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INFLUENCES OF SOME STARTER CULTURES UPON THE CHANGES IN PROTEINS OF "ŠTAJER" SAUSAGES DURING THE FERMENTATION

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INTRODUCTION

Foodstuffs produced by fermentation are well known. There, first of all, belong milk products, alcocholic drinks, bread, pastries etc. From meat products, to this group belong dry and rapidly fermented sausages and dry meat products. A common characteristic for all these products is technological process where enzymatic activities dominate, and they can be directed by setting the microclimatic factors or by additives /starter cultures, preparations on the basis of glucono-delta-lactone or sugar/. It should be pointed out that all these products, together with fresh meat, had passed through certain fermentative processes. These processes are, however, artificially stopped most frequently by thermical processes, unlike the fermented products where the products of their biochemical activity act preservatively, giving the final products specific organoleptic properties.

For the enzymatic processes in meat and, first of all, in fermented sausages enzyms in meat and in microorganisms are significant. The role of microorganisms becomes remarkably

significant if they are added in the form of so called "starter cultures". Microorganisms during the fermentation of meat change proteins, fat and carbohydrates. The intensity of these changes is directly proportional to the temperature and the length of fermentation, though the decisive role in that has the kind of the present ferments. Of special interest are biochemical changes in sausages during the short fermentation, above all, in rapidly fermented sausages. The shanges in proteins, as the most important component, are of particular influence upon the organoleptic quality, physico-chemical composition, nutritive value and keeping quality of final products.

In the past year great amounts of rapidly fermented sausages were produced, the most typical of which is "Štajer" sausage. Tentative experiments showed that by inoculation of the stuffing of these aausages by starter cultures, are obtained products of high quality. We were intrigued to study, in this work, the influence of some starter cultures /"Baktofermente" and "Duplofermente" upon the changes in proteins of "Štajer" sausage.

II. REVIEW OF LITERATURE

In French literature, in 1919., a suggestion of inoculation of the sausage stuffing by yeasts, for the purpose of getting the characteristic flawour is mentioned /1/. Further trials in the

inoculation of microorganisms into sausages were noted in 1921., and in 1928 /8, 13/. In 1940. Jensen patented the addition of lactobacillus to fermented sausages. After 1954. a number of scientists started to examine microorganisms which might be added as starter cultures to fermented sausages /6, 7, 9, 17, 18, 20, 21/.

In U.S.A. starter cultures are produced from Pediococcus cerevisiae /7, 18/, and in Europe micrococci and lactobacilli are used /19, 20, 21/.

Starter cultures are added to fermented sausages for the purpose of destruction of carbohydrates, flavouring the products and antagonistic action upon the unwanted microflora. Most microorganisms contain proteolytic ferments, which, according to the substrate, can be of endogenous or egzogenous origin. Proteolytic ferments of microorganisms decompose meat proteins. Meat also contains its proteolytic ferments. We do not know whether in the processes of fermentation dominate activities of meat ferments or those of microorganisms.

Niinivaara et al. /21/, Pezacki et al. /23/, Keller et al. /15/ and Bianchi /2/ suggest that decomposition of proteins during the fermentation comes as a result of the activity of meat ferments and those of microorganisms. On the contrary, Maillet et al. /17/ and Giolitti /11/ are of the opinion that the proteolytic activity is a result of the action of meat ferments, while the ferments in microorganisms have little

importance. In her dissertation work, Preac-Mursić /22/, has proved that the decomposition of meat proteins during fermentation of sausages is the result of enzymatic activity of meat ferments and of microorganisms ferments.

Körmendy et al. /16/ have found out that in the fermentation of winter salamy of Hungarian type, the amounts of basic and neutral aminoacids are increased till the 45th day. Above all, the amounts of leucine, phenylalanine, valine, proline, alanine, glycine, threonine, serine, methionine and tyrozine, are increased.

Reuter et al. /25/ have determined that during the fermentation of sausages, the amounts of glutaminic acid, alanine, methionine, leucine, lyzine, hystidine and phenylalanine are increased. The authors suggest that these changes might be the result of the number and the kind of the present microorganisms.

