

## **PRODUCTION OF DRY *LACTOBACILLUS RHAMNOSUS* GG PREPARATIONS BY SPRAY DRYING AND LYOPHILIZATION IN AQUEOUS TWO-PHASE SYSTEMS**

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**Background.** Drying is the oldest method of food preservation. It works by removing water from the food, which prevents the growth of microorganisms and decay. Moreover, spray or freeze drying is also used for the preservation of probiotic cultures. The aim of this study was to compare a survival rate of probiotic bacteria *Lactobacillus rhamnosus* during spray and freeze drying in ATPS. These results were also compared with survival rate of cells dried under the same conditions but suspended only in skim milk, 6% solution of PVP or 6% solution of dextran.

**Material and methods.** The bacteria *Lactobacillus rhamnosus* GG were suspended and spray or freeze dried in various types of aqueous two-phase emulsions: PVP/dextran, PEG4000/dextran and PEG8000/dextran. These emulsions consisted of different types of polymers and had varying ratio of polymers in dispersed (dextran) and dispersing (PEG and PVP) phases.

**Results.** The research demonstrated that survival rate of bacteria directly after drying depended mainly on protective reagent, rather than on drying method. After 30-day-storage of the dried bacteria cell specimens, the highest survival rate was noted in case of freeze dried cells in milk. In case of spray drying the highest cell survival rate was observed when emulsion PVP3.6%/dextran2.4% was used as a drying medium.

**Conclusions.** Finally, it has been found that cell survival rate was not strongly influenced by the storage temperature of the powder but it depended on the drying medium.

**Key words:** ATPS, freeze drying, probiotic bacteria, spray drying

## INTRODUCTION

*Lactobacillus rhamnosus* GG is a clinically documented bacterial strain which is used in many countries as a probiotic culture in different dairy products or in pharmaceutical diet supplements [Korpela et al. 1997]. This bacterium is considered safe (GRAS) microorganism. *L. rhamnosus* GG is a homofermentative L-(+)-lactic acid producer [Berry et al. 1997].

Probiotic cultures are frequently utilized in various diet supplements in dried form, as either spray-dried or freeze-dried powders [Holzapfel et al. 2001]. However, most probiotic bacteria do not survive well when they are exposed to osmotic and temperature extremes during spray or freeze drying [Anal and Singh 2007]. Moreover, when spray or freeze drying is used for the preservation of probiotic cultures, a part of their activity is lost in a relatively short time on storage.

An important step in probiotic culture drying is the selection of a protective substance that is added to the culture prior to drying in order to improve survival of the cells. For example, the incorporation of non-fat milk solids [Selmer-Olsen et al. 1999], granular starch [Crittenden et al. 2001], gum acacia [Desmond et al. 2002], and high-saccharified maltodextrin have been shown to improve cell viability during drying and storage [Dembczyński et al. 2008].

Aqueous two-phase systems (ATPS) have also been used as a protective factor during spray drying. For example, it can be formed by two partially miscible aqueous polymer solutions, such as polyethylene glycol (PEG)/dextran or polyvinylpyrrolidone (PVP)/dextran. When applying ATPS for drying purposes, the ATPS is “water-in-water” emulsion, in which one phase is dispersed in the other, continuous phase. Thanks to it, cells are double protected – they are closed in dispersed and covered by continuous phase [Elversson and Millqvist-Fureby 2006].

The aim of drying is produce easy-to-use, microbiologically stable *L. rhamnosus* GG preparations. Spray and freeze drying conserve bacterial activity and make storage possible for a long time. In the present work, dry preparations of *L. rhamnosus* GG were obtained by freeze- and spray drying of bacterial cells with milk, PVP and dextran solution, and aqueous two-phase systems as a drying carrier.

## MATERIAL AND METHODS

### Preparation of ATPS

In these experiments the following polymers were used for preparation of ATPS: polyvinylpyrrolidone (PVP; Biochemika Fluka), polyethylene glycol (PEG) 4000 and 8000 (Fluka), and dextran (Polfa Kutno, Poland).

The ATPS emulsions were prepared by mixing stock solutions (6%, 8%, 10% w/w) of top phase polymers and bottom phase polymers in different proportions (Table 1).

