Studies on the nitrate reductase and catalase activities of micrococci in connection with their freezing and freeze-drying

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The application of freeze-dried preparations of starter cultures in meat industry offers a number of advantages: a possibility of centrally controlled manufacture and easy handling (Gayer, 1979). The question arises of whether, in the processes of freeze-drying, the cultures preserve their viability and metabolic activity, and whether they can be used adequately, just like broth cultures.

The major property of the micrococci and staphylococci, used as starter cultures in the manufacture of meat products, is their nitrate reductase activity, necessary in colour formation (Junker and Liepe, 1981; Kuusela et al., 1978; Pfeil and Liepe, 1974; Liepe, 1976, 1978). Further, catalase synthesis is of great significance, since that enzyme is necessary for neutralizing the peroxides resulting from the metabolism of a number of microorganisms, including lactobacilli (Rozier, 1971; Tjaberg, 1969).

The objective of the present work is to study the changes in the nitrate reductase and catalase activities of micrococci employed as starter cultures, after freezing and freeze-drying.

Material and Methods

Micrococcus varians strains M_{16} and M_{19} and Staphylococcus saprophyticus strain M_{95}, isolated from specific Bulgarian raw-dried meat products, were used in the experiments. Work was done with 24 h broth cultures with microorganism numbers of 10^8–10^9 cells/ml. Following the addition of dry skimmed cow milk as a protector, they were frozen at -30°C or -65°C in air, under the conditions of forced air convection, and freeze-dried (Tsvetkov, 1979; 1981). The studies of the frozen and the freeze-dried preparations were made immediately after thawing (at 20°C), or the restoration of the initial volume of the dry culture and after reinoculation for repairing in liquid media to obtain 24 hour broth cultures.

The nitrate reductase activity of the samples was determined by Puolanne's (1977) modification of Egami and Taniguchi's (1970) method and is expressed in terms of micromols of nitrite/ml. The capacity of the microorganisms under investigation to reduce nitrate was determined at a nitrate concentration of 160 mg%, at pH 6,0 and a temperature of 44°C.

Catalase activity expressed as a catalase index, was determined by the method of Lück (1962), at pH 5,4, a temperature of 25°C and 0,6% H_2O_2.

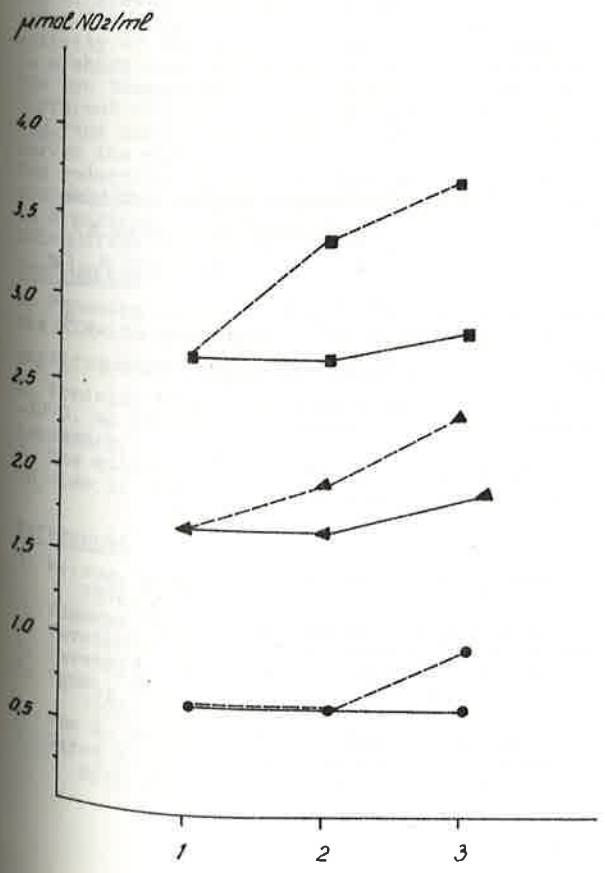

Fig. 1. Changes in the nitrate reductase activities of strains M_{16} (●); M_{19} (▲); and M_{95} (■)

1. Broth solutions
2. Frozen cultures:
   —— at -30°C
   --- at -65°C
3. Freeze-dried cultures

Results and Discussion

The results obtained in the study of the nitrate reductase activities of the three micrococcus strains in broth, following freezing and freeze-drying, are shown in Fig. 1. They present the means of 12 comparative studies.

The initial broth cultures of the three micrococcus strains, numbering 10^8-10^9 cells/ml, were found to have different nitrate reductase activities. The means for the three strains are 0,64 µmol NO_2/ml for M_{16}; 1,65 µmol NO_2/ml for M_{19}; and 2,66 µmol NO_2/ml for M_{95}.