Filipović et al. /4/ have examined the changes of free aminoacids during the fermentation of sausages produced with and without starter cultures, and have found out that during the fermentation, the amounts of threonine, methionine, isoleucine, leucine and phenylalanine are increased.

All mentioned authors have found out that the amounts of free aminoacids in sausages during certain stages of fermentation

are increased. In the quoted results, however, the differences in the kind of free aminoacids are noticed. By the analysis of the above mentioned literature data, it may be conluded that the tested sausages were different the technics of examination and interpretation of the results.

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Taking into account the literature data, and the specifity of our production, the aim of this work was to determine: the influence of starter cultures "Baktofermente" and "Duplofermente" upon the changes in proteins of the stuffing of "Štajer" sausage.

III. METHODIC AND TECHNICS OF WORK

1. The stuffing of "Štajer" sausage is composed of:

Beef of I category	35%
Pork of I category	35%
Solid fatty tissue	30%

2. The composition of the curing solution: Meat for these sausage was cured in the mixture of salts for the dry curing of the following composition:

NaCl	1 kg
$NaNO_2$	6 g
$NaNO_3$	6o g
Sugar	2o g

Of this mixture 2,3 g to 1 kg of the stuffing was added.

3. The stuffing was divided into four groups from which four kinds of samples were made.

a/ to sausages with antibiotics to 1o kg of cured meat were added: 5.6oo.ooo I.U. of penicillin, 1o g of streptomycin and 5.ooo.ooo I.U. of Nystalin.

b/ to sausages with "Baktofermente" this additive in the amount of o,o5% was added.

c/ to sausages with "Duplofermente" o,o5% of "Duplofermente was added.

d/ the sausage without the additive was made only with the addition of starter cultures.

e/ winter salami "Sibiu" of Roumanian provenience was bought at the market.

4. Determination of proteins soluble in water. Proteins soluble in water were determined in water extract by the macromethod by Kieldahl. The extraction was performed at the room temperature in 2 hours, with occasional shaking.

The results were calculated as total content of proteins in the sample.

5. Determination of α-aminonitrogen. The amount of α-aminonitrogen in the tested samples of sausages was determined by formal titration of water extract by the metod of Sörensen.

The extract was prepared by the extraction of the sample with tenfold amount of distilled water at the room temperature and with occasional shaking, for 1 hour.

The method by Sörensen is founded on the blocking of amino groups by neutral solution of formaldehyde, in which the carboxyl group of the aminoacid, i.e. the protein or protein decomposition products are released. The increase od acidity, determined by titration with NaOH solution is the measure of the amount of present α-aminonitrogen.

Calculation:

$$\alpha\text{-aminonitrogen} = \frac{1{,}4 \cdot V \cdot F \cdot 100}{G} \quad /mg\%/$$

where

V = volume o,1 n NaOH

F = factor o,1 in NaOH

G = weight of the sample

1 ml o,1 n NaOH corresponds to 1,4 mg of α-aminonitrogen.

The values are given as mg% to the content of proteins.

6. Determination of free aminoacids. Free aminoacids were determined by chromatography on the ion exchangers by the method by Moore et al. /3/ in the combination with colourimetry. The analysis were performed on the amino-analysator, Beckman-Spince, model 120B. The values are given as % to the content of proteins.

IV. RESULTS

In order to get an insight into the proteolytic processes in sausages during fermentation, there were determined the amounts of:

1. proteins soluble in water,
2. α-aminonitrogen,
3. total number of free aminoacids, and
4. free aminoacids.

1. The amount of proteins soluble in water is a simple indicator of proteolytic degradation of protein molecules. By the splitting of peptide bonds, protein chains become shorter, turning in this way into lower molecular compounds, soluble in water.

In the table 1. it is shown that:

- "Štajer" sausage, inoculated by "Baktofermente", and winter salami "Sibiu" have the highest, and the sausage

with "Duplofermente" the lowest amount of proteins soluble in water.

