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A CONTRIBUTION TO THE INVESTIGATIONS ON PURE
AND CONJOINT CULTURES OF STREPTOCOCCI AND
MICROCOCCI IN THE RIPENING OF SAUSAGES

by

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INTRODUCTION

Recently and particularly in the last few years many authors (2, 3, 6, 10, 12, 16) have studied the role of lactic acid bacteria in the ripening of sausages. Special attention has been paid to the utilization of lactic acid bacteria in the form of starter culture, either as pure or in conjoint culture with micrococci (11, 13, 14, 17). The best results, so far, have been attained from the combination of mixed lactobacilli and micrococci. Their enzymes are, therefore, being produced industrially and are also being used in our country.

In the last few years, the Industrial Microbiology Lab in the Faculty of Agriculture, Belgrade, is engaged in the investigations on the biocenotic relationships of lactic acid bacteria and micrococci as well as their application in the ripening of cheeses (19,20). A polyvalent culture consisting of Streptococcus lactis Ak-60, and Micrococcus M-104, has been obtained during these studies. This culture has been applied as starter in the ripening of White and Trapist cheeses. In a number of experiments, without any exceptions, it has been determined that by use of this conjoint culture, the ripening time is considerably reduced with a better taste and flavour as compared with the standard product. On the other hand, in the literature concerning the

field of meat technology, a large number of authors conclude that for hastening the process of fermentation in the meat products, the best combination is the lactobacilli and micrococci. NURMI (14) on the basis of his results, concluded that streptococci were not suitable for the purpose and that further studies on the possibilities of their use in the ripening of sausages were meaningless. Considering all this, we however, decided that our culture which has successfully been employed in the ripening of cheeses, should be tried on as starter culture in the production of sausages. The experimental results, till todate, have shown that our decision was not wrong. In this paper some preliminary results are being reported.

MATERIALS AND METHODS

The "turistička kobasica" (a trade name meaning tourist sausage) being produced in the Belgrade Meat Industry was used in these studies. The composition of this sausage is as follows:

Pork, category A	60 %
Beef, category A	20 %
Bacon	20 %

In 100 kg of this mixture 2.2 kg of table salt, 0.1 kg of black pepper and a mixture of spices from the factory "Kolinska", Yugoslavia, are added.

Streptococcus lactis Ak-60 (received from U.S.A.) and Micrococcus M-104 (isolated in the Industrial Microbiology Lab of the Faculty of Agriculture, Belgrade - Zemun) were used as pure cultures in these studies. The cultures were multiplied in yeast dextrose broth(YDB, 9).

The sausages were prepared in 4 different variations. For each variant 100 kg. of the raw material was used. At the time of mincing and mixing 24 hours old broth culture was added at the following rate:

- I. standard variant - without the addition of microbial culture,
- II. 1 % Micrococcus M-104
- III. 1 % Streptococcus Ak-60 and
- IV. 0.5 % Micrococcus M-104 + 0.5% Streptococcus Ak-60

Yeast dextrose agar (YDA) was used to determine the total bacterial count in the sausages; Rogosa agar (18) was used for the determination of lactobacilli; Barnes medium (1) for the streptococci; meat peptone agar (MPA) with added 10% NaCl for the micrococci; violet red bile agar (VRBA, 8) for the coliform bacteria; and MPA after heating a dilution 1:10 at 80°C for 10 minutes for the determination of sporeforming bacteria.

In addition to the microbiological analysis undertaken on the finished sausages, the following chemical analysis were also conducted:

1. pH of the sausages,
2. total moisture by drying at 105°C, and
3. loss of weight at the end of the ripening process or when the panel evaluated that the individual variant was ready for marketing.

The organoleptic evaluation of the finished products was conducted by a panel consisting of 7 members (all Food Technologists). The point system with the range from 1 to 5 points was employed. Important factors in this evaluation were consistency, cutting characters, taste, flavour and colour of the cut.

