# FLUORESCENCE MICROSCOPY OF MEAT PRODUCTS

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ABSTRACT: Fluorescence techniques were used as a means to study the microstructure of meat products. Frankfurters from several commercial establishments and raw unheated batter from various production lines were investigated. The size and distribution of fat globules in frankfurters were demonstrated by staining with Nile Blue A. Nile Blue A was used as a 0.01% aqueous solution and staining was for 4 to 5 min. The protein matrix was demonstrated by staining with Acridine Orange. A combined stain of Acridine Orange and Nile Blue A was also tested. The Nile Blue A stained sections were examined in rhodamine channel and the Acridine Orange stained sections in fluorescent channel. Bacteria were visualized in commercial summer sausage and in raw unheated batter by staining with Acridine Orange. The methods are simple, rapid and applicable for study of microstructure and for industrial quality control of the meat products.

INTRODUCTION: Transmision or scanning electron microscopy have been widely used for most of the structural studies of meat products. The high resolution provided by these techniques made it possible to study some ultrastructures such as fat globules and protein matrix in the so-called meat emulsion type products /Borchert et al.,1967; Basgall et al.,1983 / and as lactic acid-producing bacteria in fermented sausages / Katsaras and Leistner, 1987 /. The limitation in sample size and the relatively long preparation time are factors that lower the efficiency of the above techniques, making them less convenient for meat product analysis. Fat globules and protein matrix can be studied by light microscopy / Hansen, 1960; Kempton and Trupp, 1983; Cassens et al., 1977; Dimitrov et al.,1985 /. However, the light microscopy sometimes does not provide sufficient contrast in thin sections and allows only poor resolution in thick sections. A considerably higher resolution can be achieved by epi-fluorescence microscopy emploing relatively rapid and simple procedures / Yiu, 1985 /. Most fluorescence microscopic methods are provided with very sensitive and specific markers which are capable of giving both structural and chemical information. Yiu / 1985 / has used successfully fluorescence microscopy for analyzing cheese microstructures. Fulcher / 1982 / pointed out that the fluorescence microscope is one of the most sensitive instruments available for morphological and microchemical analysis of biological

material. The fluorescence microscope has advantages over conventional microscopic methods, namely improved sensitivity, contrast and, in most instances, chemical specifity. The methods are extremely simple, rapid and are readily applicable to many different types of preparations,especially whole grains and flours. The aim of this paper is to demonstrate the capability of fluorescence microscopy in visualizing and evaluating the morphology of processed meats.

MATERIALS AND METHODS: Frankfurters from several commercial establishments, raw unheated batter from various production lines and commercial summer sausage were used in this investigation. Samples of about 0,5 cm per side were fixed in I0% formalin for 24 h. Alternatively, unfixed samples were sectioned, and then the sections were fixed / for I0 to I5 min / after they had been attached to cover slips by brief drying. The fixed or unfixed samples were frozen in isopentane cooled with liquid nitrogen and then sectioned at 8 to I2 $\mu$m thickness in a cryostat at -20°C. Sections of commercial frankfurters were stained for 4 to 5 min in 0.0I% / w/v / aqueous Nile Blue A, rinsed in water for I0 to I5 min as a means to visualize fat globules. Sections of commercial frankfurters were stained for 3 to 5 min in 0.I% / w/v / aqueous Acridine Orange / Yiu, I985 / and then rinsed in water for I0 to I5 min as a means to visualize protein matrix. A combined stain of Nile Blue A and Acridine Orange was also tested. The combined staining was accomplished first with Acridine Orange as above folowed by 3 to 5 min staining with Nile Blue A. Bacteria were visualized in commercial summer sausage using Acridine Orange as described above, exept with a staining time of I to 3 min. After staining, the sections were mounted in 70% glycerol containing I mg/ml paraphenilenediamine / Johnson et al. I982 /, and the edges of the coverslip were sealed with fingernail polish. Sections were stored between viewings at - 20°C in a light tight box.
Microscopy was conducted with a Carl Zeiss Standart Research microscope equipped with an epi-fluorescence illuminating system which included a 450-490 band pass exciter filter and a 520-560 band pass barrier filter / FC I / and a 546/I0 band pass exciter filter and 590 longwave pass barrier filter / FC II /. Photomicrographs were taken with 35 mm Kodak Technical Pan film 24I5.