Varying ratio of polymers in dispersed (dextran) and dispersing (PEG or PVP) phases was tested. If emulsion was formed, it had given two phases after centrifugation (21 885 × g, 30 min).

Table 1. Concentrations of the polymers which formed emulsions of ATPS type and were used to spray and freeze drying

| Top phase polymers (continuous phase) | Bottom phase (dispersed phase) | Content of top phase polymer % | Content of bottom phase polymer % | Content of dry matter in powder after drying % |
|---------------------------------------|--------------------------------|--------------------------------|-----------------------------------|--|
| PVP                                   | dextran                        | 5.4                            | 0.6                               | 94.04 ±0.66                                    |
|                                       |                                | 3.6                            | 2.4                               | 93.57 ±0.37                                    |
|                                       |                                | 3.0                            | 3.0                               | 93.08 ±0.06                                    |
| PEG4000                               | dextran                        | 3.2                            | 2.4                               | 93.94 ±0.27                                    |
|                                       |                                | 3.0                            | 3.0                               | 94.91 ±0.47                                    |
| PEG8000                               | dextran                        | 3.2                            | 2.4                               | 89.06 ±0.59                                    |
|                                       |                                | 3.0                            | 3.0                               | 93.35 ±0.37                                    |

### Microorganism and culture conditions

For drying experiments lactic acid bacteria *L. rhamnosus* GG (ATTC 53103) were used. The bacteria were grown on MRS medium (Biocorp) in the incubator at 37°C. The pH of medium was adjusted to 6.5. Medium was autoclaved at 121°C for 30 min. The bacterial culture was concentrated two times by centrifugation at 4369×g for 5 min (Heraeus Multifuge 3S-R, Germany). Before a second centrifugation, MRS with glycerine (10%) was added. The bacteria were frozen in -70°C, stored in -20°C, and used as inoculate. One ml of inoculate was put into two test tubes with 5 ml of MRS and incubated at 37°C for 24 h. Next, cultures from test tubes were transferred to four Erlenmeyer's flasks with 200 ml of MRS (1 ml of bacteria per 100 ml of MRS medium) and grown in the incubator at 37°C for 20 h.

### Spray and freeze drying process

The bacteria (1 ml bacteria culture per 250 ml of solution) were suspended in three control substances (skim milk, 6% PVP, or 6% dextran) and in the ATPS emulsion. Before spray drying samples were stirred for an hour at 4-6°C. For spray drying a Mobile Minor 2000 pilot plant spray-dryer with a co-current two-fluid nozzle system with pneumatic atomization (Niro A/S GEA, Denmark) was used. The inlet air temperature was set at 180°C and the outlet air temperature was maintained at 80°C by adjusting the flow rate of the feed solution. The emulsion was maintained by continuously stirring the feed solution. For freeze drying, 5 ml of cell suspension was poured into sterile ampoules. The ampoule's weight with the cells was recorded. The Christ BETA 1-16 freeze-dryer (Germany) was used. Conditions of freeze drying are shown in Table 2.

### Survival rate after drying

Bacteria survival rate (%) was determined according to the formula  $(N/N_0) \times 100\%$ , where  $N_0$  represents the number of viable cells (cfu/ml) before drying and  $N$  represents the number of viable cells directly after drying (cfu/ml). After spray drying, samples

Table 2. Conditions of freeze drying

| Time<br>h | Conditions                  | Purpose          |
|-----------|-----------------------------|------------------|
| 2         | -35°C, atmospheric pressure | samples freezing |
| 17        | 15°C, 22 Pa                 | main drying      |
| 4         | 20°C, 22 Pa                 | final drying     |

were rehydrated and the adequate amounts of cell powders were suspended in water to reconstitute the initial level of solids. After freeze drying, water was added to the cell powders in the ampoules to obtain the same weight as recorded before freeze drying. Concentration of viable cells was determined by plating an appropriate cell suspension onto MRS agar medium and counting after incubation in 30°C for 72 h.

### Storage of dry cell preparations

Cells dried in skim milk, 6% PVP solution, 6% dextran solution and in the ATPS as a carrier were stored during 30 days in two different temperatures (4 and 21°C). Dried cells were hermetically closed in plastic containers. Viable cells concentration (cfu/ml) after storage was counted in the same way as after drying. Storage survival rate (%) was calculated according to the formula  $(S/S_0) \times 100\%$ , where S is a bacterial count (cfu/ml) after 30 days of storage and  $S_0$  is a bacterial count (cfu/ml) directly after drying.