After freezing broth cultures at -30°C, the nitrate reductase activities of all the three strains studied do not change substantially, while with -65°C, a certain increase is observed in the activities of strains M_{19} and M_{95}.

After freeze-drying, an increase in nitrate reductase activity is observed in all the three strains under investigation, an increase, which is more significant in freeze-dried cultures after freezing at -65°C, and constitutes an average of 1,00; 2,37; 3,70 µmol NO_2/ml, respectively, for M_{16}, M_{19} and M_{95}.

The attempts to repair the treated cultures in MPB for 24 h brought about different results, depending on freezing temperature. Freezing the cultures at -30°C results in a lowering of the nitrate reductase activities of repaired cultures, and freezing at -65°C, in its enhancement. The repairing of the cultures after freeze-drying leads to a sharp decrease in activity with both temperatures for the three strains.

A linear relationship was found between nitrate reduction and the counts of the microorganisms studied in the range from 9,0 x 10^7 to 1 x 10^{10} cells/ml, in the frozen and freeze-dried cultures, which is in conformity with the data of Kuusela et al. (1978). At higher concentrations of of microorganisms the substrate is completely exhausted, which is due, as suggested by Kuusela et al. (1978), Pfeil and Liepe (1974), to the high cell numbers, 10^{10}-10^{11} cells/ml, that reduce not only nitrate, but also nitrite.

The rise observed in nitrate reductase activity, with a preserved number of microorganisms, after freezing at -65°C, is due probably to an increase in membrane permeability. The major reason for that is the dehydration of membranes and the hyperconcentration of electrolytes exerting a dissociating effect on lipoproteid complexes (Belous et al., 1976).

The data obtained in the study of freezing and freeze-drying effects on catalase activity expressed as the catalase index of the strains under investigation, are shown in Figs. 2 and 3.

The catalase activity of broth cultures numbering 10^8-10^9 cells/ml, varies, on the average, for M_{16}, from 0,01 to 0,07 x 10^6; for M_{19}, from 0,025 to 0,045 x 10^6; and for M_{95}, from 0,006 to 0,055 x 10^6.

After freezing at -30°C (Fig. 2), differences are found in the activities of the individual strains. A strong decrease is observed in the catalase activity of strain M_{19}, and a sharp

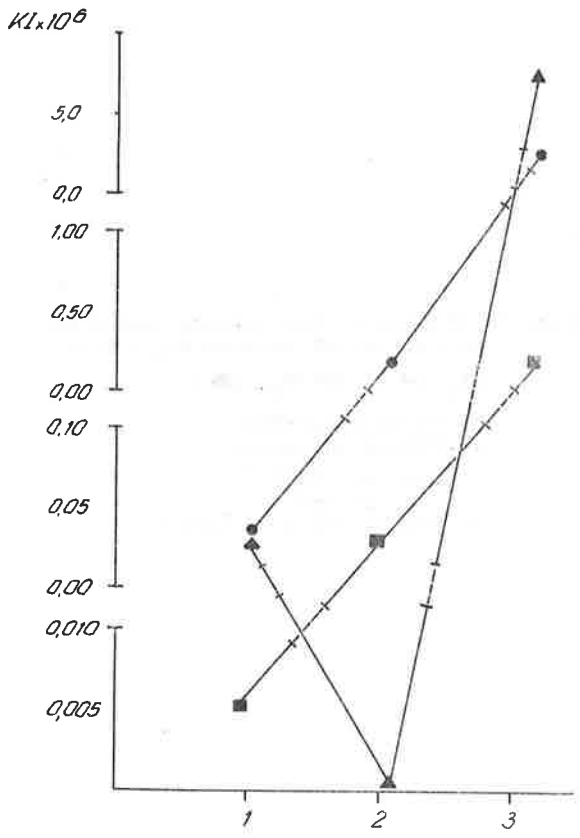

Fig. 2. Changes in the catalase activities of strains M_{16} (●); M_{19} (▲); and M_{95} (■)
1. In broth
2. After freezing at -30°C
3. After freeze-drying

