Microorganism Topography in Raw-dried Sausages

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SUMMARY: In the present study the microorganism topography in raw-dried sausages, produced by addition of starter cultures, is investigated. The possibility for accelerating the ripening of these sausages by the improvement of starter culture distribution immediately after their application to the sausage mix is studied. Microstructural and physicochemical investigations are carried out in parallel batches raw-dried sausages with and without liophilized starter cultures - Lactobacillus plantarium and Micrococcus varians. The starter cultures were applied in two ways: they were sprinkled in the form of powder and they were pulverized with dissolved in distilled water cultures. The microorganism topography in the sausages, pH variation and water content at definite moments of ripening are examined.

When the starter cultures are applied, on the fourth day of ripening, a clearly expressed "nest" clustering of microorganisms is observed in small but dense located colonies. Their location is mainly in connective tissue spaces, along the muscle fibres, but it is observed also single colonies among the fat cells. When the starter cultures are pulverized on the meat batter, they are distributed more evenly, the distance among the bacterial colonies is smaller and so the usage of substrate microorganisms is more favourable. The uniform distribution develops conditions for more uniform penetration of their metabolic products in the sausage mix, more rapidly decrease of pH and acceleration of the fermentation process in the sausages.

INTRODUCTION: The internal and external factors, the number and types microorganisms significant to the fermented sausages, are extensively studied. Leistner (1990) states, that the natural flora and added starter cultures are not distributed evenly in the fermented sausages but are immobilized in cavities or nests in the sausage mix. According Katsaras and Leistner (1988) the distance among these cavities or nests varies between 100 and 5000 μm. Leistner (1990) underlines that microorganisms are placed as in a trap. They are not able to release themselves from these nests and the ripening of the fermented sausages can be considered as a solid-state-fermentation. According to the author a relatively small distance among the bacterial nests in the sausage matrix should be advantegeous. Therefore, a more even innoculation of the sausage mix with suitable starter cultures can be more significant than it is considered before.

Leistner and Lücke (1989) emphasize that an investigation of the fermented mass topography by a scanning electron microscopy should be useful for a better understanding of the processes and for their improvements.

The purpose of our investigations is to use light and transmission electron microscopy to examine the microorganism topography in raw-dried sausages and the information obtained to be used for a more even innoculation of the starter cultures in the sausage mix.

MATERIALS AND METHODS: The experiments were carried out with raw-dried rapidly ripening semi-perishable sausages "Maljovitsa". One sort beef and semi-lean pork in 1:1 ratio was cut into pieces of 150 and 300 g and was cooled at -5 to -6°C. The cooled raw materials were treated in cutter as initially the beef was places, after 4-5 rev the semi-lean pork, then the curing mixture and at the end - seasonings and the ascorbic acid. As a starter culture we used Bulgarian liophilized preparation "Biostart". The preparation containes Lactobacillus plantarium and Micrococcus varians in 1:1 ratio, 10^9 microbial cells in 1 g preparation. The preparation was dissolved in distilled water with pH - 7,0 and temperature - 23°C in 1:50 ratio. The dissolved starter cultures were pulverized on the meat raw materials in the process of cutting. The process of cutting was done until the filling mass achieves grainy structure with piece size - 3-4 mm.

For a comparison we used samples produced according to the same technology and formulation and the difference was that the starter cultures were applied in the form of powder. One batch from the same sausages was produced without starter culture application.

The sausage mix was stuffed in synthetic casings - 50 mm. After hanging in the form of canes, the sausages were dried in automatic drying-room at 22°C and relative air humidity - 93% for 72 hours until obtaining red color. After cool smoking at 20°C for 6 hours the sausages were dried in automatic drying-room at 17°C and relative air humidity - 85% for 5 days decreased humidity to 75% for 4 days. The rest of the drying was done at 12°C at the same air humidity (75%). The drying continues about 15 days until the requirements of the parameter "water in % of the total mass" was achieved, and the value of this parameter for sausages "Maljovitsa" is no more than 40%. During the technological process we measured the pH in the meat batter and in the sausages on the fourth, seventh, eleventh and fourteenth days of the drying. At some of these periods of time we examined the water content by "Infra-Lyzer", preliminarily calibrated for raw-dried sausages.