### Determination of dry weights in cell suspension and in dry cells

Solid content in the cell suspensions and dry cell preparations was monitored gravimetrically after drying to a constant weight at 105°C. All samples were analysed in triplicate.

### Statistical analysis

Multifactorial ANOVA and HSD Tukey test were conducted to show which factor (drying method, protective medium, storage temperature) had the highest impact on bacteria *Lactobacillus rhamnosus* survival rate in fixed preparations directly after drying and after 30 days of storage. The statistical tests were performed at the significance level  $\alpha = 0.05$ .

## RESULTS AND DISCUSSION

Polymers which have formed emulsions of ATPS type and were used to spray and freeze drying are listed in Table 1. PVP, PEG8000 and PEG4000 formed emulsions with dextran in every tested ratio. However, obtaining of the powder after spray drying was impossible for several emulsions indicated in Table 1. After spray drying, the chamber and other elements of dryer were coated by a glutinous substance and no powder was found. Thus, it was not possible to dry the bacteria enclosed in these emulsions.

The ATPSs, in which concentrations of top phase were 3.6% and 3.0% and concentrations of bottom phase were 2.4% and 3.0%, were selected as a protective medium for bacteria drying: PVP3.6%/dextran2.4% (PVP3.6/D2.4), PVP3.0%/dextran3.0% (PVP 3.0/D3.0), PEG4000 3.6%/dextran2.4% (P4 3.6/D2.4), PEG4000 3.0%/dextran3.0% (P4 3.0/D3.0), PEG8000 3.6%/dextran2.4% (P8 3.6/D2.4), PEG8000 3.0%/dextran3.0% (P8 3.0/D3.0). These emulsions had a good stability and the powder was obtained for all pairs of polymers which formed two-phase systems. Additionally, the application of these emulsions was economically reasonable because the overall polymer concentration used in those systems was the lowest among tested in this work. This ratio of polymers allowed creating emulsions in the case of PVP/dextran, PEG4000/dextran, and PEG8000/dextran. Emulsion PVP5.4%/dextran0.6% (PVP5.4/D0.6) was also checked. In these emulsions, bacteria cells preferentially gathered in the dispersed phase of dextran. It was expected that after drying this state would be kept and it allows producing preparations in which cells would be closed in an internal dextran phase surrounded by the layer formed by the continuous phase [Millqvist-Fureby et al. 2000]. Moreover, the water content in powder after drying in aforementioned emulsions did not exceed 11%. Thus, obtained dry cell preparations should be microbiologically stable.

In addition to bacteria drying in the two-phase systems, the protective effect of single substances used for ATPS formation was also investigated. This included only PVP and dextran since spray drying PEG did not result in the dry powder.

Skim milk, being the most common protective agent used for drying of lactic acid bacteria, was also utilized to compare the results with other substances. Moreover, bacteria suspended in all the above-mentioned protective agents were also subjected to freeze drying to assess the effect of drying method on the viability of *L. rhamnosus* GG cells.

The cell survival rates (%) directly after drying are shown in the Figure 1. Statistical analysis revealed that the survival of *L. rhamnosus* GG cells directly after drying was mainly dependent on the type of protective medium ( $F = 256.69$ ). The drying method had less influence on cells viability ( $F = 8.55$ ).

There was no statistical difference between the survival rate of cells spray dried in skim milk and in 6% solution of PVP ( $p > 0.05$ ). Although different cell survival rates were obtained for skim milk and emulsions of PVP3.6/D2.4, P4 3.6/D2.4 and P8 3.6/D2.4 immediately after spray drying, none were found to be significantly different ( $p > 0.05$ ).

A similar situation was observed during freeze-drying of the cells suspended in milk. Viability of these cells (29%) was lower than viability of cells suspended in 6% solution of PVP (53%). However, according to the statistical analysis there was no significant effect of these additives on the cell survival rate ( $p > 0.05$ ). There was also no statistical difference on cell viability after freeze-drying when skim milk and emulsions of PVP3.6/D2.4, P4 3.6/D2.4 and P8 3.6/D2.4 were used as protective agents ( $p > 0.05$ ).

