

COMPARATIVE STUDIES ON STARTER BACTERIAL PREPARATIONS FROM  
FREEZE-DRIED ONE-SPECIES AND MIXED CULTURES OF MICROCOCCUS AND  
LACTOBACILLUS

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**ABSTRACT:** The investigations are carried out in order to determine the survival of micrococcus and lactobacillus in one-species and mixed cultures after freeze-drying and during their storage. Two industrial strains of *M. varians* and one strain of *L. plantarum* are used in one-species and in two variants of mixed starter cultures. Micrococcus and lactobacillus are cultivated separately in fermenter and freeze-dried, separately and mixed, with protector containing medium (skim milk). The survival of microorganisms after freeze-drying and during their storage of one, three and six months is studied. The micrococcus microbial count is  $5,0 \cdot 10^9 - 2,1 \cdot 10^9$ , and the lactobacillus microbial counts is  $3,8 \cdot 10^9 - 2,0 \cdot 10^9$  in one-species cultures. Their counts in mixed cultures is  $6,8 \cdot 10^9 - 1,5 \cdot 10^9$  and  $2,0 \cdot 10^9 - 1,3 \cdot 10^9$ , respectively. The received results show, that micrococcus and lactobacillus in one-species and mixed cultures as starter preparations have similar survival after freeze-drying and storage at  $3-4^\circ\text{C}$  till six months.

**INTRODUCTION:** The use of starter bacterial cultures from appropriate species of microorganisms with definite enzyme activity gives possibility to direct and accelerate the ripening processes in meat products. Starter cultures are one-species and mixed cultures of one, two and more species of microorganisms. The most frequently used are lactobacillus, pediococcus, micrococcus, staphylococcus, yeasts /9, 10, 13, 15/. The applying of such a cultures is still established itself in the great part of technologies in food industry. Starter bacterial cultures can be applied in meat batter as liquid cultures, frozen or freeze-dried preparations. The examinations show, that the most appropriate is the use of freeze-dried preparations because of handling and control convenience and the possibility for combination of different strains with definite enzyme activity /10, 11, 14/. The main criterion for the efficiency of these preparations is the preservation of enough viable counts. There are many investigations, which explain factors, that influence the survival and activity of freeze-dried microorganisms during their storage. The optimal conditions for long-term storage of freeze-dried bacterial cultures must be determined individually for every species microorganism, as the age, composition of protector containing medium, residual moisture and temperature are taken into consideration /1, 2, 4, 5, 7, 12, 14, 16/. As the most harmless for bacteria is indicated the residual moisture in the region of 1-6%, which ensures max quantity viable cells in conditions of long-term storage at temperatures not higher than  $2-4^\circ\text{C}$  /2, 7, 11/. The medium and the package in which are stored freeze-dried cultures exert also influence on the microorganisms' survival. Many authors indicate that the most effective storage is that one in gas and water impermeable packaging materials under

vacuum /3, 4, 6/. Micrococcus and lactobacillus are stable at freezing and freeze-drying. Besides this, they complement each other with their enzyme activity and are suitable combination for starter bacterial cultures /8, 13, 15/. Therefore in our investigation a great attention is paid to these two species microorganisms. The survival of particular species microorganisms which take part in starter preparations - lactobacillus and micrococcus - are the object of present examinations. The development of this problem is of especial significance for these starter preparations, at which is impossible the joined cultivation of two and more species microorganisms. In these cases usually cultivation, freezing and freeze-drying are carried out separately, after which the microorganisms are mixed and cut.

**MATERIALS AND METHODS:** Two industrial strains - M.varians - M<sub>1</sub> and M<sub>2</sub>, and one strain - L.plantarum - L<sub>2</sub>, are examined in one species cultures and in two variants of mixed cultures. Micrococcus and lactobacillus are cultivated separately in fermenter at optimal parameters for their development - culture medium, temperature, pH and aeration. The broth cultures, received at the end of exponential and primary stationary phase of development, are freeze-dried alone in one-species cultures and in mixed cultures in micrococcus/lactobacillus ratio 1:1 /variant 1/ and 2:1 /variant 2/. 8% dry skim milk is used as a protector containing medium. The freeze-dried cultures, prepackaged in polyethylene film, are stored in chilling camera at 3-4°C. The samples under examination are taken immediately after freeze-drying of 1, 3 and 6 months. The viable counts is determined according to the method of decimal reduction and plate count technique, for micrococcus is used MSA /Mannit Salt Agar/ and for lactobacillus - Rogoza medium /MERCK/. Microbiological values are expressed in colonies forming units per 1 g freeze-dried preparation.

