Comparative studies on the survival and activity of frozen and lyophilized cultures of Micrococcus varians

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During the last years, for speeding up the processes of ripening of the dry meat products are used pure bacterial cultures.They are offered as broths (6), frozen (5), or lyophilized (2,9) preparations.The latter have a number of advantages (2) and it is necessary to study the conditions, ensuring their activity and high surviva in freezing and lyophilizing drying.

The Micrococcae are a group of microorganisms very important for the ripening of the meat products (7,8).Known is their sensitivity to impact from various outside factors (3,5). Data in the literature about their survival following lyophilization are scarce,in comparison to the Lactovacillae (4) which it appears have been studied better.This fact made necessary to study the changes which appear following freezing and sublimation drying in the survival and activity of micrococcae isolated from dry sausages and evaluated as perspective for use in the meat industry.

Material and methodics

In the tests is used M.varians,st ain M_{95} isolated and differentiated in the Department for Microbiology with the Institute for Meat Industry.To freezing and lyophilization are submited 24 hour broth cultures using as a protector dry skimmed milk.As control is used a suspension of the strain in physiological solution.Freezing was effected at -30°C in nitrogen media.

The survival of the micrococcae was proved immediately following freezing and lyophilization and also subsiquent storage for 1 and 3 months of the cultures as frozen and lyophilized.

The activity is determined after indices which are of importance when micrococcae are used as starter cultures - catalase and nitratreductase activity (following traditional methods)(1) and acid formation from glucose and sacc arose - determined titrimetricaly on liquid nutritive media after a 10 days cultivation and expressed by the differences (pH) of the acidity of the media.

Results and Discussion

Survival. The results obtained with freezing and storage in a frozen state of Micrococcae varians strain M_{95} are reflected on fig.1A. As seen,in the presence of a protector a higher number of microbial cells survived,when the freezing is made in nitrogen media, than at -30°C.In the storage however,are observed a quicker recovery and keeping of the frozen in air media microorganisms.The number in the treated in nitrogen media microorganisms gradualy decreases.

The sen itivity of micrococcae frozen in suspension in physiological solution without a protector is higher at -30°C,but the storage is better.In spite of the fact that the cells survive better the treatment in nitrogen media,in storage for more than 1 month and lack of a protector,their number decreases very fast.

The lag phase in the develpment of the initial culture is longer, when the innoculation material is suspended in physiological solution, than with the existence of a protector,dry milk.Observed is an elongation of the lag phase in the development of strain M_{95} in the recovery following freezing of the culture,which is best expressed immediately after freezing of the culture,especialy at -30°C.Following 1 month of storage this period is shortened and the recovered culture develops faster.

The changes in the number of the micrococcae after sublimation drying and storage in a lyophilized stage are given on fig.1B. In spite of the fact that immediately after the lyophilization is observed a certain decrease in the number of the treated cells,during storage in the presence of a protector,their initial number is restored,while without the

presence of a protector the number gradualy decreases.

Activity. The tests for establishing the changes in the acid formation ability from glucose and saccharose demonstrate that the presence of a protector does not influence significantly the fermentative activity in the begining,but after a storage of 3 months the presence of a protector is of importance.Fig.2 shows that the activity not only is preserved well after freezing and storage in the frozen condition during 3 months, but that there might be established a certain increase in the acid formation. The means and temperature of freezing,used in the tests, do not appear to have any major impact.

After the lyophilization drying is observed a certain activation in the degradation of glucose and saccharose to acids,but in storage to 3 months it decreases unsignificantly for the glucose and more considerably for sacchar se (fig.3).

The changes in the catalase and nitrareductase activieties of microccocus strain M_{95} after freezing and lyophilization, and 1 and 3 months of storage in a frozen or lyphilized state are presented on table 1.The intensivity of the reaction is given by four + denominations.As seen from the table,in storage the frozen cultures keep better the catalase activity,and the lyophilized ones - the nitrareductase activity.

In the presence of a protective media of dry milk,after freezing at both temperatures and after lyophilization,slightly are decreased the possibilities for reduction of the nitrates.Following one month of storage,better expressed is the decreased ability to the nitratreductase activity of the frozen at -30°C culture.The lyophilized culture keeps better this ability and with the presence of a protetor media it is totaly restituted. With ut a protector and after 3 months of storage,the nitratreductase activity decreases.