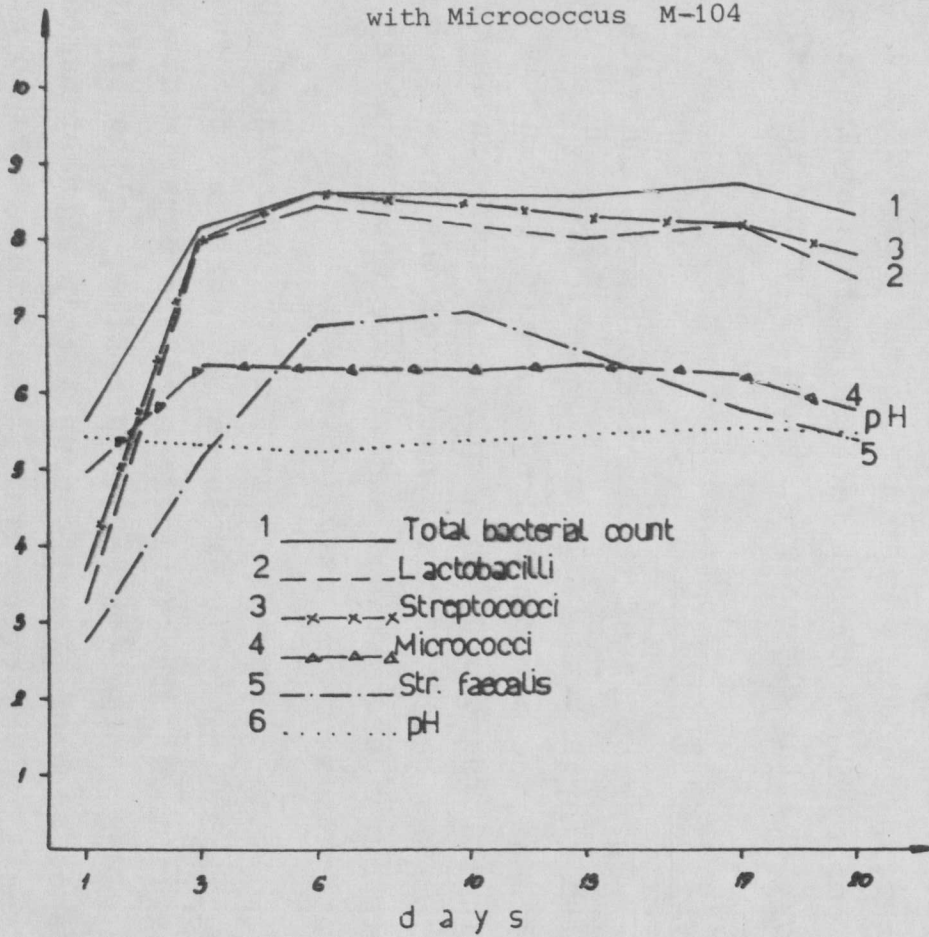
RESULTS AND DISCUSSION

The results of the bacteriological analysis are presented in the graphs 1 to 4. It may be seen from these graphs that the growth of the natural microflora was abundant in all the sausage variants. Therefore, no significant differences can be concluded on the basis of the dynamics of the growth of microorganisms in the standard variant and the variants in which pure and conjoint cultures were used.

It is, however, clear that the dynamic of the growth of total bacteria followed the same course as the number of lactobacilli in all the variations of the experiment (highest number was found on the 6th day). Meanwhile, in the III variant with pure culture of Streptococcus lactis highest number was found on the 13th day of growth. It is interesting to note that the