RESULTS AND DISCUSSION: When examining raw batter it was best to take small cubes / about 0.5 cm per side / and fix them for I0 to I5 min in I0% formalin. The fixation gave some rigidity on the surface of the samples and made it easier to handle. Nile Blue A, a common fluorochrome used for staining fat-containing structures have been used by Yiu ( I985 ) in a fluorescence microscopic study of cheese and by Fulcher ( I982 ) in fluorescence microscopy of cereals. The relatively simple and rapid procedure

was found most appropriate for studying the size and distribution of fat in frahkfurters and in unheated batter. The results for Nile Blue A staining are shown in figures I and 2. The fat globules are in white and the protein matrix is in black but when are viewed directly with the microscope the fats fluoresce a brilliant orange-red color and stand out clearly and distinctly against the dark backgreund. The dye stains the fat rapidly and the possibility false identification of trapped air bubbles or pockets is eliminated besause they do not fluoresce. Intensity of staining was controlled by the length of staining time and the rinsing procedure. If samples are overstained, the fluorescence is so bright that the image is clouded and detail is lost. The most appropriate time of staining and rinsing are given in the Materials and Methods section. Figures I and 2 are photographs of sections of frankfurters from different manufacturers. The size and the distribution of the fat globules are clearly observable. The heated or raw products are stained very well with Nile Blue A. A typical result for Acridine Orange staining of frankfurters sections is demonstrated in figure 3. The protein matrix and the muscle fibres are in white and the lipids are in black. The staining is very simple and rapid. A combined staining for both protein and fat may be accomplished using Acridine Orange and Nile Blue A. The two components of the samples can be examined on the same section by switching the channels on the microscope. A typical result for Acridine Orange staining of bacteria " lactobacilli " in commercial summer sausage is shown in Figure 4. The bacteria fluoresce brightly and are clearly visible. Using scanning electron microscopy Katsaras and Leistner ( I987 ) illustrated nestings of bacteria in fermented sausage. According to Leistner ( I987 ) studies on the topgraphy of the fermentation process in meat is a promising research area. The fluorescence technique we have described is much simpler and faster. The method can be used for illustration of bacteria in raw batter ( figure 5 )

CONCLUSIONS: Our investigations show that fluorescence microscopy of meat products is a very useful for the study of meat microstructure and have the advantage of identifying components such as lipid, protein and bacteria. The procedures are simple and rapid and may be coupled easily to an automated image processing system which allows the possibility of applying the technique to on-line quality control;

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Council of the National Live Stock and Meat Board and Wisconsin Beef Council.

LIST OF FIGURES: Figure I.Section of frankfurter stained with Nile Blue A. The fat globules appear as bright, white areas against a dark background. It can be seen a rather uniform distribution of small fat globules. Scale bar is I00 µm.
Figure 2. Section of frankfurter stained with Nile Blue A. Large fat globules and large variation in the size distribution of fat globules. Scale bar is I00 µm.
Figure 3. Section of frankfurter stained with Acridine Orange. The protein matrix and the muscle fibres are stained as bright, white areas and the fat is dark. Scale bar is 50 µm.
Figure 4. Section of summer sausage stained with Acridine Orange. Bacteria are stained bright and appear in a nest. The scale bar is I00 µm.
Figure 5. Section of raw batter stained with Acridine Orange. Bacteria are stained bright and appear in nests. The scale bar is 50 µm.