Observed is a decrease of the catalase activity immediately following freezing at both temperaturesin physiological solution and after lyophilization wity protector dry milk.The activity is preserved in freezing with protective media.After 1 month of storage with protective media and freezing at -30°C the activity is restored totaly,while with the other means of freezing this is to a smaller degree.

The lyophilized culture expresses a smaller catalase activity following 1 month of storage.Keeping of the activity in relation to acid formation and nitratreductase has a major importance in the preparation of the lyophilized preparations of starter cultures for the industry.

Consclusions.

1.The survival of M.varians strain M_{95} is lower in storage in the frozen status, than in the lyophilized status.

2.Freezing and lyophilization do not have a negative influence on the acid formation from glucose immediately and after treatment of the cultures following 3 months of storage. Acid formation from glucose is decreased following storage.

3.Frozen micrococcus culture is better preserved in the way of catalase and the lyophilized in the way of nitratreducate activity.

Table 1

Changes in the catalase and nitratreductase activities of micrococcus strain M_{95} following freezing and lyophilization

Culture	Freezing	Protector	Activity	
			catalase	nitratreductase
I. Initial	-	Milk	+++	++++
After freezing	-30°C	"	++++	+++
1 month storage	"	"	++++	+
3 months storage	"	"	++	+
After freezing	nitrogen	"	++++	+++
1 month storage	"	"	+++	+++
3 months storage	"	"	+++	++
II. Initial	-	phys.sol.	++++	++++
After freezing	-30°C	"	++	++++
1 month storage	"	"	+++	++
3 months storage	"	"	+++	+

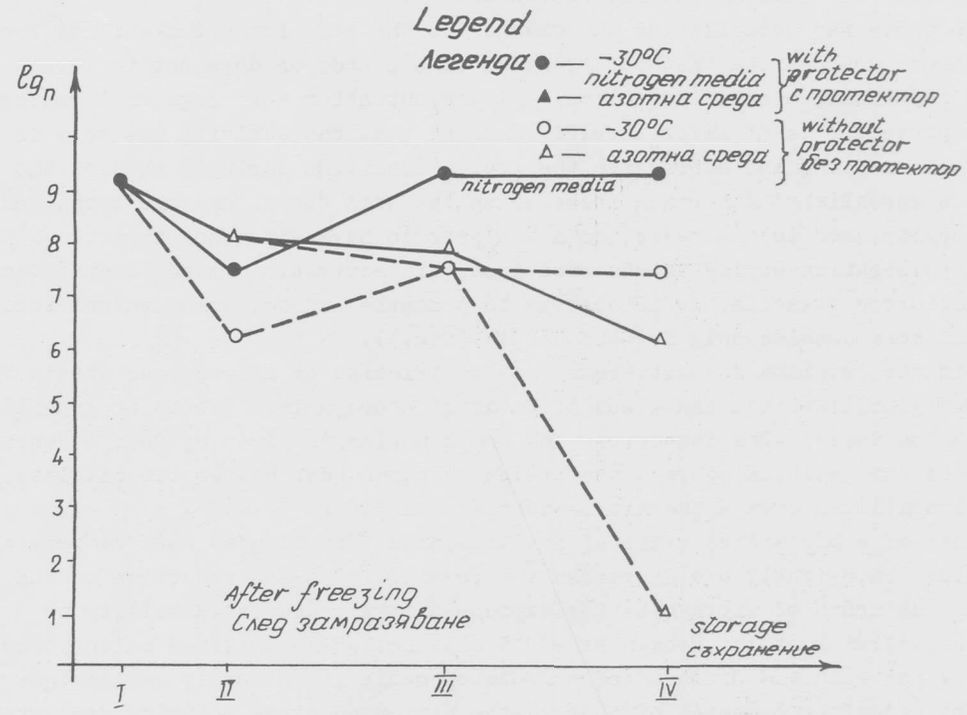

Fig.1 A. Changes in the number of micrococcae after freezing and sublimation drying and storage in frozen and lyophilized status.

