# LIPOLYTIC ACTIVITY OF SELECTED MICROCOCCAL STRAINS AND THEIR INFLUENCE ON THE FREE FATTY ACIDS COMPOSITION OF THE BULGARIAN RAW-DRIED SAUSAGE

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## SUMMARY

The lipolytic activity of 56 micrococcal strains isolated from Bulgarian raw-dried sausage 'lukanka' was studied. Their abilities to hydrolyse fat were first determined on triolein and then by quantitative determination of the free fatty acid number and the free non-volatile fatty acids on sub-cutaneous pork fat after incubation at 30°C for 48h. Strains M160 and M483 were finally selected for using as starter cultures in the production of the raw-dried sausage and the quantity of the free non-volatile and volatile fatty acids were determined during different periods of the production process and in the finished products, respectively. It has been observed that the free non-volatile fatty acids increased gradually from the initial fermentation process to the finished products. Sausages prepared with the strain M483 contained significantly higher free non-volatile fatty acids (0.380mg/g fat) compared to sausages prepared with the strain M160 (0.335mg/g fat) and the control samples (0.289mg/g fat) prepared under natural fermentation process.

Similarly, the quantities of the free volatile fatty acids were higher in sausages prepared with the strain M483 (1.793 $\mu$g/g fat) than in sausages prepared with the strain M160 (1.723 $\mu$g/g fat) and the control samples (1.191 $\mu$g/g fat).

## INTRODUCTION

Different species of micrococci have long been used in the meat industry as starter cultures mainly due to their ability to reduce nitrate for improving the colour characteristics of the cured meat products. These microorganisms are also endowed with proteolytic (Selgas *et al.*, 1993) and lipolytic activities (Cantoni *et al.*, 1967; Talon *et al.*,1992) which contribute to the flavour development of the product.Many micrococci show lipolytic activity against triglycerides, including long chain fatty acids. However, most of these work have been done on micrococci isolated from dairy origin (Stadhouders, 1974).

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In the present work, the lipolytic activity of few selected micrococcal strains isolated from Bulgarian raw-dried sausage was studied. Their influence as starter cultures on the free fatty acids content of the sausage was also investigated.

## MATERIALS AND METHODS

A total of 56 micrococcal strains possessing 'strong' and 'intermediate' nitrate reductase activity (Borpuzari and Boschkova, 1993) were used in this study.

Lipolytic activity of these strains was first determined qualitatively on triolein as per method of Kouker and Jaeger (1987) - experiment I. In experiment II the titratable free fatty acids on subcutaneous pork fat was determined as per method of Nielsen and Kemner (1989), and in experiment III, the quantity of the free non-volatile fatty acids released by the strains from subcutaneous pork fat was deter mined as per method of Nielsen and Kemner (1989) with modifications. Methylation was done as per method described by Hartman and Lago (1973) and the conditions of the gas chromatography were as follows :

Gas chromatograph - Fractovap 2407 T, Karlo Erba, Italy; detector - flame ionization detector; columns - stainless steel, length 2000 mm and $\phi$ 2mm packed with 10% LAC 886 on chromosorb G silylated 60/80 mesh; temperature of columns - initial temperature programmed at 140°C, rate of increase 5°C/min upto 210°C; gas carrier - nitrogen with the rate of flow 30 ml/min; rate of chart speed- 10 mm/min.

The quantity of the free non-volatile fatty acids of sausages during different stages of ripening was determined as follows :

0.5 g of fat extracted by diethyl ether as per method of Soxhlet was dissolved in small quantity of diethyl ether and placed on thin layer chromatographic plates of silica gel ( Merck, 60 F 254 ). The plates were developed in a mixture of hexane, diethyl ether and glacial acetic acid ( 80 : 20 : 1 ) for about 35 min. Fatty acid fractions were scrapped off and methylated as per method of Hartman and Lago (1973) and the quantity of the free non-volatile fatty acids was determined gas chromatographically as above.

The quantity of the free VFAs in the finished product of the sausages was determined by steam distillation of the samples as per method of Halvarson (1972) with modifications. The collected distillate was evaporated under vacuum and the dried residue was dissolved in 1 ml of 1 N trichloroacetic acid. The quantity of the free VFAs was determined gas chromatographically as above with modifications in the conditions of the gas chromatography - initial temperature was programmed at 120°C upto 160°C.

## RESULTS AND DISCUSSION

The qualitative determination of the lipolytic activity of the selected 56 strains of micrococci on triolein showed that 4 strains, namely, M334, M537, M623, and M634 did not possess lipolytic activity. These strains also did not exhibit lipolytic activity in

experiments II and III.

The results of the experiment II are presented in Table 1. From the table it is seen that the strain M483 showed the highest lipolytic activity (11.24 mg KOH/g fat). Strains M33, M411,M456, M56, M591, and M593 also showed good lipolytic activity (9.68, 9.07, 6.98, 6.51, 6.47, and 6.44 mg KOH/g fat, respectively).The quantities of the titratable fatty acids released by the micrococcal strains in our experiment were somewhat higher than those reported by Montel *et al.* (1992) on *Staphylococcus saprophyticus* M31. Similarly, Demeyer *et al.* (1974) also reported lower values of titratable fatty acids for strains of micrococcaceae.

The results of the experiment III are presented in Table 2. From the results it is seen that strain M483 released the maximum quantity of free non-volatile fatty acids (5.544 mg/g fat) followed by strains M411 (4.50 mg/g fat), M33 (4.398 mg/g fat) and M456 (3.469 mg/g fat). Strains M483,M33,M411,M593 released the maximum quantity of oleic acid ($C_{18:1}$).

Cantoni *et al.* (1967), however, reported that the strain *Staphylococcus aureus* ATCC 78 released preferentially oleic acid from subcutaneous pork fat. The control sample also contained 0.4672 mg free nonvolatile fatty acids per g fat which showed that the lipolytic enzymes of the adepose tissues might influence the accumulation of the free fatty acids in the sausages.

On the basis of the above results, strains M483 has been selected for use as starter culture. Strain M160 which has best proteolytic and somewhat weaker lipolytic activity (details on the proteolytic activity of the strain are presented in an accompanying paper) has also been selected for similar purposes.

Results on the influence of these 2 strains of micrococci as starter culture on the free volatile fatty acids (VFA) in the finished products of lukanka are presented in Table 3. The quantity of the VFA in both the treated samples was significantly higher ($P < 0.05$) than the control samples. Of the VFAs, acetic acid was found in maximum concentration. Halvarson (1972) also reported increase in the VFAs of the finished products of sausage.

The results on the influence of the micrococcal strains on the free non-volatile fatty acids of sausages during stages of their production are presented in Table 4. From the results it is seen that the quantity of total non-volatile fatty acids increased from the sausage mix to the finished product in both the treated and the control samples. Sausages prepared with M483 contained maximum amount of non-volatile fatty acids than those prepared with M160 and the control sample. In the finished products, concentration of oleic acid ($C_{18:1}$) was the maximum followed by palmitic + palmito-oleic acids ($C_{16:0}$ + $C_{16:1}$).

These increase of the free volatile and non-volatile fatty acids in the finished product, particularly in case of the sausages prepared with strain M483 is in agreement with its

good lipolytic activity.

## CONCLUSION

Micrococcal strains isolated from Bulgarian raw-dried sausage exhibited lipolytic activity on triolein and subcutaneous pork fat. Use of micrococcal strains possessing lipolytic activity as starter cultures in the production of raw-dried sausages increased the concentration of free volatile and non-volatile fatty acids in the finished products.

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