

COMPARATIVE STUDIES ON THE SURVIVABILITY AND THE ACTIVITY OF FROZEN AND FREEZE-DRIED CULTURES OF *L. plantarum*

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The pure bacterial cultures used in the manufacture of raw-dried sausages, have to be up to a number of requirements. One of the important qualities of the cultures is that they have a high survivability on freezing and freeze-drying, with a preservation of activity.

The viability and the activity of different freeze-dried microorganisms depends on the preparation of the cultures, the growth media and the protecting means added (6, 7, 8), on the age and the species of the bacteria (2, 12), on the concentration of the bacterial cells (10, 11), on the regimes of the technological processes of freezing, freeze-drying, etc.

Microorganisms have different freezing sensitivities (2, 4, 3, 8). There are data in literature on the survivability on freeze-drying of lactic acid bacteria applied in dairy industry (1, 5, 9). The data on the preparation of freeze-dried pure cultures for meat industry are scarce. That is why we set ourselves the objective to study the changes in the survivability and the activity, upon freezing or freeze-drying, of a culture of *L. plantarum*, used as a bacterial starter in the manufacture of raw-dried sausages.

MATERIAL AND METHODS

THE EXPERIMENTS were conducted using *L. plantarum* strain L₄, isolated and differentiated in the Microbiology Department (Meat Techn. Res. Inst.) and applied in the manufacture of raw-dried sausages. The *Lactobacillus* was grown on a suitable nutrient medium, an optimum one for its growth. Dry skimmed milk was used as a protective medium, and a suspension in physiological solution, as a control. Freezing was performed at -30°C, and in a nitrogen medium.

Studies were conducted to determine the survivability and the activity of the *lactobacillus* immediately after freezing or freeze-drying and after one or three months of storage of the frozen or the freeze-dried cultures.

The count of the microbial cells that survived was determined by dilutions in a liquid nutrient medium, and their probable count was found using McCready's table.

The *lactobacillus* activity was determined pH-metrically according to changes in the acidity of nutrient media containing glucose or sucrose after a 10-day growth period; the acid-producing capacity, in skimmed milk after a month's growth, by Törner's method.

RESULTS AND DISCUSSION

SURVIVABILITY. When dry skimmed milk was used as a protective medium, a 100% survivability was found for *L. plantarum* after freezing in a nitrogen medium or at -30°C and after freeze-drying, with a storage for 1 or 3 months. The lag-phase in the growth of the recovered freeze-dried and frozen cultures is shorter than the one of the initial culture (Fig. 1).

A strong reduction in the percentage of survivability is observed in the control (an *L.* suspension in physiological solution). Immediately after freezing in a nitrogen medium, 100% of the bacterial cells survive; after a storage for 1 month, only 1%, and after 3 months, 0,00024%. The suspension frozen at -30°C has a survivability of 10% immediately after freezing, 0,24% after 1 month of storage, and 0,000001% after 3 months. Out of the freeze-dried cultures, only 1% remain viable immediately after freeze-drying, 0,1% upon a one month storage, and 0,000001% after 3 months (fig. 2).

These results confirm that dry skimmed milk can be used successfully as a protector, in order to achieve a high per cent survivability of the *lactobacillus*.

The *Lactobacillus* L₄ cultures frozen in a nitrogen medium, are more resistant than the ones frozen at -30°C.

Activity. The analyses for acid production from glucose and sucrose immediately after the freezing of *Lactobacillus* L₄ indicate a rise in the acidity with both carbohydrates, regardless of the presence or the absence of a protective medium and of the manner of freezing. After freeze-drying, the amount of acid produced from glucose, is less than in the initial culture, and the acid from sucrose is close to the one in the initial culture. After 1 month of storage, a higher acid productivity is found in all variants. Among the freeze-dried cultures, the ones

with a protector are more active than those without a protector, but in all cases they are more active than the initial cultures (Fig. 3 and 4). After 3 months of storage, a considerable increase in the activity of the cultures is shown,

