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INFLUENCE OF pH AND DIFFERENT CONCENTRATION OF NaCl ON LIPASE
ACTIVITY OF SOME STARTER CULTURES (MICROCOCOCCUS STRAIN 199/10
AND LACTOBACILLUS STRAIN 4669/6) USED IN THE PRODUCTION OF
RAW DRIED MEAT PRODUCTS

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The study of enzymatic activity of microorganisms have an essential theoretical and practical significance.

Microbial lipases represent enzymes from the group of esterases, which have not as yet been studied totally (2,3,5,7). The studies already carried out are mainly dealing with industrial and medical enzymatic preparations (1, 4).

A definite interest represent the studies of microbial lipase activity, used in meat industry as starter cultures, because of their active part in the forming of taste and flavour of meat products; at the same time if their action on fats is stronger they induce some disagreeable changes on their quality. The action of lipase is qualified by the factors of environment: pH, substrates, temperature and others (1).

The scope of this paper is investigations on the changes in the lipase activity of starter cultures as related to pH and different concentrations of NaCl, practically used in the production of raw dried meat products.

MATERIAL AND METHODS

Two starter cultures are used, namely *Micrococcus* strain 199/10 and *Lactobacillus* strain 4669/6. The material for the studies is taken during the active exponential phase (12th hour after inoculation for strain 199/10 and 16th hour for strain 4669/6) after cultivation on a shuttle apparatus at 37°C in 500 ml nutritive medium (MPB+ KH_2PO_4). The studies are made with washed resting microbial cells and medium cultures (6).

Lipase activity was determined after the following methodics: in an Ehrlenmeyer flaske are introduced 2 ml olive oil and 1,5 ml phosphate puffer having different pH (5,2; 5,8; 6,2; 7,0 and 8,0). The contents is well mixed and the control flasks are separated, while in the test ones is introduced 4,5 ml microbial suspension or cultural medium. After intensive shaking for 3 minutes the flasks are put into an incubator at 37°C for 24 hours, after which in the controls immediately before titration is added a quantity of the investigated material (cultural medium or microbial cells). In all flasks is introduced 18 ml ethanol and 15,5 ml ether and are titrated with 0,1N NaOH in the presence of phenolphtholein.

To determine the influence of NaCl on lipase activity, the investigations were made with concentrations actually used in practise - 1,8; 2,8; 3,5%. The NaCl is introduced in the system along with the phosphate puffer of pH 5,8.

The activity of the enzyme is expressed in ml 0,1N NaOH, necessary for neutralisation of the formed fatty acids, evaluated for 100 ml culturale medium or 100 mg cell carbon.

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RESULTS AND DISCUSSION

The pH of medium influence on lipase activity for the investigated starter cultures is given in table 1.

Lipase activity with different pH of medium for micrococcus strain 199/10 and lactobazillus strain 4669/6

TABLE 1

pH	Lipase activity in ml 0,1N NaOH			
	Strain 199/10		Strain 4669/6	
	100 ml of culturale medium	100 mg of cell carbon	100 ml of culturale medium	100 mg of cell carbon
5,2	12,0	0	24,0	0
5,8	19,8	1,83	28,00	4,62
6,2	13,2	0	14,0	0
7,0	6,0	0	10,0	6,18
8,0	22,9	1,98	3,8	0

Data show that lipase activity of the cultural medium is higher than that of microbial cells for both strains. From the investigations of different pH values, the lipase activity for micrococcus is higher in the alcalic zone with pH 8,0 in the culture medium (22,9 units) and the microbial cells (1,98 units); in the acid zone with pH 5,8, in the culture medium it attains 19,8 units, in the microbial cells - 1,83 units. For other pH value (5,2; 6,2 and 7,0) to lipase activity was evident in the microbial cells. Highest lipase activity possesses the lactobacillus, attaining in the acid zone with pH 5,8 - 28 units. With pH 7,0 is observed highest lipase activity of microbial cells, which is not present with pH 5,2; 6,2 and 8,0.

The influence of different NaCl concentrations used in meat processing, on lipase activity of microbial cells and culture medium of both strains is given in table 2.

Data show, that the presence of NaCl in the medium inhibits lipase activity of the culture medium for both strains. The concentration 1,8% of NaCl gives the slightest inhibition, with a 2,8% NaCl concentration the inhibiting action is increased to 32,8% (for strain 199/10) and 21,4% (for strain 4669/6). The inhibition process was highest with concentration for NaCl - 3,5% . Salt exhibits a stronger inhibition effect on lipase activity of micrococcus strain 199/10 (to 49,5%).

TABLE 2

Concentration of NaCl in %	Lipase activity in ml 0,1			
	Strain 199/10		Strain 4669/6	
	100 ml of culture medium	% of inhibition	100 ml of culture medium	% of inhibition
1,8	15,8	20,4	23,9	14,6
2,8	13,3	32,8	22,0	21,4
3,5	10,0	49,5	19,9	28,9
Control	19,8	0	28,0	0

The results of these investigations have scientific significance with practical aspects in the studies and application of starter cultures in meat processing.

RESULTS

1. It is established that the lipase activity of microbial cells of micrococcus strain 199/10 and lactobacillus strain

4669/6 is significantly weaker than that of the culture medium.

2. The lipase activity of microbial cells and culture medium of lactobacillus strain 4669/6 is higher than that of micrococcus strain 199/10.

3. The lipase activity of microbial cells and culture medium of the investigated starter cultures is highest with pH 5,8. Micrococcus strain 199/10 possesses a second optimum of lipase activity with pH 8,0, while lactobacillus strain 4669/6 with the other pH values shows a decline of the lipase activity.

4. NaCl exhibits well expressed inhibiting action on the lipase activity of both strains, which action is increased with the increase of the NaCl concentration, and this has a practical significance.

L I T E R A T U R E

1. Arsids, I. M. - Fermentnaia i spirtovaia promishlenost, 8, 27, 1967
2. Bours, J.A., D. Mossel - Microbiol. a. Serology, 25, 1, 29, 1969
3. Narasaki, T., J.A. Tamura, K. Arima - Agric. Biol. Chem., 32, 12, 1453, 1968
4. Okunev, O.N. Lipasa salatistova stafilokoka. Dis. Wolgograd, 1970
5. Ota, V.A., K. Vamada - Agric. Biol. Chem. 30, 4, 351, 1966
6. Stoychev, M., G. Djejeva, F. Niinivaara - Symposium für Starterkulturen, Helsinki, 1972
7. Voshida, F., N.A. Motal, E. Ichima - Appl. Microbiol., 16, 6, 845, 1968