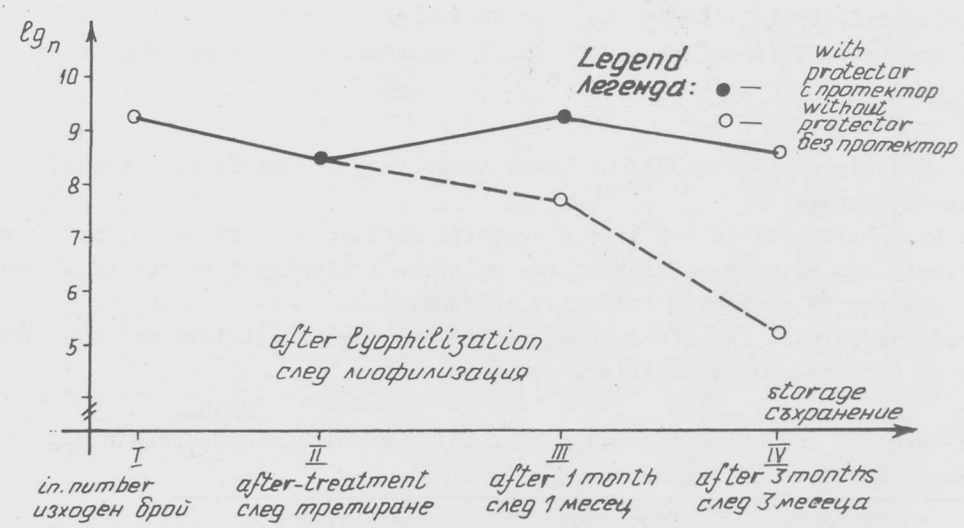

Fig.1 B. Changes in the number of micrococcae after freezing and sublimation drying and storage in frozen and lyophilized status.

