned in cryostat at -20°C. Frankfurters were sectioned at 40 µm thickness and summer sausage at 50-60 µm thickness. Sections of frankfurters were stained in a 0.01% (w/v) aqueous solution of Nile Blue A for 5 to 10 min as a means to visualize fat globules. Sections of summer sausage were stained for 1 to 3 min in 0.1% (w/v) aqueous Acridine Orange (Yiu, 1985) as a means to visualize bacteria. After stining the sections were rinsed briefly in water to remove excess stain, dried and mounted under sealed coverslips in 70% glycerol containing 1 mg/ml para-phenylendiamine (Johnson et al., 1982) to limit fading. Slides were stored at -20°C in a light tight box. Stained sections were examined using a MRC - 500 Confocal Imaging System (Bio-Rad Microscience). For Nile Blue A stained sections, filter system FC II (high sensitivity green excitation at approximate wavelenght 515 nm), and for Acridine Orange stained sections, filter system FC I (high sensitivity blue excitation at approximate wavelenght 490 nm) was used. Photomicrographs were taken with 35 nm Kodak T-Max 400 professional film.

RESULTS AND DISCUSSION: The optical sectioning of frankfurters by Confocal Scanning Laser Microscopy is shown in Figure 1. The fat globules appear as intense well defined light areas within the protein matrix. The internal structure of the frankfurters is visualized and revealed the size and arrangements of the fat globules by optical sectioning at 0, 5, 10, 15 µm of depth of the section. It is seen a very fine emulsion of small fat globules uniform distributed throughout the protein matrix. The numbers of the fat globules increased at 5, 10 and 15 µm of depth of the section. Microscopy was done on thick sections, and so images were taken away from the surface thereby avoiding disruption of the internal structure. The result from optical sectioning of the summer sausage is shown in figure 2. The optical sections are at 0, 6, 12 and 18 µm. The bacteria appear as intensely stained particles. The lactobacilli are elongated and they have taken the characteristic shape described by Leistner and Lucke (1989) in the fermented products. The point illumination and pinhole detection system effectively suppresses contribution from off-focus levels of the objects. This is a great advantage of CSLM over conventional light microscopy (Heertje et al., 1987). Shotton (1989) pointed out that CSOM has the ability to reduce out-of-focus blur, and thus permit accurate non-invasive optical sectioning, that makes confocal scanning microscopy so well suited for the

imaging and three-dimensional tomography of stained biological specimens. According to Heertje et al., (1989) depending on the optical density of the sample, optical sectioning can be applied up to depths of about 100 μm from the surface and the image is obtained without disruption of the structures and laborious sample preparation. The whole operation takes only a few minutes. The opportunities for scanning electron microscopy are considerably less.

CONCLUSIONS: Our investigations show that CSLM is a very powerful tool for the study of meat microstructure. The optical sectioning and the disturbance-free observation of the internal structures offer new possibilities for study of the technological processes of meat processing and for an effective quality control of the meat products.

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LIST OF FIGURES: Figure 1. Optical sections of frankfurter showing size and distribution of fat globules as follows: 1A, 0 μm; 1B, 5 μm; 1C, 10 μm; 1D, 15 μm. Scale bar is 250 μm. Figure 2. Optical sections of summer sausage showing bacteria as bright objects as follows: 2A, 0 μm; 2B, 6 μm; 2C, 12 μm; 2D, 18 μm.