The materials for the microstructural investigations in the form of blocks - 0,5 x 0,5 x 0,5 cm - were frozen in isopentan, precooled in liquid nitrogen to -196°C. Sections with thickness 10 μm, produced by cryostat Minotome - USA, was treated regarding the classical methodics for staining with hematoxilin-eosin and methyl-blue according Leofler. The observations were carried out under light microscope Docuval - Karl Zeis.

The materials for the ultrastructural investigations were fixed by glutaraldehyde and postfixed by osmium tetraoxide and embedded in Dorkupan - Fluka. Ultrathin sections made on ultramicrotom LKV - III, were contrasted with uranyl acetate and lead nitrate and observed under transmission electron microscope TESLA BS 613 at 80 kV.

RESULTS AND DISCUSSION: In the microstructural observations on the development of the starter cultures from Lactobacillus plantarium and Micrococcus varians in the filling mass of the sausage batches with powder starter cultures, on the fourth day of drying we observed the typical for this product microstructure of nest distribution of the microorganisms in

well formed colonies, located mainly in the spaces of muscle tissue modified during the technological treatment (fig. 1). In the sausages with pulverized starter cultures at the same period of ripening the same nest formation of the colonies was observed, but they are relatively small and are located at significantly smaller distances (fig. 2). This eventually is a result from the more even initially distribution of the pulverized starter cultures, which ensures the ensimination of big parts in the sausage mix. Therefore, the greater part of the microbial colonies obtained germs in the process of ripening. According to the ultrastructural investigations it is also observed nest microorganism distribution from starter cultures in the connective tissue among the muscle fibres (fig. 3). In the sausages without starter cultures on the fourth day of drying it is difficult to find under microscope single greater colonies of microorganisms from the natural microflora (fig. 4).

Katsares and Leistner (1988) indicate as a condition for improvement and stabilization of the raw sausage ripening the shortening of the distances among the bacterial colonies. According to them this is due to the agitation of the fine fragmented batter during cutting. In the production of our raw-dried sausages the cutting can not continue for a long time because it is limited by the necessary grainy structure of the batter. Therefore, an even bacterial distribution in the filling mass can not be achieved. And we are oriented to the improvement of starter culture innoculation in the sausage mix by a pulverization during cutting.

On the seventh day of drying we established clearly expressed difference in pH of the sausage batches with starter cultures (4.60 and 4.80) in comparison with the batches without starter cultures (5.57) on fig. 11. This is an objective parameter for the development of the applied starter cultures in the products, which is found by micro and ultrastructural analysis.

On the seventh day of ripening the differences in the density and distribution of the colonies in the sections from the tested sausages are the same. The sausages with pulverized starter cultures (fig. 5) have the greatest number of microbial nests, distributed at small distances. When the starter cultures are applied in the powder form, the microbial nests are relatively less and located at greater distances (fig. 6). The sausages without starter cultures have great, dense colonies, located at greater distances among the muscle fibres (fig. 7).

On the fourteenth day of drying it is observed a decrease in the differences of the density of microbial nests in the sausages with pulverized and powder starter cultures (fig. 8 and 9), which is reflected on the relatively same pH values (fig. 11). Regarding the ultrastructure it is observed clearly expressed cavities in the protein matrix, filled with densely located microorganisms (fig. 10). We accent that these cavities have been formed as a result of the influence of the metabolic products of the microorganisms on the protein matrix. The evidence for this is the lay-out of the marginal surface of the cavities, which has the form of the respective microorganisms, and sporadically among them the isles of still whole miofibrilar proteins are formed. The conducted micro and ultrastructural investigations were integrated with the physicochemical data during the drying of the produced sausage batches. The pH data are shown on fig. 11. It is observed replacing of the steap part of pH decrease at the beginning of sausage drying with starter cultures, as this is most clearly expressed in the pulverized starter cultures. This can be explained by the acceleration of the fermentation process due to the better distribution of starter cultures and more densely located microbial nests. The water content decrease in the tested sausages follows in general the observed tendency of pH values for a more rapid decrease on the first days of sausage drying with pulverized starter cultures (fig. 12).