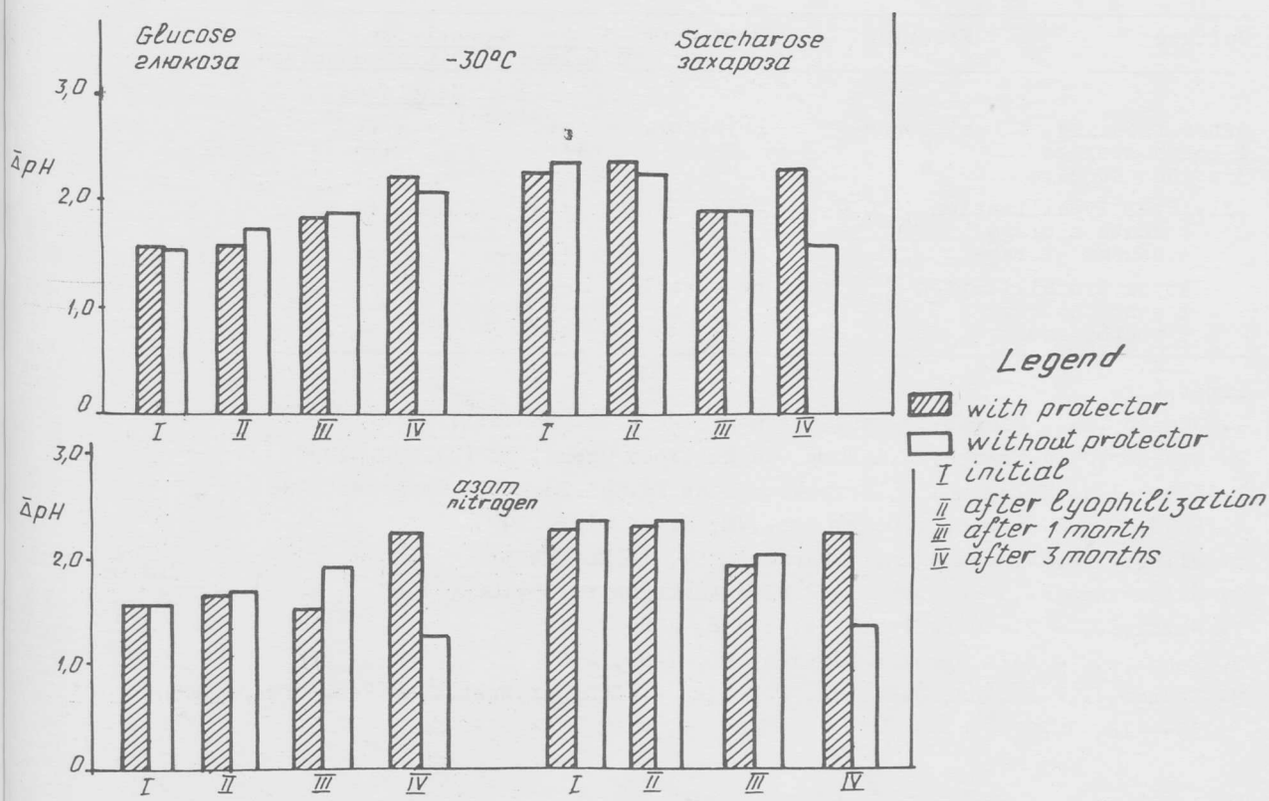

Fig.2 Changes in the acid formation of M.varians after freezing in nitrogen and at -30°C

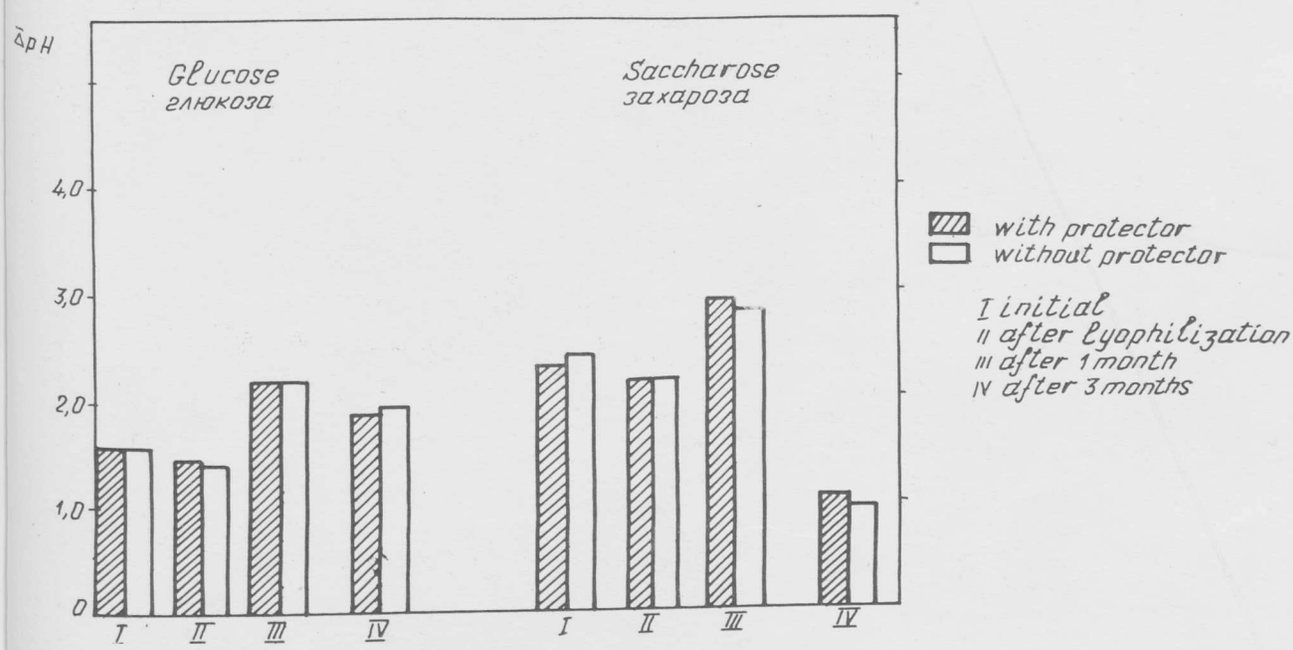

Fig.3 Changes in the acid formation of M.varians after lyophilization.

Culture	Freezing	Protector	Activity	
			catalase	nitratreductase
			Continued	
After freezing	nitrogen	Phys.sol.	++	++++
1 month storage	"	"	+++	+++
3 months storage	"	"	+	+
III.After lyphilization	-30°C	Milk	++	+++
1 month storage	"	"	+	++++
3 months storage	"	"	++	+++
After lyophilization	"	Phys.sol.	++	+++
1 month storage	"	"	++	+++
3 months storage	"	"	++	++

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