Surprisingly, directly after spray and freeze drying the highest cell survival was obtained when 6% dextran was used as a protective solution. In this case, the cell viability ranged from 57 to 67% and was significantly higher than that obtained for skim milk as a protective medium (18-29%). These results differ from the reports of other authors. Capela et al. [2006] applying milk and natural and synthetic polymers such as dextran, hydroxyethyl starch, polydextrose, and oligofructose, observed the highest cell survival rate (nearly 86%) in preparations dried in the presence of milk. Ananta et al. [2005] concluded that variations in survival rate can be explained by different temperature

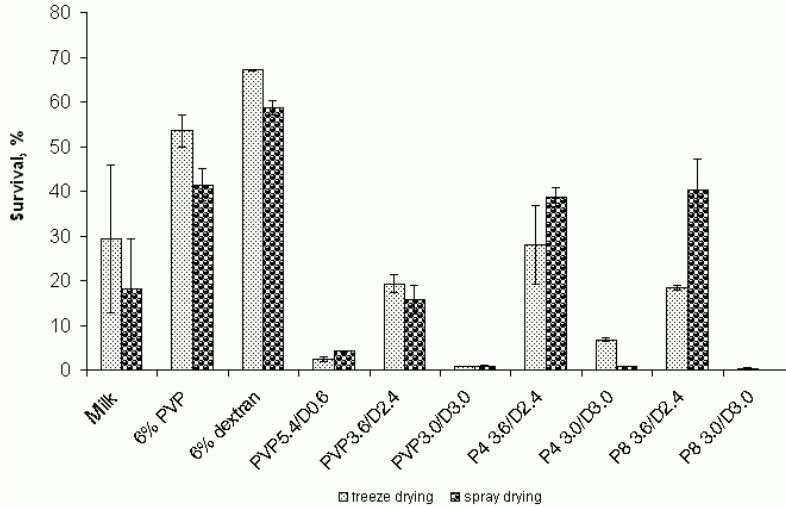


Fig. 1. Effect of freeze and spray drying on the viability of *Lactobacillus rhamnosus* GG cells suspended in different protective agents. Protective agents: cell suspended before drying in: PVP5.4%/dextran0.6% (PVP5.4/D0.6), PVP3.6%/dextran2.4% (PVP3.6/D2.4), PVP3.0%/dextran3.0% (PVP3.0/D3.0), PEG4000 3.6%/dextran2.4% (P4 3.6/D2.4), PEG4000 3.0%/dextran3.0% (P4 3.0/D3.0), PEG8000 3.6%/dextran2.4% (P8 3.6/D2.4), PEG 8000 3.0%/dextran3.0% (P8 3.0/D3.0)

resistance of bacteria, which depends on the species. Palmfeldt and Hahn-Hagerdal [2000] obtained 80% cell viability of *Lactobacillus reuteri* protected by the skim milk in freeze-dried preparations. However, Castro et al. [1997] reported 25% survival rate for *Lactobacillus bulgaricus* in a freeze-dried milk preparations, which was almost the same as that obtained in the present paper for *Lactobacillus rhamnosus* spray dried in a skim milk.

Ananta et al. [2005] noticed that spray drying is not a perfect method for production of dry cell preparations because a survival rate of cells during drying was low (due to high temperature) and stability during storage was also low. During freeze drying temperature is much lower and there is less thermal stress imposed on the cells [Morgan et al. 2006]. Nevertheless, some lactic acid bacteria strains might be dried without drastic decrease of viability. It is possible when the temperature in spray dryer is as low as possible. This research showed that bacterium *L. rhamnosus* are not prone to high temperature during spray drying. For most of the tested protective agents the cell survival rate was not statistically different after spray drying than viability after freeze drying ( $p > 0.05$ ). The exceptions from this rule were only 6% solution of dextran and emulsions of P8 3.0/D3.0 and P4 3.0/D3.0. In this case there was a significant statistical effect on the cell viability when different methods of drying were utilized ( $p < 0.05$ ).

