# PROTEOLYTIC ACTIVITY OF SELECTED MICROCOCCAL STRAINS AND THEIR INFLUENCE ON THE FREE AMINO ACID COMPOSITION OF BULGARIAN RAW-DRIED SAUSAGE

R.N.BORPUZARI*, K.BOSCHKOVA, S. KATSAROVA, K. VALKOVA
Higher Institute of Food and Flavour Industries,
26, Maritza Blvd., Plovdiv, Bulgaria-4000 (Fax: 032 - 44-01-02).

## SUMMARY

The proteolytic activity of 56 micrococcal strains selected on the basis of their nitrate reductase activity out of a total of 401 strains isolated from Bulgarian raw-dried sausage were studied at 12 and 30°C after 6 and 72h of incubation by spectrophotometric determination of the tyrosine value in meat extracts and in casein. Presence of $NaNO_3$ (0.016%) and NaCl (2.4%) improved the proteolytic activity of the selected 8 micrococcal strains. Sausages prepared with the use of starter culture of micrococcal strain M160 (this strain possessed the maximum proteolytic activity) contained the maximum free amino acids (4520.11 mg/100g dry matter) followed by sausages prepared with strain M483 (3457.27 mg/100g dry matter) and the least was in the control samples prepared under natural fermentation.

## INTRODUCTION

During the process of ripening, the sausage mix of fermented raw-dried sausage undergoes complex physico-chemical and biochemical changes as a result of the actions of the muscle tissue enzymes and those released by the bacterial cells. The proteolytic enzymes of the starter cultures cause partial breakdown of the meat protein and the quantity of the non protein nitrogen (NPN) and free amino acids are increased which improve the organoleptic properties and nutritional quality of such products.

Different species of *Micrococcus* are used as starter culture in the production of 'European - style' dry sausages. Eventhough several authors have reported on the proteolytic activity of micrococci isolated from dairy origin and their influence on the eating qualities of dairy products (Bhowmik and Marth, 1988, 1990; Nath and Ledford, 1972), very little information is available on the proteolytic activity of micrococci in the meat mass. However, Selgas *et al.* (1993) have reported recently on the proteolytic activity

* Lakhimpur College of Veterinary Science, Assam Agricultural University, North Lakhimpur, India, PIN 787 001.

of micrococci isolated from Spanish dry fermented sausages.

In the present work, the proteolytic activity of few selected micrococcal strains isolated from Bulgarian raw-dried sausages 'lukanka' was studied and based on their proteolytic and other activities few strains were selected for further use as starter culture in the production of such sausages. The influence of the strains finally selected for use as starter culture on the changes in the composition of free amino acids of lukanka during different stages of its production was also investigated.

## MATERIALS AND METHODS

A total of 56 micrococcal strains possessing 'strong' and 'intermediate' nitrate reductase activity (Borpuzari and Boschkova, 1993a) were used in this study. Their proteolytic activity was determined first on casein (2%) and also in a model system containing meat extract. *Longissimus dorsi* muscle of freshly slaughtered beef and pork carcasses was cut into small pieces and were mixed at the ratio of 60 : 40, respectively. This meat mix was then homogenised with the addition of 3 parts of distilled water for 22h at 4 ± 1°C, after which the homogenate was filtered through cheese cloth and sterilised by Seitz filtration. The filtrate was aseptically transferred in 2ml quantities into test tubes and 2ml of the active broth cultures of the tested strains containing approximately $10^6$ cfu/ml were added and incubated at 12 and 30°C for 6 and 72h. In case of the control sample, the bacterial culture was substituted with 2ml of 1% NaCl solution.

After the incubation period, unhydrolysed protein fractions were precipitated with 4ml of 5% trichloroacetic acid solution for 20 min. The precipitated solution was then filtered through filter paper (Filtrak 388) and the tyrosine value ($\mu$mol/ml) was estimated spectrophotometrically (Spectrophotometer PYE Unicam PU 8800 uv/vis, Philips) at 670 nm as per method of Pearson (1968).

The influence of curing salts (NaCl 2.4% and $NaNO_3$ 0.016%) on the proteolytic activity of 8 selected micrococcal strains was also investigated in the meat extract after incubation at 12°C for 48h and 7d.

Based on the proteolytic and lipolytic activity ( details of the lipolytic activity of the micrococcal strains are presented in an accompanying paper), 2 strains were selected for use as starter cultures. Three batches of lukanka were produced with the use of these 2 strains as monoculture at the rate of $10^7$ cfu/g sausage mix with a simultaneous control batch under standard processing conditions (Borpuzari and Boschkova, 1993b). The quantity of the free amino acids in the fat free sausage mass were determined during different stages of production ( viz., sausage mix, 24h after the initial fermentation phase, 3rd, 6th,13th d of ripening and the finished product) as per method of Benson *et al.*

(1967) in the amino acid analyser (model AAA-881, Mikrotechna, Praha) with the length of the bigger column- 60cm and the smaller- 5cm using sodium citrate buffer with pH 3.25, 4.25 and 5.28. The quantity of the free amino acids was expressed as mg/100g dry matter.

