Effects of freezing and freeze-drying on lactic acid production by starter cultures

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One of the most important properties of the starter cultures employed in meat industry is the disintegration of carbohydrates down to lactic acid. Freezing and freeze-drying, as methods of preserving the viability and the activity of starter cultures, affect the individual microorganism species and strains in different ways (Khorolsky et al., 1981; Blankov and Klebanov, 1961). Their survivability depends on the species and the condition of the bacterial culture subjected to freezing and freeze-drying, on the rates and the regimens of applying these treatments (Jabarit, 1969a; 1969b), on protectors in the medium (Tsvetkov, 1979; Dolinov, 1969), etc.

The studies of Kuusela et al. (1978), Comi and Cantoni (1980) indicate, that the enzymatic activity of a given bacterial culture is not always proportional to the numbers of bacterial cells.

The purpose of the present work is to study the changes occurring following freezing and freeze-drying in the acid production of lactobacilli and micrococci isolated from raw-dried meat products characteristic of Bulgaria, where the microorganisms are used as starter cultures.

Material and Methods

The experiments made use of Lactobacillus plantarum strain L_4, Micrococcus varians strains M_{16} and M_{19}, and Staph. saprophyticus strain M_{95}. Studies were made of broth cultures, frozen and freeze-dried preparations. The preparations were prepared using dry skimmed cow milk as a protector (Tsvetkov et al., 1981). Freezing was performed at two different temperatures: $-30^{\circ}C$, and $-65^{\circ}C$, in a low temperature chamber with forced air convection in the chamber.

Lactic acid production was determined by a method described by Kuusela (1978): the culture is incubated in a buffer solution of a pH of 5,5 containing 1% glucose, at $44^{\circ}C$ for 2 h. Acid amount is determined by titration using 0,01 N NaOH and estimated in μmol/ml.

The activities of frozen and freeze-dried cultures were determined immediately after thawing and rehydration and after repairing in MPB for 24 h or for 48 h and 72 h.

Results and Discussion

The results obtained indicated that acid production in the initial broth cultures varied, on the average, between 8 and 11 μmol/ml for micrococci, and 13 and 16 μmol/ml for the lactobacilli. The cultures used are in the beginning of the stationary phase, when their enzymatic activity has not yet reached its maximum. The numbers of viable cells constitute 10^8 to 10^9 cells/ml. With this cell concentration in the broth cultures, the acid productions of the three micrococcus strains do not differ significantly.

Fig. 1 presents the changes in the activity of Lactobacillus plant. L_4 after the different treatments. After freezing, acid production rises slightly and reaches an average of 14-18 μmol/ml, and after freeze-drying the average is 30 μmol/ml, respectively.

It is observed that, unlike micrococci, this strain's acid production is enhanced significantly after repairing in a broth of the frozen and freeze-dried cultures. This could be explained by a shorter lag-phase in the growth of lactobacilli, in which the necessary amount of biomass is accumulated faster and it ensures an enhancement of the enzymatic activities in the concentrations shown above.

The storage of a frozen L_4 preparation for up to 15 days guarantees a preservation of activity. An acid production of up to 40 mol/ml has been found.

Following freezing, no significant changes are observed in the activity of strain M_{16} (mean lactic acid from 11,5 μmol/ml in the broth to 12,8 μmol/ml after freezing), and strains M_{19} and M_{95} demonstrate an enhancement of acid production: from 8-10 to 13-14 μmol/ml (Fig.2). This could be explained by the enhanced enzymatic activity after the accumulation of a sufficient amount of bacterial cells, a principle, demonstrated already by Pasteur. The attempts at repairing the frozen cultures by inoculation in MPB and incubation for 24 hours at $30^{\circ}C$, showed a decrease in activity. It seems that, after repairing the frozen culture, a lengthening of the lag-phase results, upon which a slower accumulation occurs of the necessary amount of biomass ensuring an enhancement of the acid production activity.

The freeze-drying processes lead to a further enhancement of the activities of the three strains under investigation: up to 13-18 μmol/ml. The values obtained are higher than the ones for frozen cultures and this could be explained by the less harmful, to the cell, repairing of the freeze-dried suspensions, than of the frozen ones.