2. The amount of α -aminonitrogen shows the aminogroups in α position. Their quantity changes are proportional to the changes in aminoacids. The data in the table 1. show that:

   - the highest amounts of α -aminonitrogen have sausages inoculated by"Baktofermente" and winter salami;

   - the lowest of α -aminonitrogen has "Štajer" sausage with antibiotics.

3. Total number of free aminoacids is the highest in "Štajer" sausage inoculated by "Baktofermente", and the lowest in sausages with antibiotics, /picture 1/.

4. The amount of free aminoacids. Picture 2 shows that:

   - the highest amounts of glycine, alanine, methionine, isoleucine, leucine, phenylalanine, hystidine, threonine and glutaminic acid are found in "Štajer" sausage inoculated with "Baktofermente", and the lowest in sausage with antibiotics;

   - thyrozine, arginine and succinic acid are found in the highest amounts in sausages with antibiotics;

   - the highest amount of lyzine is found in sausages without starter cultures;

- the highest amount of proline is found in winter salami "Sibiu".

V. DISCUSSION

All results given in /table 1, pictures 1 and 2/ show that during fermentation of sausages meat proteins are changed. The changes in "Štajer" sausage differ from those in winter salami and in "Štajer" sausage they depend on added starter cultures, antibiotics, i.e. on "home" microflora.

From all given data in the chapter "Results of experiments" it is clear that most intense proteolytic processes occur in "Štajer" sausage inoculated with "Bakterofermente" and in winter salami "Sibiu" and the lightest in sausages with antibiotics and with "Duplofermente" /table 1/, which undoubtedly indicates the enzymatic activity of microorganisms in fermented sausages. These data are in conformity with the opinions of Niinivaara et al. /21/, Pezacki et al. /23/, Keller et al. /15/, Bianchi /2/ and Preac-Mursić /22/.

The obtained differences in the amounts of proteins soluble in water, in α-aminonitrogen /table 1/, and in total number of free aminoacids /picture 1/ show that the changes in meat proteins are dependable on the present microflora. Preac-Mursić /22/ has found out that lactobacilli, out of eight tested groups of microorganisms, are the least proteolytically active.

It completely agrees with our results, for "Duplofermente" contains a considerable percentage of lactobacilli. Micrococci, however, are highly proteolytically active /22/, and also the sausages inoculated with "Baktofermente" /composed of micrococci/ in our tests showed the most intensive changes in proteins.

Changes in meat protein are also particularly explicit in winter salami. That is only logical, because in those products the fermentation lasted for 1oo days, unlike "Štajer" sausages, where fermentation lasted for only 25 days. This sausage, however, in spite of high amounts of α-aminonitrogen /table 1/, does not have a corresponding total number of free aminoacids /picture 1/. This occurence is accountable by decarboxylation of free aminoacids during the long fermentation process. Körmendy et al. /15/ have noticed a similar manifestation. During the fermentation of winter salami of Hungarian type, they registred a decrease in the amount of glutaminic acid, which, due to decarboxylation, turned into aminobutyric acid. It is most interesting that in our experiments also the amount of glutaminic acid in winter salami was the lowest of all /picture 2/. It is very characteristic that in winter salami there is the highest amount of proline.

From all above mentioned results that starter cultures "Baktofermente" among athers, express also proteolytic activity upon

meat proteins. These changes are in sausages produced with "Duplofermente" considerably weaker. That might be explained by specific characteristics of microorganisms, of which starter cultures are composed, as well as by their biochemical activity. "Baktofermente" are startercultures composed of micrococci which are, as it is well known, proteolytically more active than lactobacilli found in "Duplofermente". "Baktofermente" however, in the first, most decisive days for biochemical changes caused by fermentation of sausages do not add to a decrease of pH of the stuffing, which is the basic characteristic of "Duplofermente" /5/. A changed pH of the sausage stuffing might be an important element for stimulation or inhibition of the activity of proteolytic meat ferments, and the present microorganisms.

All above mentioned indicates to the fact that the composition of microflora is a very important factor for the changes in meat proteins during fermentation of sausages.

VI. CONCLUSIONS

1. Sausages produced with "Baktofermente" show the highest amounts of proteins soluble in water, α-aminonitrogen and free aminoacids.