The lowest cell survival was obtained with P8 3.0%/D3.0 as a protective medium. After spray drying the cell viability of 0.08% was recorded. A slightly higher result of 0.28% was obtained after freeze drying. When PVP3.0/D3.0 and P4 3.0/D3.0 were used as the protective media, the survival rate was also very low. It was nearly 7% for the

cells freeze-dried in the emulsion of P4 3.0/D3.0. For emulsions of PVP3.0/D3.0 and P4 3.0/D3.0, the cell survival rate was much lower, close to 0.80%. Probably, this resulted from a phenomenon called phase inversion of the emulsion. During phase inversion, a dispersed phase becomes a continuous phase and vice versa [Merchuk et al. 1998]. Thus, the cells which were initially enclosed in dextran phase were released to PEG or PVP phase. Another tendency in a viability of the cells suspended in ATPS, in which polymers concentrations were close to phase inversion, was observed by Millqvist-Fureby et al. [2000] for spray-dried preparations of *Enterococcus faecium* M74. These bacteria were spray-dried in emulsion where PVP content was 40% of overall polymer concentration. High cell survival rate in this emulsion surprised even the authors of this research. There was no further research to explain this result. Moreover, in literature there is no information about high cell survival during spray drying in emulsions where concentration of polymers is close to phase inversion. In turn, higher survival rates obtained for bacteria spray-dried in emulsions formed by P8 3.6/D2.4, P4 3.6/D2.4 and PVP3.6/D2.4 can be probably explained by the optimal droplets size of dispersed dextran in a continuous phase.

Dry cell powders were stored in 4°C and 21°C for 30 days. Cells survival was counted as a ratio of viable cells in the preparation after storage and directly after drying (Fig. 2).

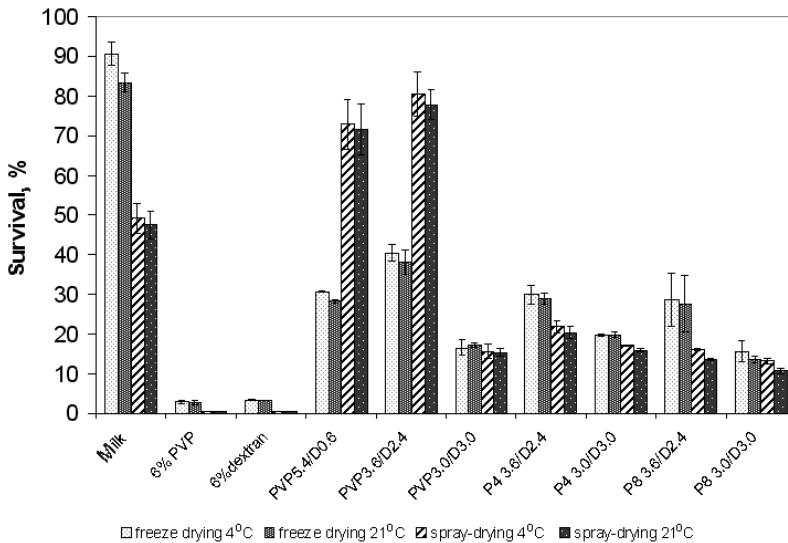


Fig. 2. Viability of dry cell preparations of *Lactobacillus rhamnosus* GG after 30-day-storage at 4°C and 21°C. The same abbreviations were used as in Figure 1

All examined factors had an influence on the cell viability after storage ( $\alpha = 0.05$ ). The cell survival was strongly dependent on a protective substance ( $F = 1991$ ). The cell viability was less influenced by a drying method ( $F = 381$ ) and storage temperature ( $F = 19.5$ ).

The highest cells survival rate of 83-91% after storage was observed in the freeze-dried preparations with skim milk used as a protective substance. The temperature of storage had no effect on cell viability ( $p > 0.05$ ). These results were not statistically different from those obtained for bacteria spray-dried in emulsions of PVP3.6/D2.4 and PVP5.4/D0.6 ( $p > 0.05$ ). In this case, the temperature of storage also did not influence the cell survival rate ( $p > 0.05$ ). For the cells dried in PVP3.6/D2.4 and PVP5.4/D0.6 the percentage of viable cells after storage was 78-81% and 72-73%, respectively. These results were statistically different from those obtained for the cells spray-dried in skim milk where the survival rate reached nearly 50% of ( $p < 0.05$ ).