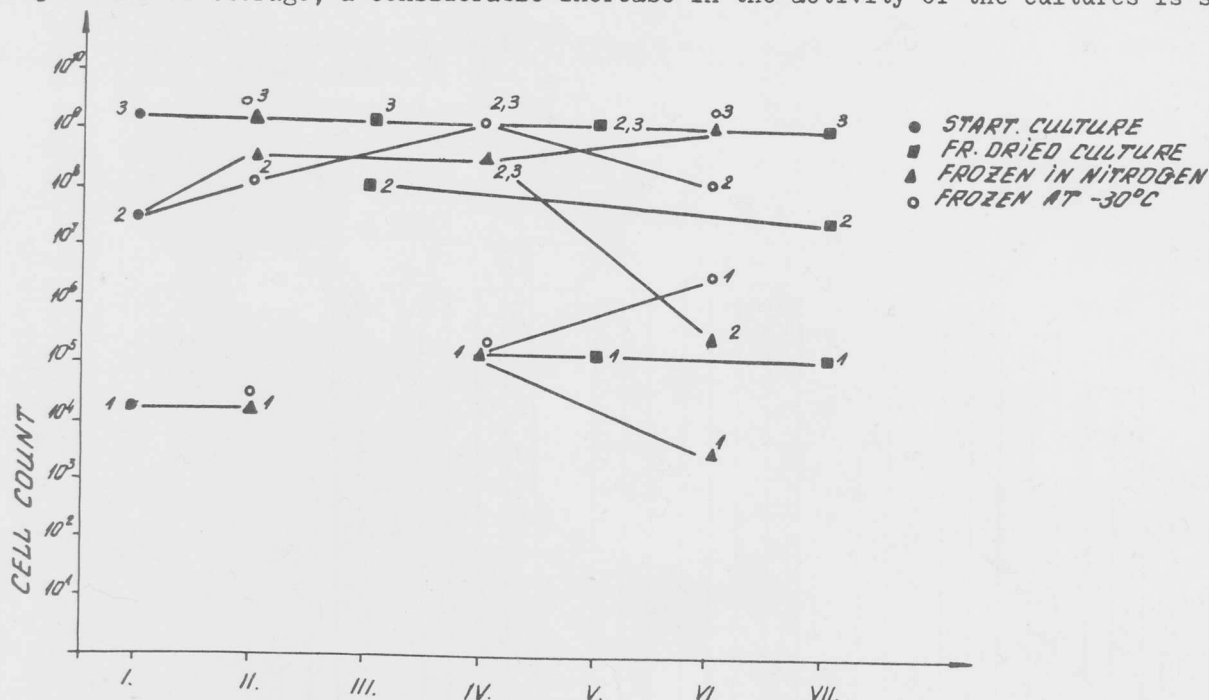


Fig. 1. Survivability of *Lactobacillus* strain L_4 in frozen and freeze-dried cultures
I - Initial (broth) culture; II - After freezing in a nitrogen medium and at -30°C ; III - After freeze-drying; IV - After 1 month, frozen; V - After 1 month, freeze-dried; VI - After 3 months, frozen; VII - After 3 months, freeze-dried.