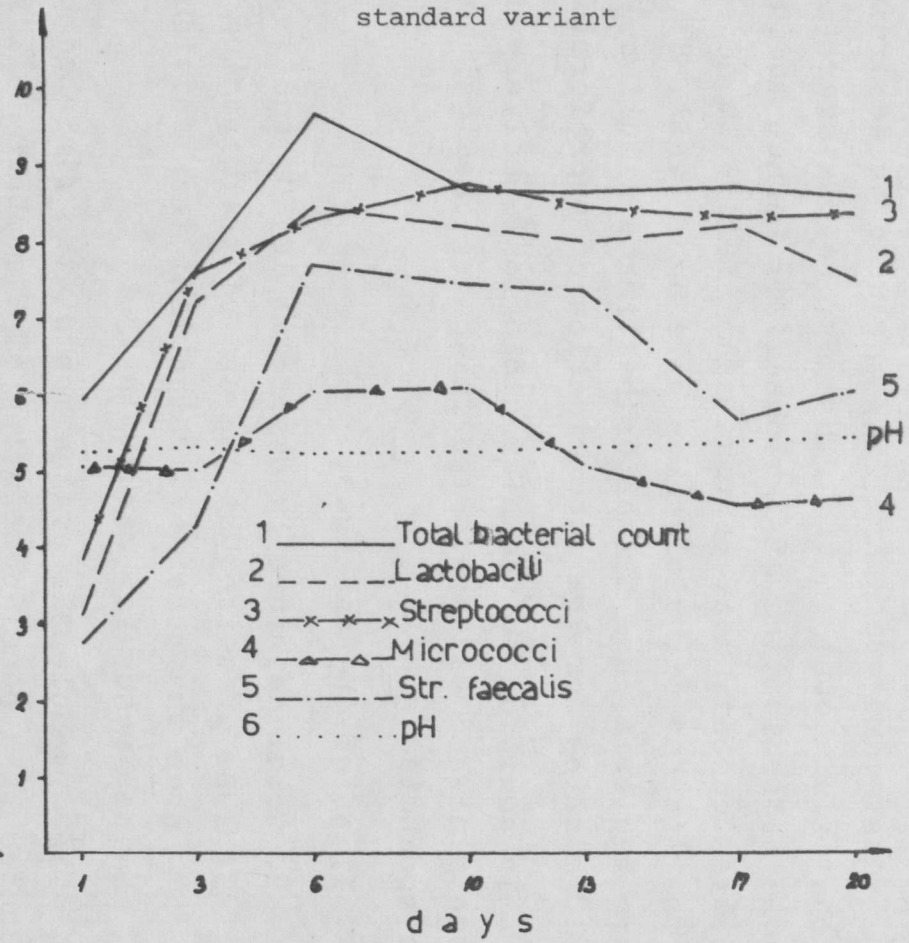
Log N

Graph. 2. Dynamic of the development of microflora in the ripening of sausages with *Micrococcus M-104*



Log N

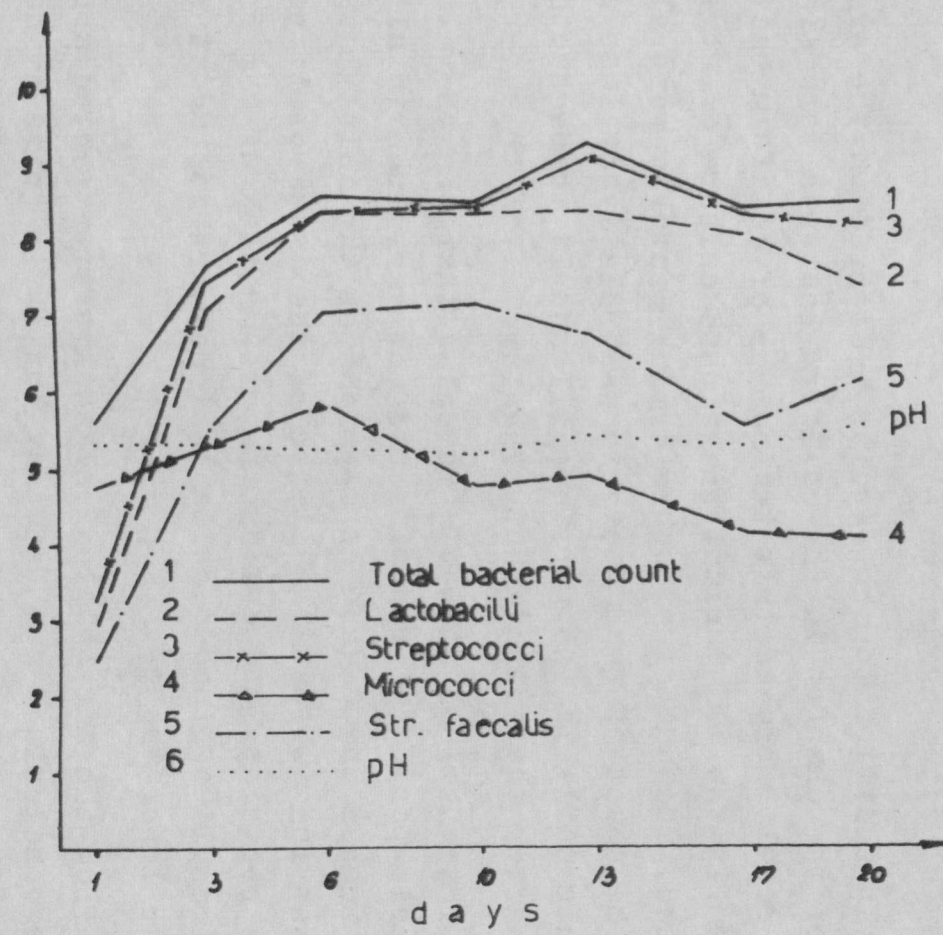
Graph. 1. Dynamic of the development of microflora in the ripening of sausages standard variant