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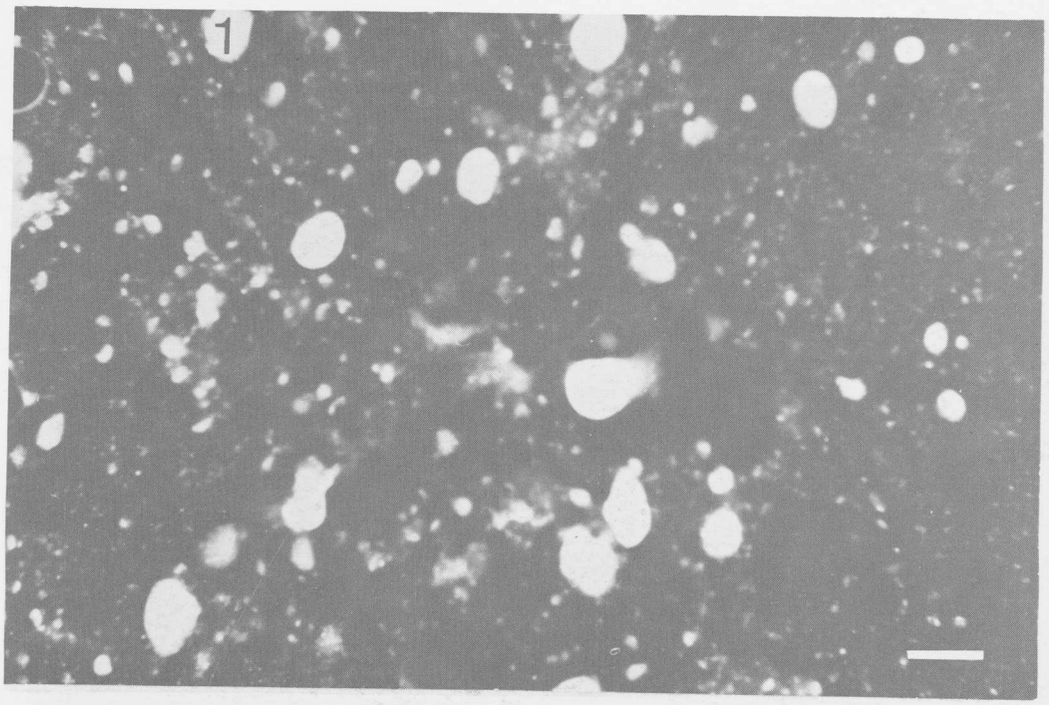


Figure 1.- Section of frankfurter stained with Nile Blue A. The fat globules appear as bright, white areas against a dark background. It can be seen a rather uniform distribution of small fat globules. Scale bar is 100µm.

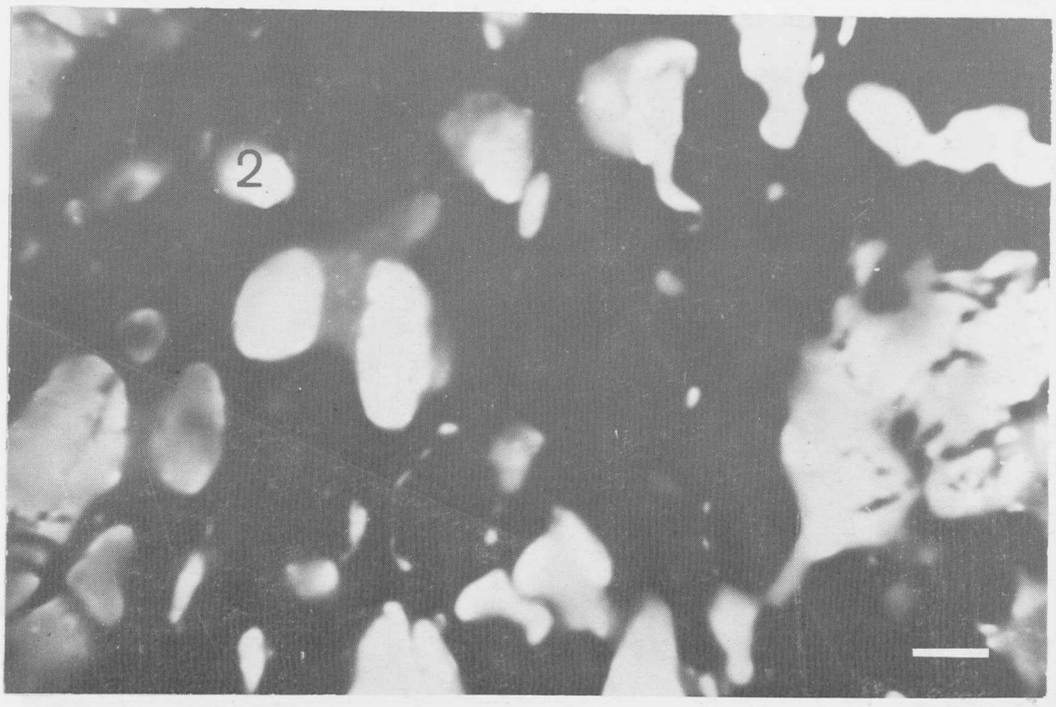


Figure 2.- Section of frankfurter stained with Nile Blue A. Large fat globules and large variation in the size distribution of fat globules. Scale bar is 100 µm.