In most of the experiments, the repairing of the freeze-dried preparation in a broth culture results in a reduction in activity. An increased activity, up to 20-30 μmol/ml, is obtained, when the broth culture is repaired for 48-72 hours.

No substantial differences have been found in the acid production of the strains studied, on account of the different freezing temperatures, $-30^{\circ}C$ and $-60^{\circ}C$.

Samples of the micrococci cultures were stored frozen at $-65^{\circ}C$ for 28 to 45 days, following which they were thawed at $+20^{\circ}C$. Analyses indicate that the said storage period guarantees a preservation of the activity of micrococci in the range of 13-15 μmol/ml, i.e., higher than in the initial 24-h broth cultures.

After a storage for 25 to 40 days in the freeze-dried state, the activity of micrococci constitutes 16-18 μmol/ml, and the attempts at their repairing in broth cultures showed again a decrease in activity.

The results obtained demonstrate, that frozen and freeze-dried preparations have a good acid

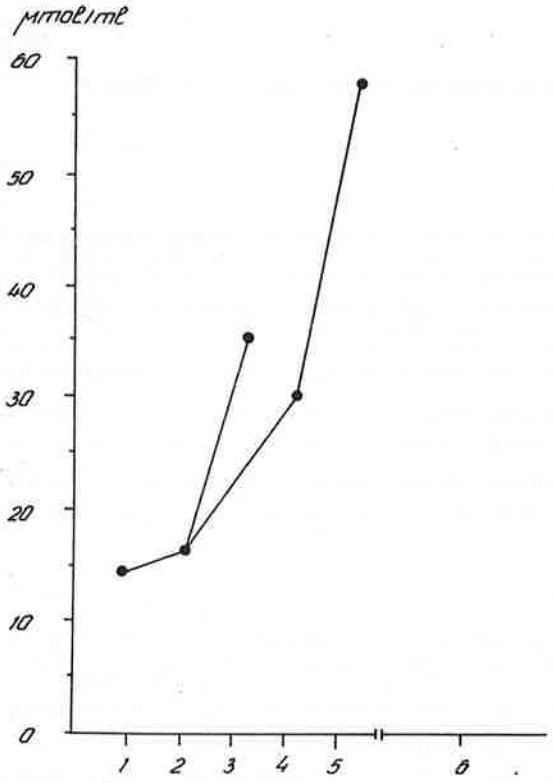

Fig. 1. Changes in the acid production of Lactobac. plantarum strain L_4 following freezing and freeze-drying
1. Broth culture
2. Frozen culture
3. Culture repaired after freezing
4. Freeze-dried culture
5. Culture repaired after freeze-drying

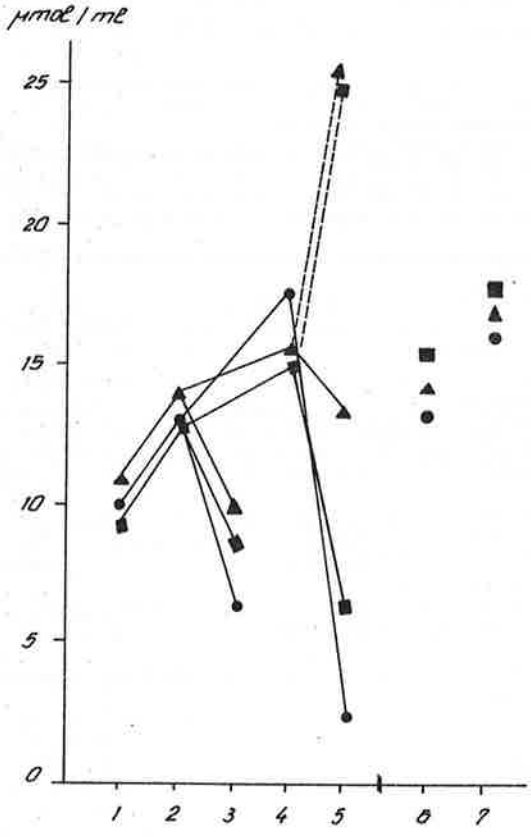

Fig. 2. Changes in the acid productions of strains M_{16} (●—); M_{19} (▲—); M_{95} (■—), after freezing and freeze-drying
1. Broth cultures
2. Frozen cultures
3. Cultures repaired after freezing
4. Freeze-dried cultures
5. Cultures repaired after freeze-drying
   —— after 24 h
   --- after 48-72 h
6. Stored frozen cultures
7. Stored freeze-dried cultures