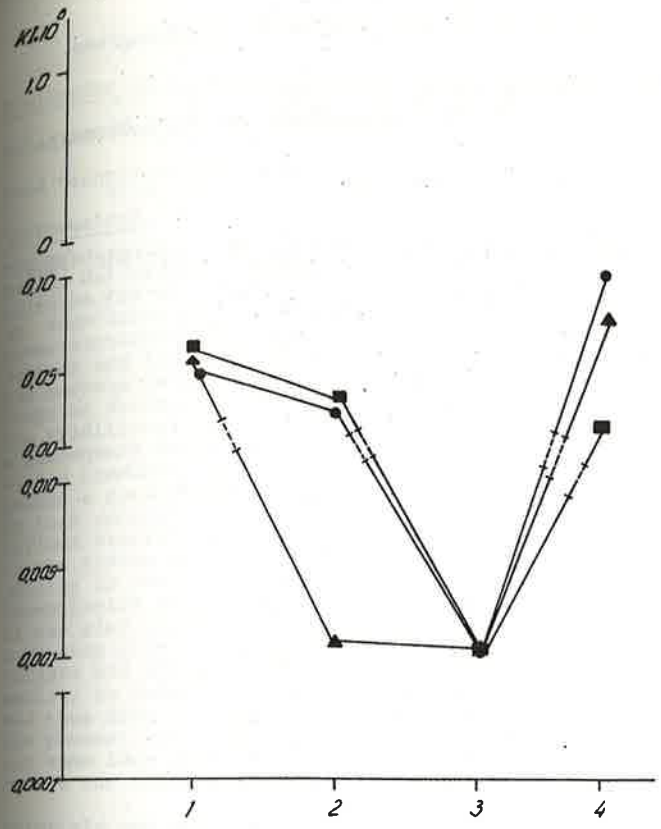

Fig. 3. Changes in the catalase activities of strains M_{16} (●); M_{19} (▲); and M_{95} (■)

1. In broth
2. After freezing at -65°C
3. After freeze-drying
4. After repairing for 48 h.

increase in strains M_{16} and M_{95}, with a cell number ten times lower.

After freeze-drying, catalase activity rises significantly in all three strains. The repairing of freeze-dried preparations results in a still better activity.

After freezing at -65°C and a subsequent freeze-drying, a reduction is found in the catalase activity of the strains (Fig. 3). The repairing of the cultures in MPB is observed to result in a sharp rise of catalase index value in all the three strains.

The two temperatures of freezing in the presence of a protector, dry skimmed cow milk, have different effects on the cultures under investigation. At -30°C, with a subsequent freeze-drying, the number of surviving microorganisms is reduced some 10 times, and at -65°C, it is preserved the same.

The reduction in the catalase activity of preparations frozen and freeze-dried at -65°C, is probably due to structural changes in the enzyme itself (Pushkar and Belous, 1981), consisting in a partial dissociation of catalase into subunits, while microbial cells are completely viable. After repairing in MPB they reproduce rapidly and demonstrate a high catalase activity.

Conclusions

1. Freezing at -30°C and -65°C with a subsequent freeze-drying preserves and even enhances the nitrate reductase activities of Micrococcus varians strains M_{16} and M_{19} and Staph. saprophyticus strain M_{95}. No repairing of the preparations in broth cultures is necessary.
2. Freezing temperature affects the catalase activity of the strains under investigation. With -30°C, an enhanced catalase activity is observed, and after freezing at -65°C, repairing is necessary.
3. The micrococci studied can be used as frozen or freeze-dried preparations in meat industry, in view of the preservation of their metabolic activity.

References

1. Belous, A.M., V.N. Lugovoi, V.V. Lemeshenko et al. Problemy kriobiokhimii, Vestn. AN USSR, 1976, 2.
2. Pushkar, N.S., A.M. Belous. Aktual'nye problemy kriobiologii, Kiev, Naukova Dumka, 1981.
3. Tsvetkov, Ts. Kriobiologiya i liofilizatsiya, Sofia, Zemizdat, 1979.
4. Tsvetkov, Ts. D. et al. Mesopromishlenost Bulletin, 1981, 1.
5. Egami, F.S., S. Taniguchi. Nitrat in Bergmeyer Methoden der Enzymatischen Analyse, Band II, 1970, 2179.
6. Fem A. et al. Biochim. Biophis. Acta, 24, 1957, 2, 306.
7. Gayer P. Vyuziti mikrobiálních startovacích kultur k výrobe tepelné neopracovávaných masných výrobku - II, Zpravodaj MP CSR, 1979, 1-2, 96-100.

8. Junker M.H., U. Liepe. Fleischwirtsch., 61, 1981, 5, 791.
9. Kuusela, K., E. Puolanne, E. Petäja, F.P. Niinivaara. 24 Eur. Fleischforscherkongress, 1978.
10. Liepe, H.U. Fleischwirtsch. 56, 1976, 178-180.
11. Liepe, H.U. Ernährungswirtschaft Lebensmitteltechnik, 1978, 4, 26-30.
12. Lück H. In: Methoden der enzymatischen Analyse. Bergmeier (ed.), Weinheim, West Germany, 1962.
13. Pfeil, E., H.U. Liepe, Fleischwirtsch. 54, 1974, 11, 1717.
14. Puolanne E., P. Törma, G. Djedjeva. Lebensm. Wiss. und Technologie, 1977, 10, 7-11
15. Rozier J. Fleischwirtsch. 51, 1971, 1063.
16. Schormüller J., M. Schilling, Nahrung, 1961, 5, 18.
17. Tjaberg T.B., M. Haugum, E. Nurmi. Proc. 15th Eur. Meet. Meat Res. Workers, Finland, 1969.