CONCLUSIONS: The microscope methods for investigation give us invaluable, unique information about the topography of the natural microflora and added starter cultures in fermented sausages. Therefore, they are significant to the better understanding of the processes and for their improvements.

The pulverization of dissolved starter cultures during cutting lead to even distribution in the sausage mix, which is reflected favourably on the process of ripening.

The uniform location of added starter cultures applied by pulverization develops prerequisites for evenly running of the technological process and for equalization of the quality of the end products.

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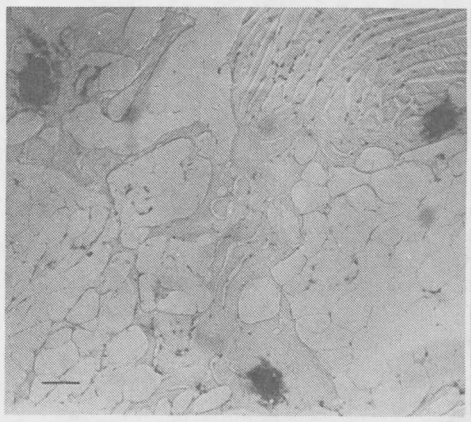


Fig. 1

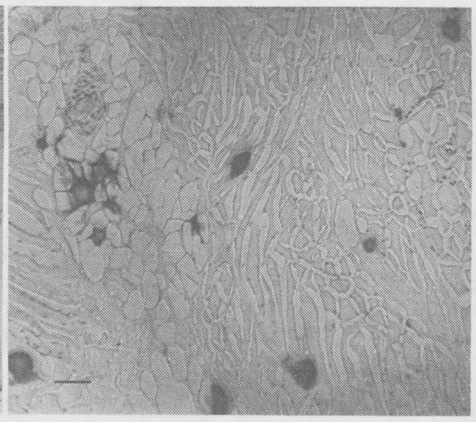


Fig. 2

Fig. 1 - Microstructure of sausage "Maljovitsa" with powder liophilized cultures. The fourth day of drying. Bar = 0,1 mm.

Fig. 2 - Microstructure of sausage "Maljovitsa" with pulverized solution of liophilized starter cultures. The fourth day of drying. Bar = 0,1 mm.

Fig. 3 - Ultrastructure of sausage "Maljovitsa" with pulverized solution from liophilized starter cultures. The fourth day of drying. Bar = 200 nm.

Fig. 4 - Microstructure of sausage "Maljovitsa" without starter cultures. The fourth day of drying. Bar = 0,1 mm.

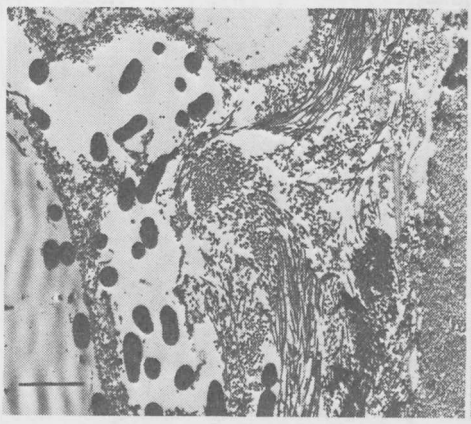
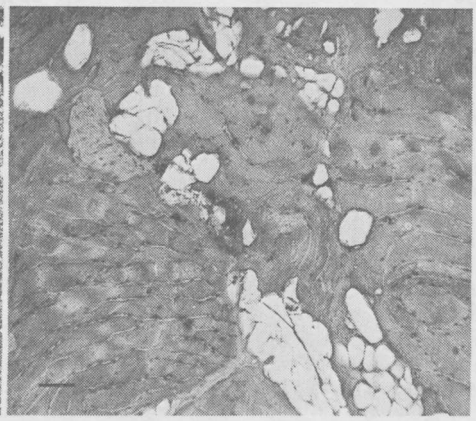