This effect demonstrated that emulsion consisting of PVP and dextran had optimal properties for drying and storing bacteria cells and guaranteed a low loss of the cells viability. Similar good protective effect of PVP/dextran emulsions on the spray-dried cells was reported by Millqvist-Fureby et al. [2000]. The PVP/dextran emulsion was used because comparing this emulsion to PEG/dextran system of similar molecular weights, it was found that the PVP rich phase contained more dextran than the PEG rich phase in a corresponding system [Millqvist-Fureby et al. 2000]. What is more, in the PVP/dextran system higher molecular weight was required to achieve phase separation at low polymer concentration than in PEG/dextran. This observation indicated that PVP and dextran interacted more favourably than PEG and dextran. Thus, probably the stronger binding of outer and inner part of particles after drying in case of PVP and dextran could be expected. This phenomenon could be responsible for a better cell protection during storage.

Low number of viable cells was noted in dried preparation after storage when the concentration of polymers of top and bottom phase was 3.0%. This probably resulted from phase inversion and coating on the dried cells was not completely formed. The cells could protrude the outer surface of the dry particles and resulting percentage of viable cells was in the range of 11-20%. All these preparations were statistically the same ( $p > 0.05$ ), independently of the method of drying and temperature of storage. Similar cell viability of 14-22% after storage was achieved when emulsions of P8 3.6/D2.4 and P4 3.6/D2.4 were used for spray drying. However, the level of cell protection directly after spray drying for the coatings made of P8 3.6/D2.4 and P4 3.6/D2.4 was the same as for the coatings formulated from the emulsion of PVP3.6/D2.4, as shown by the statistical analysis. This observation can suggest the cell protruded from dry matrix and the contact with oxygen was possible during storage from particles produced by spray drying of P8 3.6/D2.4 and P4 3.6/D2.4 emulsion systems. This can also explain the comparable survival rate with cells dried in the emulsions in which phase inversion occurred.

The lowest level of the cell protection during storage was ensured by PVP and dextran used as a drying agent alone. However, the cells dried in these substances had the highest survival rate directly after drying. The storage survival rate for PVP and dextran was dependent on the drying method ( $p < 0.05$ ) and independent on the temperature of storage ( $p > 0.05$ ). In the stored cell preparations produced by freeze drying the cell viability of about 3% was recorded. When spray drying was used, the cell survival rate decreased to 0.5%.

The concentration of viable cells (cfu/g of dry cell culture) for all the types of dried preparations directly after drying and after storage is presented in Table 3.



Table 3. Viable cell concentrations and solid contents in the *Lactobacillus rhamnosus* GG dry preparations directly after drying and after 30-day-storage