**RESULTS AND DISCUSSION:** The viable counts of micrococcus and lactobacillus are examined immediately after freeze-drying and during storage at 3-4°C of 1, 3 and 6 months. The residual moisture of preparation is retained in the region of 2-3%, which favours the survival of freeze-dried bacterial cultures at long-term storage /2/. Microbial counts in freeze-dried one-species cultures of micrococcus is  $5,0 \cdot 10^9$  -  $2,1 \cdot 10^9$  and of lactobacillus -  $3,8 \cdot 10^9$  -  $2,0 \cdot 10^9$ . In mixed cultures their counts is  $6,8 \cdot 10^9$  -  $1,5 \cdot 10^9$  of micrococcus and  $2,0 \cdot 10^9$  -  $1,3 \cdot 10^9$  of lactobacillus. The results of examinations are presented on fig. 1. The comparative investigations show, that different combinations of micrococcus and lactobacillus can be composed immediately after their cultivation, before freezing and freeze-drying, which does not influence their survival.

**CONCLUSIONS:** 1. Micrococcus and lactobacillus in one-species and mixed cultures as starter preparations have similar survival after freeze-drying and storage at 3-4°C till six months. 2. After 1, 3 and 6 months storage at 3-4°C, the viable counts is preserved in necessary quantity, which ensures the efficiency of starter preparations. 3. Preliminary mixing of bacterial cultures as starter preparations before their freezing and freeze-drying creates a possibility for shortening the duration

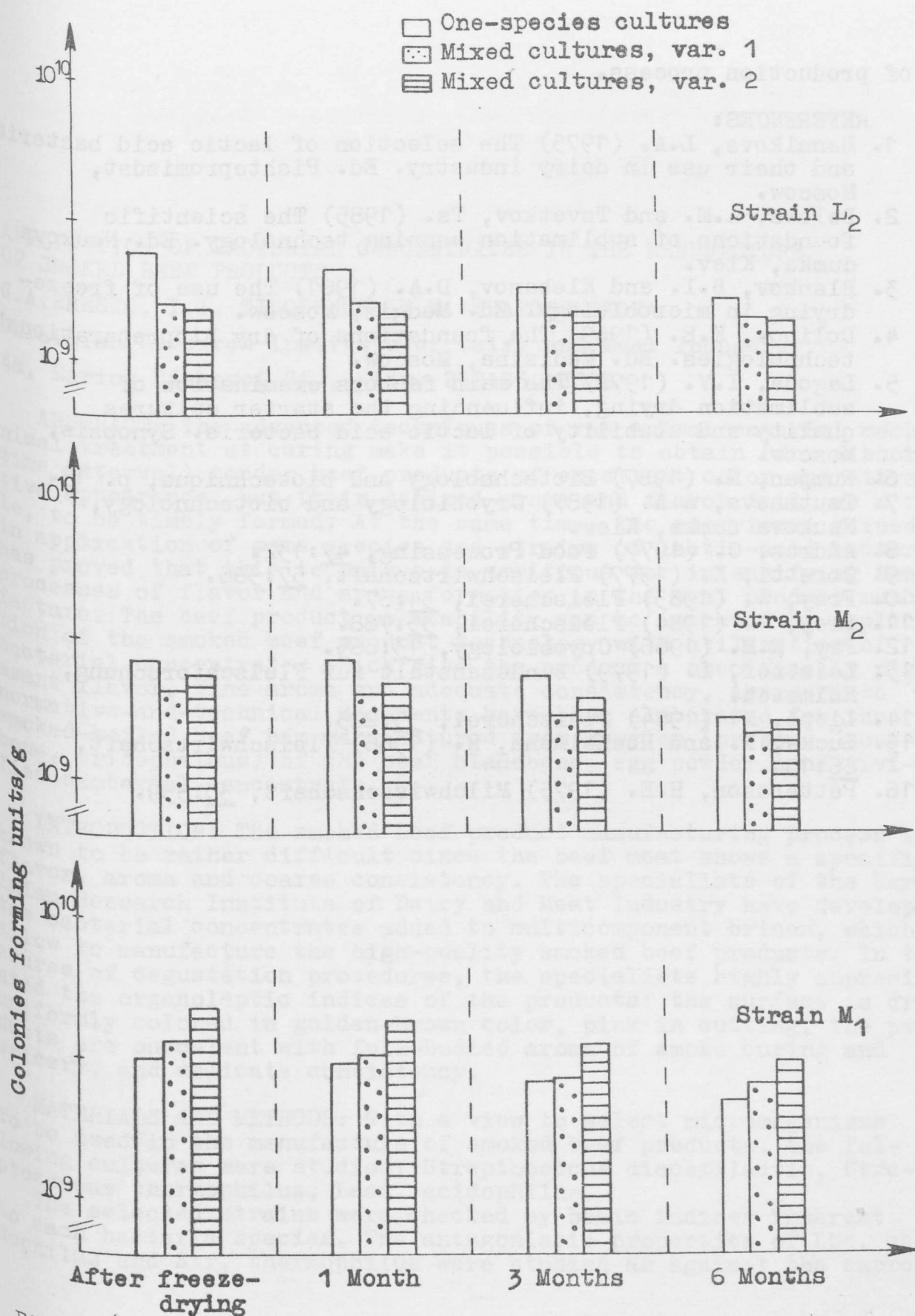


Fig. 1.—Variations in the number of starter microorganisms in one-species and mixed cultures at different terms of storage



of production process.

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