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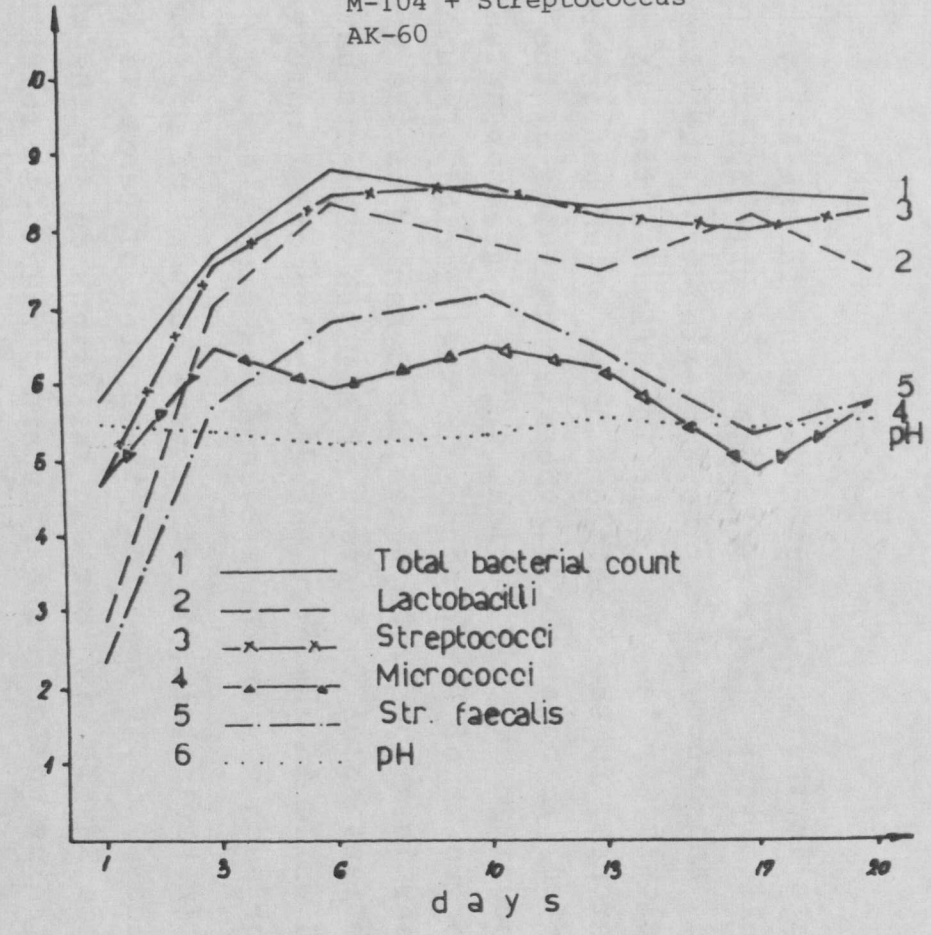
Graph. 3. Dynamic of the development of microflora in the ripening of sausages with Streptococcus Ak-60