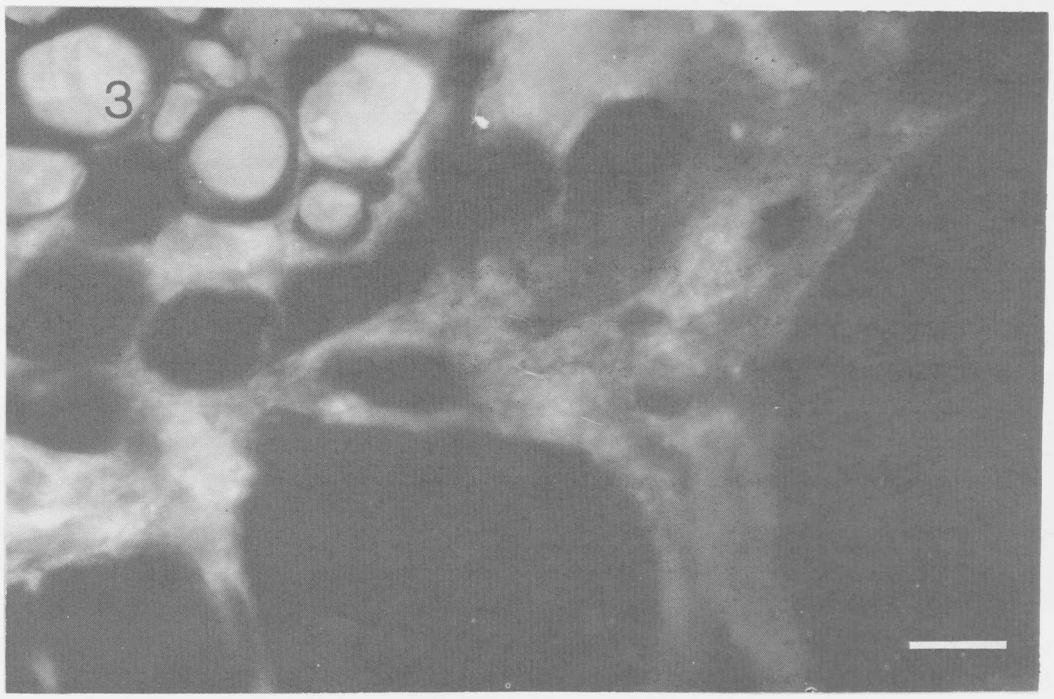


Figure 3.- Section of frankfurter stained with Acridine Orange. The protein matrix and the muscle fibers are stained as bright, white areas and the fat is dark. Scale bar is 50 µm.