| Protective agent              | Spray drying  |   |   |   | Freeze drying   |   | Content of dry matter in powder % |
|-------------------------------|---|---|---|---|---|---|-----------------------------------|
|                               | viable cell concentration after drying<br>cfu/g of dry cell culture | viable cell concentration after 30-day-storage<br>cfu/g of dry cell culture |   | viable cell concentration after drying<br>cfu/g of dry cell culture | viable cell concentration after 30-day-storage<br>cfu/g of dry cell culture |   |                                   |
|                               |   | 4°C   | 21°C  |   | 4°C   | 21°C  |                                   |
| PVP 5.4%/<br>dextran 0.6%     | 1.06·10 <sup>10</sup><br>±1.22·10 <sup>10</sup>                     | 7.74·10 <sup>9</sup><br>±8.84·10 <sup>9</sup>                               | 7.53·10 <sup>9</sup><br>±8.59·10 <sup>9</sup>   | 4.38·10 <sup>9</sup><br>±4.91·10 <sup>9</sup>                       | 1.33·10 <sup>9</sup><br>±1.50·10 <sup>9</sup>                               | 1.23·10 <sup>9</sup><br>±1.38·10 <sup>9</sup>   | 93.86<br>±0.00                    |
| PVP 3.2%/<br>dextran 2.4%     | 2.01·10 <sup>10</sup><br>±9.97·10 <sup>9</sup>                      | 1.08·10 <sup>10</sup><br>±1.08·10 <sup>10</sup>                             | 1.05·10 <sup>10</sup><br>±1.06·10 <sup>10</sup> | 2.14·10 <sup>10</sup><br>±1.46·10 <sup>10</sup>                     | 1.86·10 <sup>9</sup><br>±6.42·10 <sup>9</sup>                               | 8.50·10 <sup>9</sup><br>±6.36·10 <sup>10</sup>  | 96.50<br>±3.11                    |
| PVP 3.0%/<br>dextran 3.0%     | 8.08·10 <sup>8</sup><br>±3.41·10 <sup>8</sup>                       | 1.21·10 <sup>8</sup><br>±4.06·10 <sup>7</sup>                               | 1.25·10 <sup>8</sup><br>±6.21·10 <sup>7</sup>   | 7.27·10 <sup>8</sup><br>±3.07·10 <sup>8</sup>                       | 1.15·10 <sup>8</sup><br>±3.77·10 <sup>7</sup>                               | 8.34·10 <sup>9</sup><br>±1.17·10 <sup>10</sup>  | 94.38<br>±0.09                    |
| PEG8000 3.2%/<br>dextran 2.4% | 1.01·10 <sup>11</sup><br>±1.40·10 <sup>10</sup>                     | 1.70·10 <sup>10</sup><br>±2.83·10 <sup>8</sup>                              | 2.01·10 <sup>10</sup><br>±1.43·10 <sup>10</sup> | 4.88·10 <sup>10</sup><br>±7.07·10 <sup>9</sup>                      | 1.42·10 <sup>10</sup><br>±5.30·10 <sup>9</sup>                              | 1.37·10 <sup>10</sup><br>±5.37·10 <sup>9</sup>  | 91.98<br>±0.46                    |
| PEG8000 3.0%/<br>dextran 3.0% | 6.44·10 <sup>7</sup><br>±1.08·10 <sup>7</sup>                       | 7.95·10 <sup>6</sup><br>±1.66·10 <sup>6</sup>                               | 6.43·10 <sup>7</sup><br>±1.43·10 <sup>6</sup>   | 1.44·10 <sup>8</sup><br>±4.03·10 <sup>7</sup>                       | 2.32·10 <sup>7</sup><br>±1.00·10 <sup>7</sup>                               | 1.97·10 <sup>7</sup><br>±6.50·10 <sup>6</sup>   | 94.86<br>±0.49                    |
| PEG4000 3.2%/<br>dextran 2.4% | 3.37·10 <sup>11</sup><br>±9.69·10 <sup>10</sup>                     | 6.53·10 <sup>10</sup><br>±8.77·10 <sup>9</sup>                              | 6.06·10 <sup>10</sup><br>±8.77·10 <sup>9</sup>  | 2.64·10 <sup>11</sup><br>±1.55·10 <sup>11</sup>                     | 4.85·10 <sup>10</sup><br>±6.71·10 <sup>9</sup>                              | 4.65·10 <sup>10</sup><br>±4.81·10 <sup>9</sup>  | 93.97<br>±0.03                    |
| PEG4000 3.0%/<br>dextran 3.0% | 4.06·10 <sup>9</sup><br>±5.65·10 <sup>9</sup>                       | 1.24·10 <sup>7</sup><br>±2.05·10 <sup>6</sup>                               | 5.93·10 <sup>7</sup><br>±6.60·10 <sup>7</sup>   | 6.30·10 <sup>8</sup><br>±9.83·10 <sup>7</sup>                       | 6.33·10 <sup>7</sup><br>±7.02·10 <sup>7</sup>                               | 6.11·10 <sup>8</sup><br>±6.62·10 <sup>8</sup>   | 94.38<br>±0.09                    |
| PVP 6%                        | 2.01·10 <sup>11</sup><br>±2.76·10 <sup>10</sup>                     | 9.99·10 <sup>8</sup><br>±1.41·10 <sup>6</sup>                               | 5.26·10 <sup>8</sup><br>±6.10·10 <sup>8</sup>   | 3.00·10 <sup>11</sup><br>±7.07·10 <sup>8</sup>                      | 7.01·10 <sup>9</sup><br>±3.11·10 <sup>8</sup>                               | 6.27·10 <sup>9</sup><br>±7.57·10 <sup>8</sup>   | 99.46<br>±0.01                    |
| Dextran 6%                    | 2.69·10 <sup>11</sup><br>±4.45·10 <sup>10</sup>                     | 1.63·10 <sup>9</sup><br>±2.83·10 <sup>7</sup>                               | 1.57·10 <sup>9</sup><br>±7.07·10 <sup>6</sup>   | 3.37·10 <sup>11</sup><br>±2.12·10 <sup>9</sup>                      | 6.45·10 <sup>10</sup><br>±7.55·10 <sup>10</sup>                             | 1.05·10 <sup>10</sup><br>±3.53·10 <sup>8</sup>  | 99.53<br>±0.02                    |
| Skim milk                     | 2.13·10 <sup>10</sup><br>±8.22·10 <sup>9</sup>                      | 5.21·10 <sup>9</sup><br>±4.81·10 <sup>9</sup>                               | 2.66·10 <sup>9</sup><br>±3.21·10 <sup>9</sup>   | 1.06·10 <sup>11</sup><br>±3.87·10 <sup>10</sup>                     | 9.65·10 <sup>10</sup><br>±1.27·10 <sup>10</sup>                             | 9.98·10 <sup>10</sup><br>±1.43·10 <sup>10</sup> | 98.00<br>±1.12                    |