Log N



Graph. 4. Dynamic of the development of microflora in the ripening of sausages with Micrococcus M-104 + Streptococcus AK-60

Log N



increase in the total bacterial count in the III variant compares well with the increased number of streptococci in the same period, and hence, it may be suggested that the streptococci grow slowly, or they reach their maximum after the other bacterial groups, in the conditions under which our experiments were conducted. In other words, the maximum value of the total bacterial count in the III variant is, to a greater extent, the result of the growth of streptococci in that period. This phenomenon is clear in graph 5. in which the dynamics of the growth of only streptococci in respect of all the variants have been presented while in the previous graphs the total number of bacteria for each variant have been shown separately.

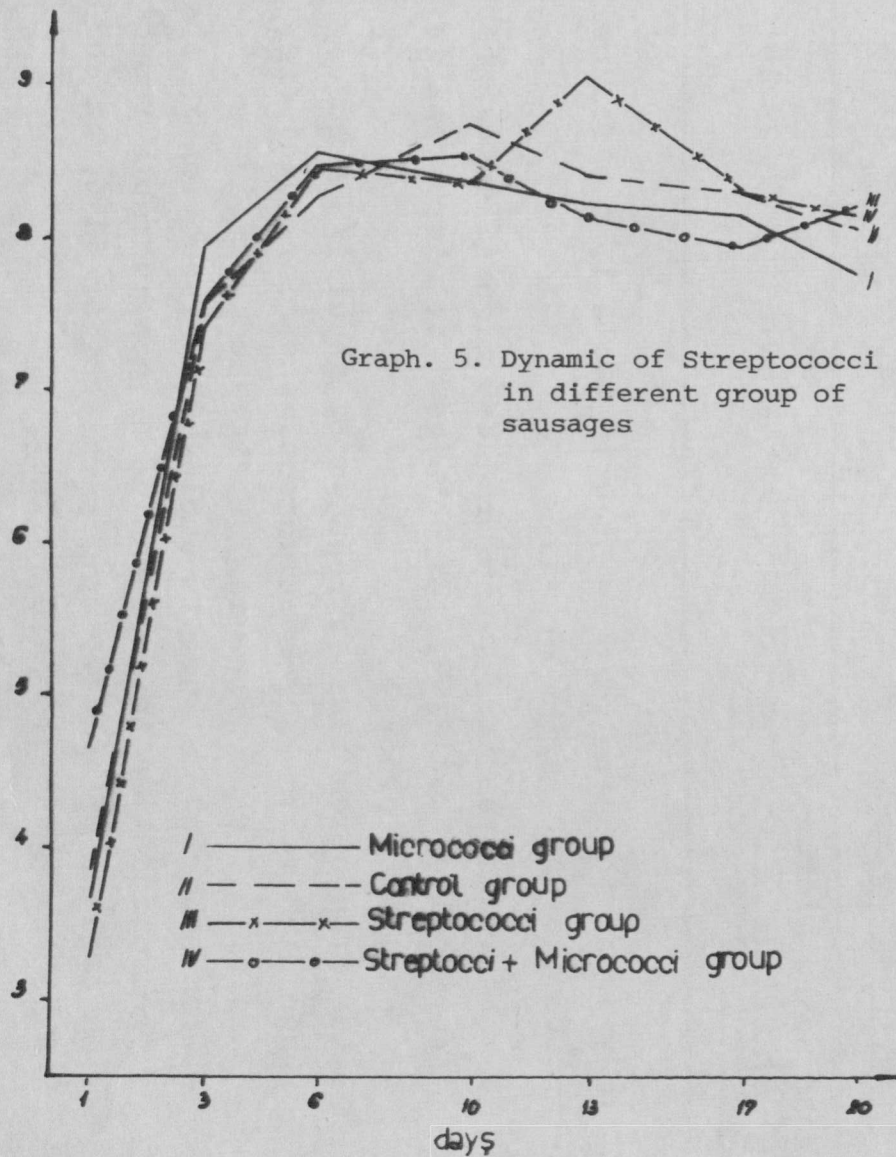
In variant II, in which pure culture of micrococci has been added, it is seen that the number of bacteria remain in the same level, with certain variations during the whole period of ripening while in the standard variant (I) relatively lesser number of micrococci were observed which, however, fall after 9th day of ripening (graph 6).