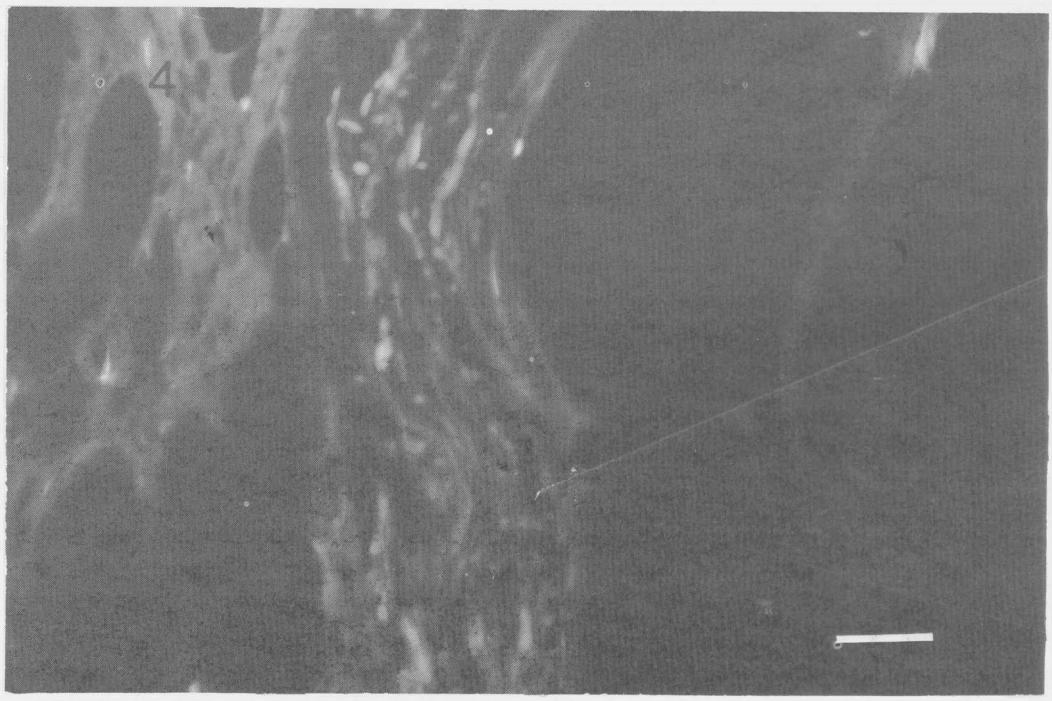


Figure 4.- Section of summer sausage stained with Acridine Orange. Bacteria are stained bright and appear in a nest. The scale bar is 100 um.

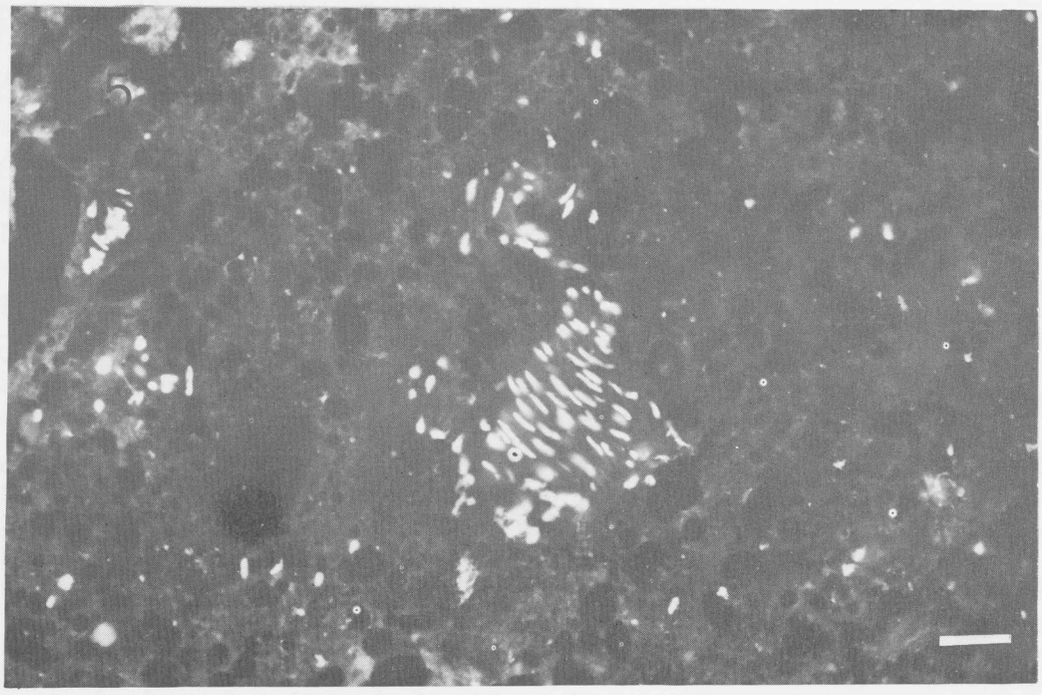

Figure 5.- Section of raw batter stained with Acridine Orange. Bacteria are stained bright and appear in nests. The scale bar is 50µm.