1 - 24-hour count
2 - 48-hour count
3 - 72-hour count

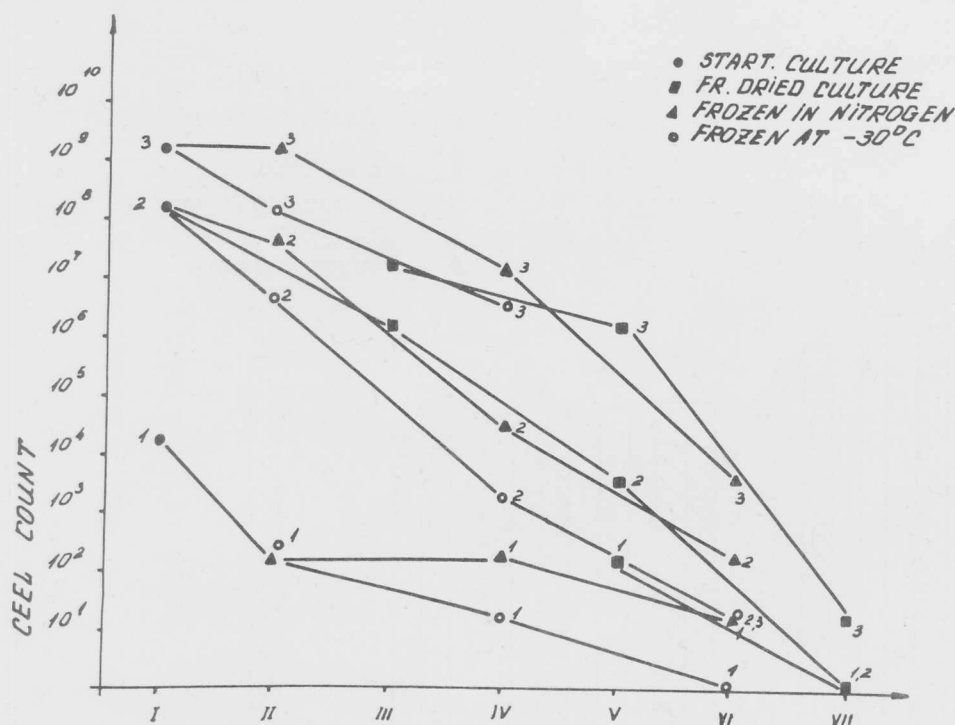


Fig. 2. Survivability of *Lactobacillus* strain L_4 in frozen and freeze-dried cultures (control)

I - Initial (broth) culture; II - After freezing in a nitrogen medium at -30°C ; III - After freeze-drying; IV - After 1 month, frozen; V - After 1 month, freeze-dried; VI - After 3 months, frozen; VII - After 3 months, freeze-dried.

1 - 24 hour count
2 - 48 hour count
3 - 72 hour count

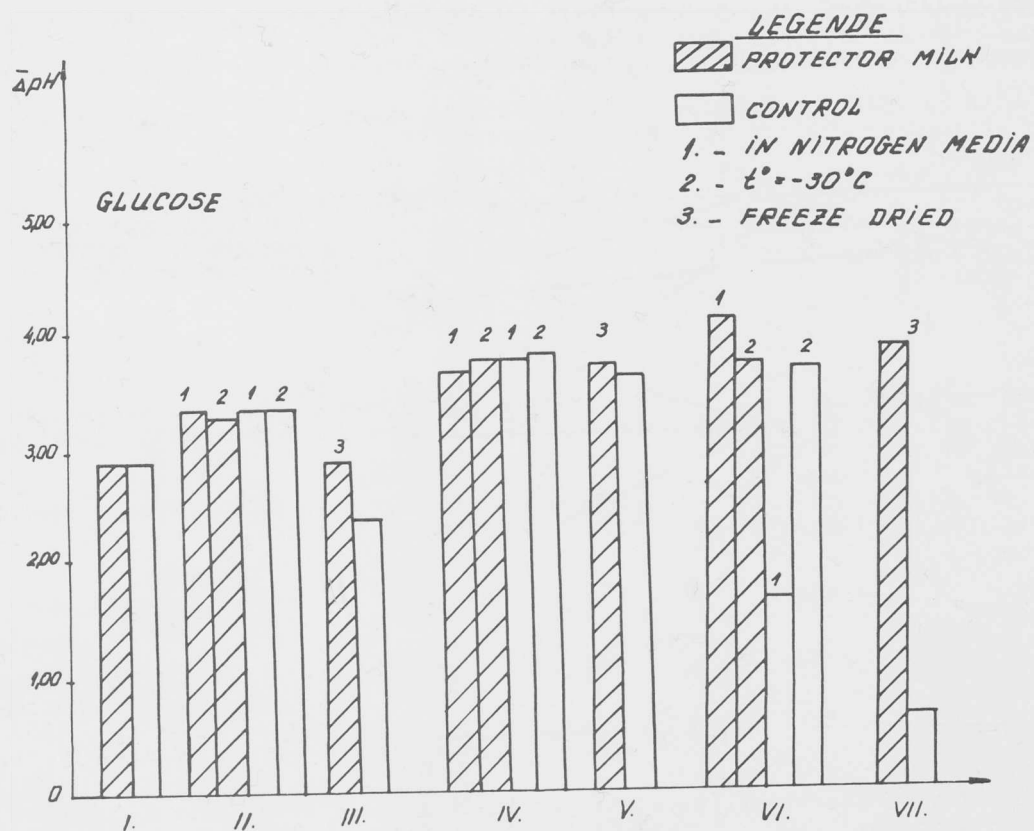


Fig. 3. Changes in the activity of Lactobacillus strain L_4 in frozen and freeze-dried cultures