Fig. 3



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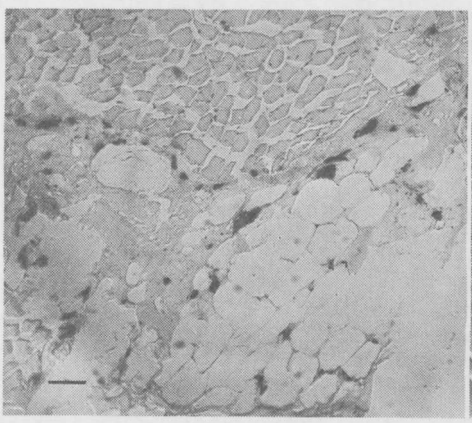

Fig. 5

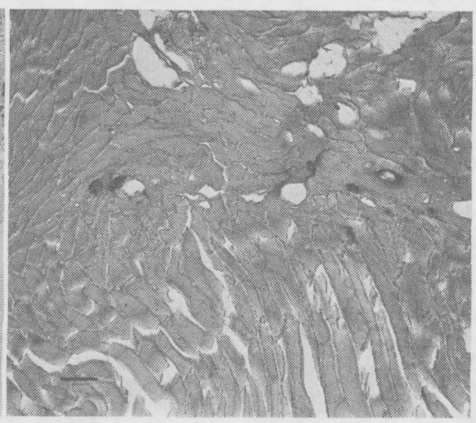

Fig. 6

Fig. 5 - Microstructure of sausage "Maljovitsa" with pulverized solution of liophilized starter cultures. The seventh day of drying. Bar = 0,1 mm.

Fig. 6 - Microstructure of sausage "Maljovitsa" with powder liophilized cultures. The seventh day of drying. Bar = 0,1 mm.

Fig. 7 - Microstructure of sausage "Maljovitsa" without starter cultures. The seventh day of drying. Bar = 0,1 mm.

Fig. 8 - Microstructure of sausage "Maljovitsa" with pulverized solution of liophilized starter cultures. The fourteenth day of drying. Bar = 0,1 mm.