In addition to the results presented in the graphs, dynamics of the changes in the coliform bacteria were also followed. These results showed that the number of coliform bacteria was minimum in the III variant where pure culture of streptococci was used. The number of sporeforming bacteria were also determined. The count of such bacteria was relatively less in all the variants while in the variants III and IV these bacteria were not found after the 13th day of ripening.

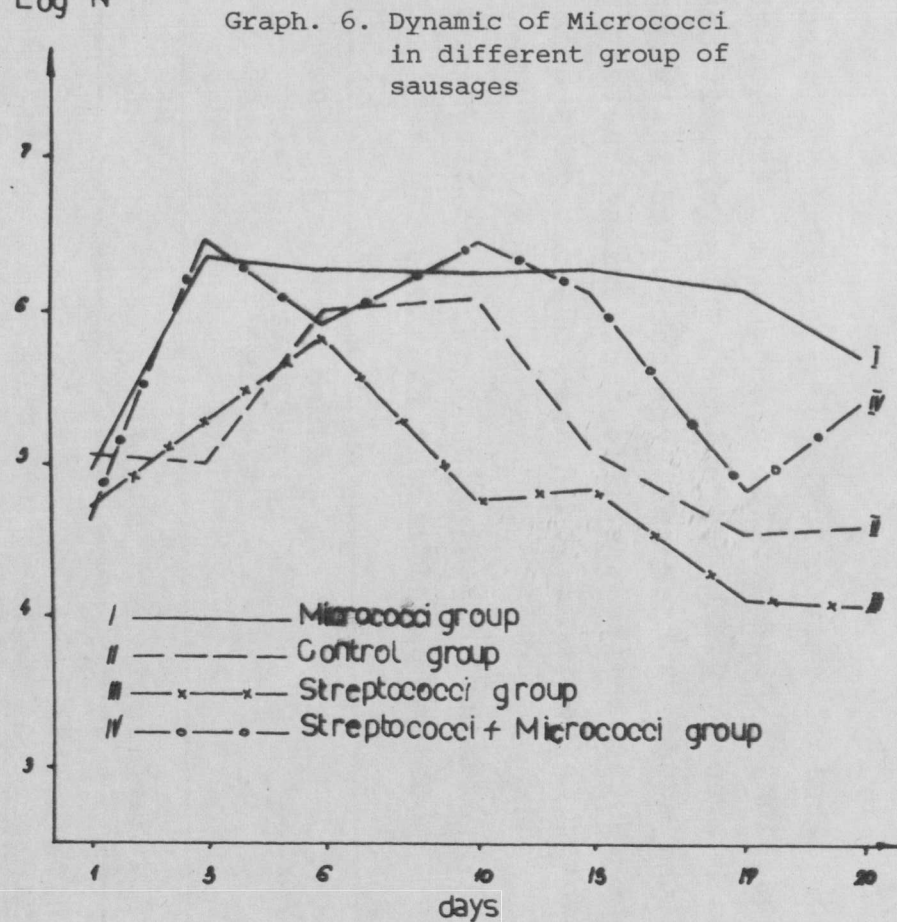
The chemical analysis revealed that the pH values during the whole period of ripening did not vary much in all the variants. Such a phenomenon is apparent as the initial pH of the meat was low (within the limits of the isoelectric point of the muscle proteins) and during the preparation of the sausages additives like glucono-delta-lactone were not added which could further lower the pH.

From the microbiological analysis presented above, no definite conclusions can be made, but it is quite evident that the

Log N



Log N



microbiological cultures which were added influenced the total composition of the microflora and, to an extent, the changes in certain groups of microorganisms. Therefore, it may be suggested that the added starter cultures conducted the ripening process of the sausages with specific biochemical factors. The results presented in tables 1 and 2 also support such conclusion.