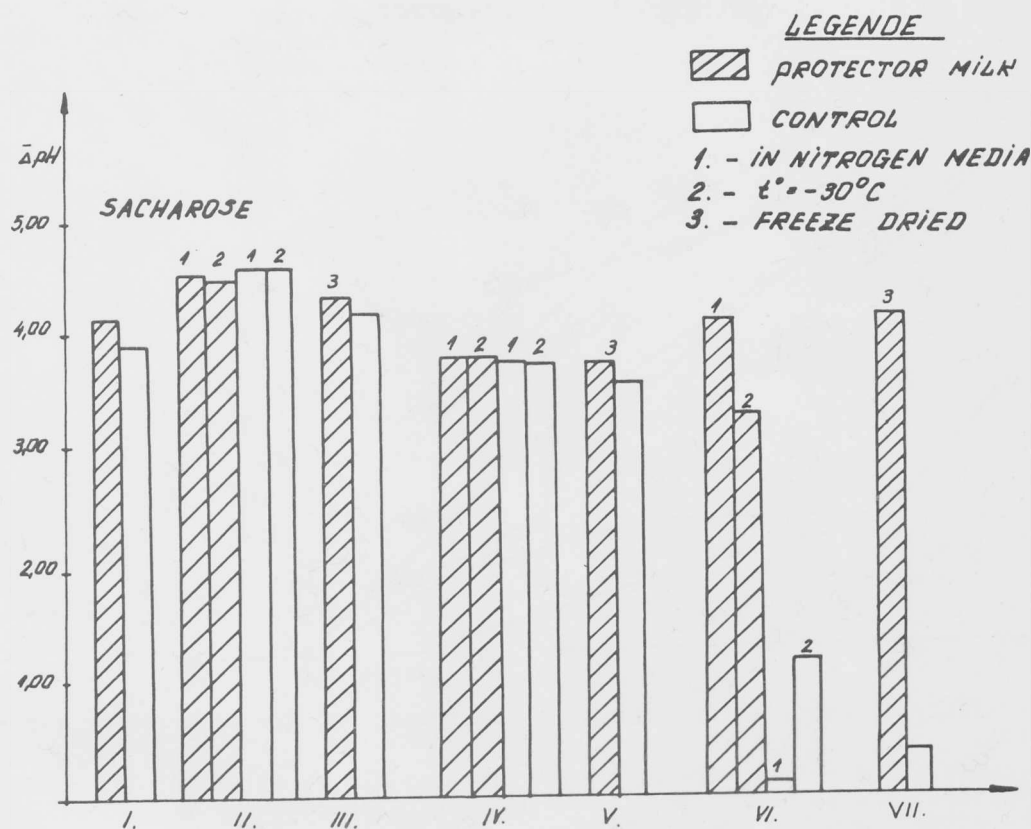


Fig. 4. Changes in the activities of Lactobacillus strain L_4 in frozen and freeze-dried cultures

which is particularly expressed in the ones frozen in a nitrogen medium, and less so in the freeze-dried ones. In the control lots (excluding the ones frozen at -30°C), a great decrease in activity is observed, especially in the freeze-dried cultures.

After freezing by both of the methods experimented, with or without a protective medium, an increased activity of acid production in milk is observed. Acid production is usually reduced after freeze-drying.

The results obtained indicate, that *L. plantarum* strain L_4 , with a protective medium of dry skimmed milk, preserves its survivability and activity in a frozen or a freeze-dried state, in studies lasting up to 3 months. That is of great importance for production, in its application as a bacterial starter.

CONCLUSIONS

1. A 100% survivability was found of *L. plantarum* strain L_4 after freezing in a nitrogen medium or at -30°C , after freeze-drying and upon storage for 3 months, on condition that dry skimmed milk was used as a protection means.
2. The activity of the frozen and freeze-dried bacterial cultures in terms of acid production from glucose and sucrose is preserved and even increased versus the initial one.

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