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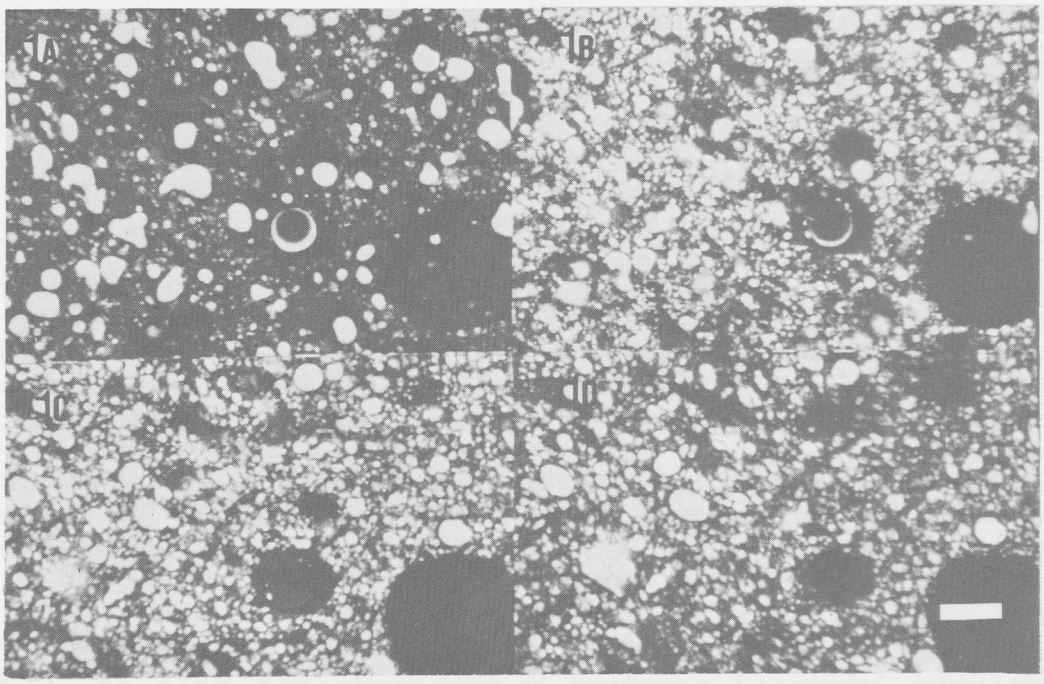

Figure 1.- Optical sections of frankfurter showing size and distribution of fat globules as follows: 1A, 0 µm; 1B, 5 µm; 1C, 10 µm; 1D, 15 µm. Scale bar is 250 µm.

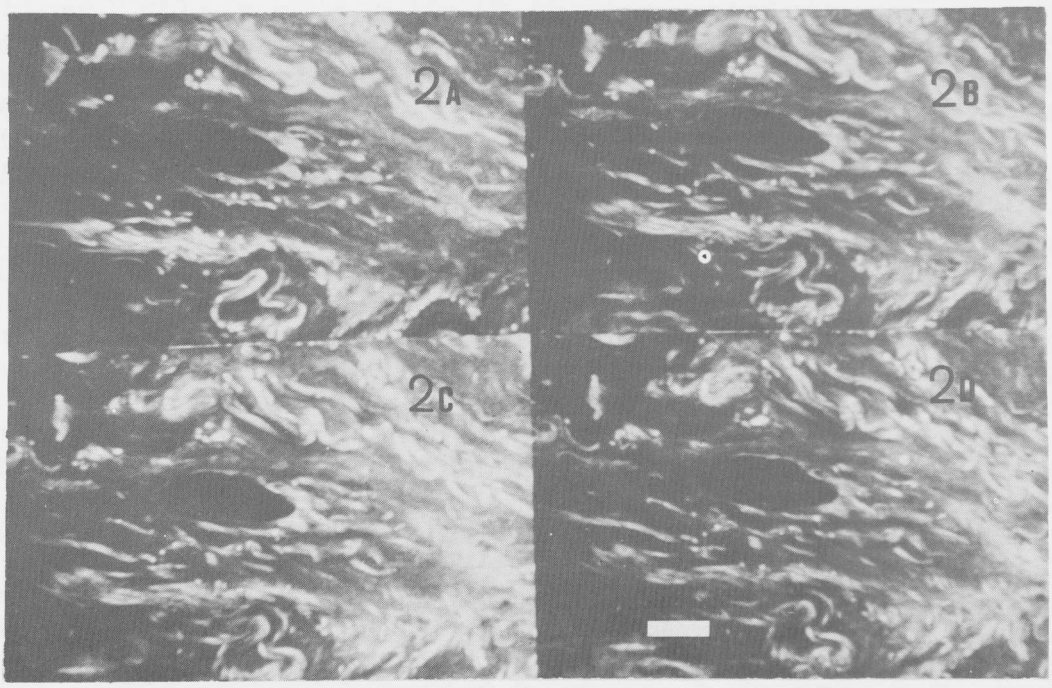

Figure 2.- Optical sections of summer sausage showing bacteria as bright objects as follows: 2A, 0 µm; 2B, 6 µm; 2C, 12 µm; 2D, 18µm.