Tab. 1. Average fermentation time, loss of weight and moisture content in different sausages

Variant	Average production time in days	% loss of weight	% moisture content
I. Standard variant	21	36.66	22.96
II. With added <u>Micrococcus</u> M-104	18 - 19	34.98	22.82
III. With added <u>Streptococcus</u> Ak-60	17 - 18	34.02	23.15
IV. With added mixture of <u>Streptococcus</u> + <u>Micrococcus</u>	14	16.18	29.15

In view of the results presented in table 1, the following important conclusions may be drawn:

1. the production time is not reduced significantly when pure culture of either streptococci or micrococci is used in the process,
2. the production time is reduced by about 1/3 when conjoint culture of streptococci and micrococci are employed for the ripening of sausages,
3. when conjoint culture is employed, a reduction in the production time is accompanied by considerable reduction of loss of weight, upto an extent of 50 %, as compared with the standard variant,

4. the loss of weight varies in the sausages prepared with pure cultures of either streptococci and micrococci in almost the same limits as in case of standard variant, and
5. the total moisture content is highest in the variant where conjoint culture has been used, but is within the limits which guarantees the shelf life to the product.

The results of the organoleptic evaluation, presented in table 22, do not coincide with the conclusions drawn by NURMI (14) from the standpoint of negative effect of streptococci on the quality of the sausages. On the contrary, our results show that the variant prepared with conjoint culture of streptococci and micrococci had a better taste and aroma than the rest of the variants. On the basis of the other organoleptic characteristics, there were lesser differences, so that it could be said that all the variants were practically alike. However, it may be added that the colour in variant with added streptococci was light in the first few days and later the colour started developing so that at the end of the ripening it was attractive and to an extent permanent as compared with the other variants.

In concluding this preliminary report, we would like to point out that the detailed investigations on this problem are under way. The results of two more experiments in the industrial conditions of production have encouraged us to continue these studies. The ripening process in the sausages in these experiments have been followed by organoleptic evaluation only. The tendency, quite similar to the one reported above, has been observed. In one of these experiments we have used glucono-delta-lactone and found that it influenced positively, specially in the variant in which conjoint culture of streptococci and micrococci have been added. Such a behaviour is in accordance with the results of CORETTI (4, 5), KOTTER et al. (7), NURMI (15) and other authors.

On the basis of the above report we are unable to bring definite conclusions, but we hope that our further investiga-

Tab. 2. AVERAGE VALUES OF ORGANOLEPTIC EVALUATION TEST (point)

V a r i a n t	Appearance	Appearance of the composition & colour cut	Consistency	Aroma	Taste	Colour stability
I Standard	4.48	4.38	4.51	3.57	4.10	3.98
II With added <u>Micrococcus</u> M-104	4.63	4.23	4.12	3.73	3.81	4.03
III With addes <u>Streptococcus</u> Ak-60	4.50	4.15	4.36	3.90	4.12	4.01
IV With added mixture <u>Micrococcus</u> M-104+ <u>Streptococcus</u> Ak-60	4.57	4.61	4.34	4.78	4.92	4.26

tions on the streptococci and micrococci, specially when con-
joint, will be a supplement to improve the technology of fer-
mented meat products.

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CONTRIBUTION À L'ÉTUDE DES CULTURES PURES ET
ASSOCIÉES DES STREPTOCOCCUS ET MICROCOCCUS
PENDANT LE MÛRISSEMENT DES SAUCISSES !

par

Marija Šutić et J. Joksimović

R é s u m é

Le travail comprend les résultats, obtenus dans les études d'application des cultures pures et associées de Streptococcus Ak-60 et Micrococcus M-104 pendant la préparation des saucisses, connus comme les saucisses " touristiques".

L'application de ces cultures dans les saucisses était étudiée dans les combinaisons suivants: 1. Contrôle, sans cultures; 2. Culture pure de Streptococcus Ak-60 ; 3. Culture pure de Micrococcus M-104 et 4. Les cultures associées de Streptococcus Ak-60 et Micrococcus M-104.