2. Sausages with "Duplofermente" have explicitly the lowest amounts of α-aminonitrogen, and considerably less free aminoacids than the sausages produced with "Baktofermente".

3. Winter salami has the highest amounts of proteins soluble in water and α-aminonitrogen, and the lowest amounts of free aminoacids.

4. During the production of "Štajer" sausages, high amounts of glycine, methionine, isoleucine, leucine, phenylalanine, hystidine, threonine and glutaminic acid are released in sausages with "Baktofermente".

5. In winter salami there are explicitly the lowest amounts of glutaminic acid.

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RESTLICHE WASSERLÖSLICHE EIWEISSSTOFFE UND α-AMINOSTICKSTOFF IN STEIRERWURST UND WINTERSALAMI

BENENNUG DER WURST	ZUSÄTZE	% WASSERLÖSLICHE EIWEISSSTOFFE	mg % α-AMINO-STICKSTOFF
STEIRERWURST	ANTIBIOTIKA	0,21	3,36
—"—	„BACTOFERMENTE"	0,23	5,00
—"—	„DUPLOFERMENTE"	0,20	3,89
—"—	———	0,21	4,21
WINTERSALAMI „SIBIU"		0,23	5,57

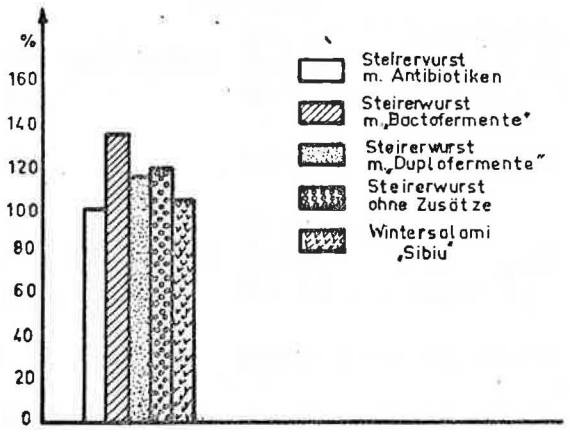

Abb.1. FREIE AMINOSAUREN IN STEIRERWURST UND WINTERSALAMI

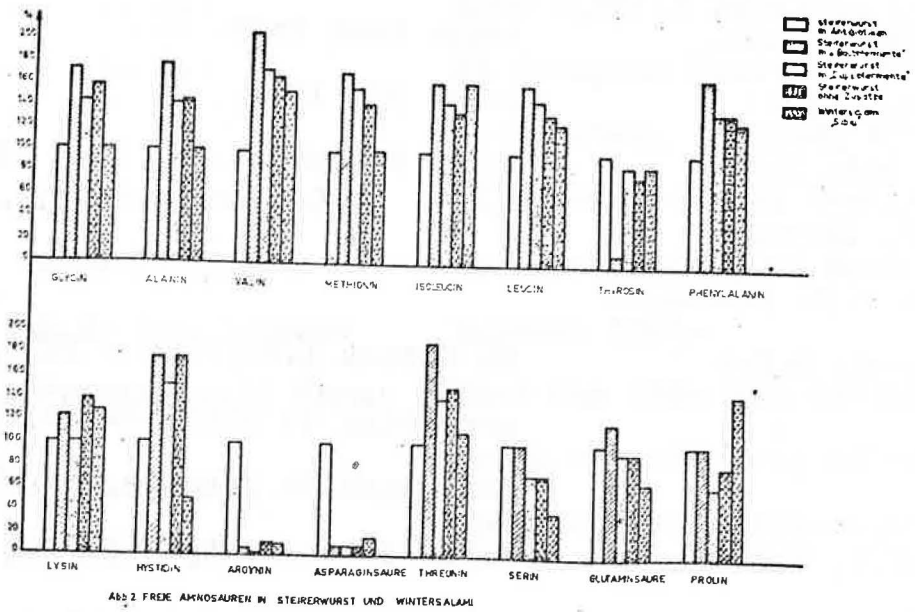

Abb.2 FREIE AMINOSAUREN IN STEIRERWURST UND WINTERSALAMI

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