production activity upon the respective treatments and on storage. They can be used in practice without an imperative preliminary repairing in broth cultures. This was found by us also in comparative experiments with the introduction of starter cultures in the manufacture of raw-dried sausages, in the form of broth cultures or frozen or freeze-dried preparations (unpublished data), and it was confirmed also by the data of Gayer (1979).
In our studies, neither in broths nor after freezing and freeze-drying is observed any positive correlation between the counts of microorganisms and the enzymatic activity produced (Table 1).

Table 1. Correlation between the amount of lactic acid produced and the counts of microorganisms in the broth culture

Strain	Microorganism counts, cells/ml	Lactate, µmol/ml	r*
1	2	3	4
	I. Broth Cultures		
L_4	$3,9 \times 10^9 - 1,5 \times 10^{10}$	12,9 - 16,0	+0,536
M_{16}	$9,1 \times 10^8 - 8,1 \times 10^9$	7,7 - 11,1	+0,790
M_{19}	$2,6 \times 10^8 - 8,0 \times 10^9$	10,8 - 11,2	+0,947
M_{95}	$3,5 \times 10^8 - 7,4 \times 10^9$	8,2 - 11,4	+0,653
	II. Frozen Cultures		
L_4	$3,1 \times 10^7 - 6,4 \times 10^{10}$	17,6 - 13,6	-1,000
M_{16}	$4,0 \times 10^6 - 9,3 \times 10^9$	21,4 - 8,5	-0,509
M_{19}	$8,0 \times 10^9 - 6,1 \times 10^{11}$	13,2 - 13,5	+0,956
M_{95}	$1,2 \times 10^8 - 7,2 \times 10^9$	8,1 - 13,6	+0,677
	III. Freeze-dried Cultures		
L_4	$4,6 \times 10^9 - 7,0 \times 10^{10}$	33,7 - 13,2	-0,910
1	2	3	4
M_{16}	$1,5 \times 10^8 - 3,2 \times 10^{11}$	22,1 - 12,5	-0,791
M_{19}	$9,0 \times 10^7 - 7,3 \times 10^{12}$	14,3 - 17,2	+0,374
M_{95}	$1,8 \times 10^8 - 1,0 \times 10^{12}$	12,6 - 17,1	+0,830

* r - Correlation coefficient.

These data conform to the results of Comi and Cantoni, according to who the optimum conditions for enzymatic activity usually differ from the ones for the growth of microorganisms. Enzymatic activity is not a function of the total growth of cells (Alford, 1960).

Inferences

1. Freezing in most cases exerts an activating effect on the acid production of the micrococci and lactobacilli under investigation.
2. On freeze-drying, the enzymatic activity of the microorganisms used in the experiments increases.
3. The repairing of frozen and freeze-dried preparations to obtain 24-h broth cultures does not result in an increase in the activity of micrococci. For this purpose, 48 to 72 hours are necessary. On the repairing of frozen and freeze-dried lactobacilli, an increase in acid production is observed.
4. A positive correlation has been found, in broth cultures of micrococci, of bacterial cell concentrations of $10^8 - 10^9$ cells/ml, between lactic acid production and the numbers of microorganisms.

Conclusion

The freezing and freeze-drying of Lactob. plantarum - L_4 and Micrococcus strains M_{16}, M_{19} and M_{95} do not affect negatively their activity in producing lactic acid. In most cases an activation is observed immediately after the treatments and after storage in the frozen or freeze-dried state for 2-3 months.

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