## RESULTS AND DISCUSSION

Results of the proteolytic activity of the selected micrococcal strains on casein (2%) and meat extract expressed as tyrosine value are presented in Table 1. All the 56 strains showed proteolytic activity on casein; however, 18 strains were non proteolytic on meat extract. This may be due to the substrate specificity of the proteolytic enzymes of the strains. In general, the proteolytic activity of the strains were higher at 30°C than at 12°C. In case of casein, exceptions were noted in case of strains M325, M328, M630 and M634 which showed higher proteolytic activity at 12°C. Only 9 and 5 strains showed proteolytic activity after 6 h of incubation at 30°C on casein and meat extract, respectively. However, their activity was comparatively lesser at 6h of incubation than after 72h of incubation at both the temperatures. None of the tested strains showed proteolytic activity on casein as well as on meat extract after 6h of incubation at 12°C.

Results of the influence of $NaNO_3$ (0.016%) and NaCl (2.4%) on the proteolytic activity of 8 selected micrococcal strains are presented in Table 2. All the strains showed higher proteolytic activity in meat extract containing these curing ingredients after 48h and 7d of incubation at 12°C as compared to their activity on meat extract alone. In both the substrates, the proteolytic activity of the micrococcal strains was more pronounced after 7d of incubation with the exception of strain M318 which showed lesser activity on meat extract after 7d of incubation as compared to 48h. This may be due to the presence of tyrosine decarboxylase. Similar observations were also made by Selgas *et at.* (1993). This strain, however, did not show similar tendency in meat extract containing the curing ingredients in our experimental conditions.

On the basis of the above results it has been established that the strain M160 possessed the most desirable proteolytic activity. Similarly, strain M483 possessed the best lipolytic and good proteolytic activity (details on the lipolytic activity of this strain are presented in an accompanying paper). As such these 2 strains were selected for potential use as starter cultures in the production of Bulgarian raw-dried sausages.

Changes in the composition of the free amino acids of lukanka produced by the use of the abovementioned 2 micrococcal strains as starter cultures are presented in Table 3. From the results presented in Table 3 it is seen that the quantity of the total free amino acids of both the treated and the control samples increased during the process of ripening. Sausages prepared with the starter cultures had significantly ($P < 0.05$) higher free

amino acids content than the control samples. Between the treated samples, the one prepared with the use of the strain M160 had the maximum free amino acids content.

No definite pattern of changes in the quantity of the individual free amino acids during different stages of ripening was observed. Certain amino acids (lysine, histidine, serine, threonine, aspartic acid etc.) showed an increase in their concentration with the advancement of the process of ripening, while the concentration of some other fluctuated (methionine, isoleucine, proline etc.), and in case of arginine and tyrosine there was a decrease in their concentration.

In the finished product, concentration of histidine was the highest in all the 3 groups of sausages which was, however, the maximum in sausages prpepared with M160. In case of sausages prepared with M160, next to histidine, the quantity of lysine, glutamic acid and leucine was at the maximum and the concentration of cysteine, arginine and tyrosine was the lowest. Glutamic acid, lysine and leucine were at the maximum concentration after histidine in sausages prepared with M483 and the least were the concentrations of arginine, cystine, and aspartic acids. In case of control samples, alanine, serine and glutamic acids were next to histidine and arginine, cystine, aspartic acid, tyrosine, and valine were found in much smaller quantities.

The increase in the concentration of the free amino acids in the treated samples may be explained due to the proteolytic activity of the micrococcal strains. Therefore, the quantity of the free amino acids in the finished products of sausages prepared with M160 was more as compared to M483.

Kormendy and Gantner (1962) and DeMasi *et al.* (1990) also reported increase in the concentration of free amino acids in sausages with the use of starter cultures. However, Niinivaara *et al.* (1964) observed that the increase in the concentration of free amino acids in sausages was chiefly due to the muscular tissue enzymes than to the proteolytic activity of the microbial enzymes.

## CONCLUSION

Micrococcal strains isolated from Bulgarian raw-dried sausage exhibited proteolytic activity on casein and meat extract and their activity was dependent on incubation temperature. Some strains exhibited substrate specificity in their proteolytic activity. Presence of curing salts appeared to improve the proteolytic activity of the micrococcal strains. Use of selected micrococcal strains as starter cultures in the production of raw-dried sausage increased the concentrations of free amino acids. Out of the individual free amino acids, concentration of histidine was the highest in the finished products.

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