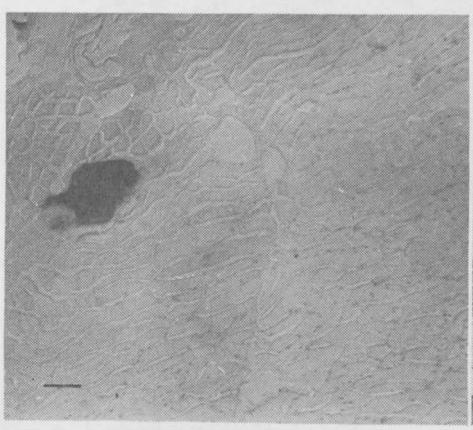

Fig. 7

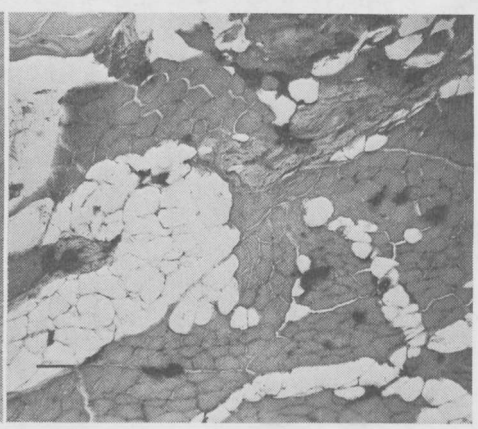

Fig. 8

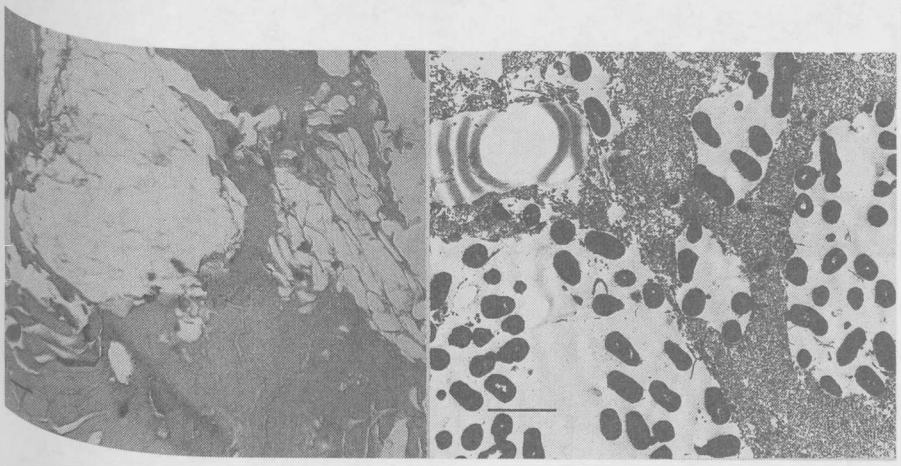

Fig. 9

Fig. 10

Microstructure of sausage "Maljovitsa" with powder liophilized starter cultures The fourteenth day of drying. Bar = 0,1 mm.
Fig. 9

Ultrastructure of sausage "Maljovitsa" with pulverized solution from liophilized starter cultures. The fourteenth day of drying.
Bar = 200 nm.

Fig. 10

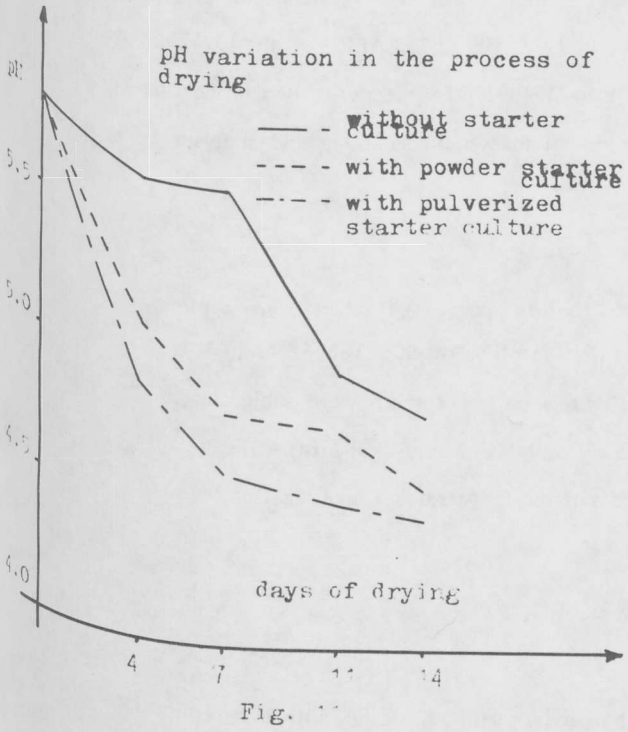

Fig. 11

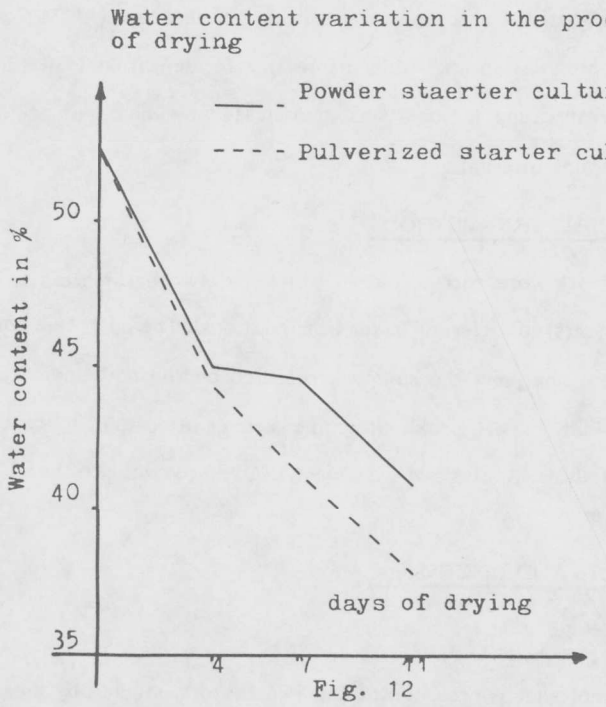

Fig. 12