Pendant le mûrissement des saucisses les changements étaient étudiés concernant le développement des bactéries, chimiques, physiques et organoleptiques caractéristiques des saucisses.

Les résultats de ces études montraient que la meilleure qualité des saucisses était obtenue en les préparant avec les cultures associées de Streptococcus Ak-60 et Micrococcus M-104. La qualité des saucisses, faits par les cultures pures, était variable; quelquefois elle était meilleure mais quelquefois pire que les saucisses de la contrôle.

BEITRAG ZUR FORSCHUNG DER REINEN UND VEREINIGTEN
KULTUREN DER STREPTOCOCCUS- UND MICROCOCCUSBAKTERIEN
WÄHREND DER WURSTREIFUNG

von

Marija Šutić und J. Joksimović

ZUSAMMENFASSUNG

Die Arbeit enthält die Ergebnisse, bekommen in der Anwendungsforschung der reinen und vereinigten Kulturen der Streptococcus Ak-60 und Micrococcus M-104 Bakterien, während der Reifung der Wurst, die als "Touristwurst" bekannt ist.

Die Anwendung dieser Kulturen in der Wurstvorbereitung wurde in der folgenden Kombinationen untersucht: 1. Kontrolle, ohne Kulturen; 2. Reine Bakterienkulturen der Streptococcus Ak-60; 3. Reine Bakterienkulturen der Micrococcus M-104 und 4. Vereinigten Bakterienkulturen der Streptococcus Ak-60 und Micrococcus M-104.

Im Verlauf der Reifung die Veränderungen in der Wurst untersucht waren, die Entwicklung der Bakterien, chemische, physische und organo-leptische Wurstcharacteristik betreffen.

Die Ergebnisse dieser Forschungen haben gezeigt, dass die beste Wurstqualität erreicht wurde, wenn die vereinigte Bakterienkulturen von Streptococcus Ak-60 und Micrococcus M-104 für die Wurstvorbereitung gebraucht wurden. Die Wurstqualität vorbereitet von reinen Bakterienkulturen variiert; manchmal ist sie besser oder schlimmer als die Wurstkontrolle.

ПРИЛОГ К ИЗУЧЕНИЮ ЧИСТЫХ И СМЕСИ КУЛЬТУР СТРЕПТОКОККИ
И МИКРОКОККИ ПРИ СОЗРЕВАНИИ КОЛБАС

Мария Шутич и Я. Иоксимович

Р е з ю м е

В этой работе приведены результаты исследования применены чистые и смеси культур с Streptococcus Ak-60 и Micrococcus при созревании, так называемых "туристских" колбас.

Эксперимент производился в четырех вариантах: 1. контроль; 2. чистая культур Streptococcus Ak-60; 3. чистая культур Micrococcus M-104 и 4. смесь культур Streptococcus Ak-60 + Micrococcus M-104.

В процессе созревания колбас наблюдалась динамика развития бактерий, физическо-химическая и органолептические свойства.

Результаты этих исследований показывают, что самое лучшее качество колбас получается способом употребления смеси культур Streptococcus Ak-60 + Micrococcus M-104. Колбасы у которых употреблены чистые культуры были в некоторых случаях лучшими, а в некоторых худшими в отношении к контролю.

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S u m m a r y

In this report the results of investigations on the application of pure and conjoint cultures of Streptococcus lactis Ak-60 and Micrococcus M-104 in the fermented sausages known as "touristic" sausage are presented.

The experimental work was carried out with 4 variants: 1. control, without cultures; 2. pure culture a Micrococcus M-104; pure culture a Streptococcus Ak-60 and 4. conjoint cultures of Streptococcus Ak-60 and Micrococcus M-104.

During the ripening of sausages the dynamics of bacterial growth, chemical - physical and organoleptical properties were investigated.

The results from this investigations indicate that the best quality of sausages were with conjoint cultures Streptococcus Ak-60 and Micrococcus M-104. The sausage prepared with pure cultures were sometimes better or similar to the standard variant.