The number of viable cells per unit mass of dry bacterial culture preparations is an important factor in commercial applications. A daily therapeutic minimum of 10<sup>8</sup> cfu per 1 gram of dry cells is proposed to ensure probiotic effects on consumers' health. These aspects must be considered in the production of dried probiotic preparation for use as a food supplement or starters cultures [Ananta et al. 2005]. Table 3 shows that, in most preparations, condition of minimum cell concentration (10<sup>8</sup> cfu/gram) is met. When emulsions P8 3.0/D3.0 and P4 3.0/D3.0 were used as protective agents in both freeze and spray drying, there were fewer living cells per gram than 10<sup>8</sup>, in dry preparations. On the other hand, P8 3.0/D3.0 emulsion ensured high concentration of viable cells only in freeze-dried preparations. Directly after spray drying the highest viable cell concentrations, ranging to 10<sup>11</sup> were observed for the cells suspended in P8 3.6/D2.4 emulsion, and in 6% PVP, and 6% dextran solutions, respectively. Similar results were observed in freeze-dried preparations when 6% PVP solution, 6% dextran solution, and skim milk were used as protective agents. In all tested preparations, the storage temperature had small influence on the amount of viable cells.

## CONCLUSIONS

There was no difference in cells viability in dry preparations obtained by freeze and spray drying when the *Lactobacillus rhamnosus* GG was dried in PVP/dextran, PEG4000/dextran and PEG8000/dextran. However, the *L. rhamnosus* GG cells survival rate strongly depends on the protective agent in which bacteria is suspended before drying. The concentration of viable cells after storage also depends mainly on a protective medium utilized in the process of drying. Storage temperature and drying method had low impact on cell viability. The aqueous two-phase systems used in this work provide a sufficient environment to protect bacteria cells during freeze and spray drying and guarantee high cell stability during storage. For economical reasons, spray drying of *L. rhamnosus* GG cells suspended in the ATPS emulsions can be successfully used as a method to produce dry bacteria preparations.

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## PRODUKCJA PREPARATÓW *LACTOBACILLUS RHAMNOSUS* GG METODAMI SUSZENIA ROZPYŁOWEGO I LIOFILIZACJI W WODNYCH EMULSYJNYCH UKŁADACH TYPU ATPS

**Wstęp.** Suszenie jest najstarszą metodą konserwowania żywności. Polega na usuwaniu wody z produktów spożywczych, co zapobiega rozwojowi mikroflory bakteryjnej i gnicciu. Ponadto suszenia: rozpyłowe i liofilizacyjne są stosowane także do zabezpieczania kultur probiotycznych. Celem pracy było porównanie przeżywalności bakterii probiotycznych *Lactobacillus rhamnosus* poddanych procesowi suszenia rozpyłowego i liofilizacji w wodnych emulsyjnych układach dwufazowych. Przeżywalność była także porównywana z przeżywalnością bakterii w tych samych warunkach, ale zawieszonych w od tłuszczonym mleku, 6-procentowym roztworze PVP lub 6-procentowym roztworze dekstranu.

**Material i metody.** Hodowla bakterii *Lactobacillus rhamnosus* GG była zawieszona i suszona rozpyłowo oraz liofilizacyjnie w różnych typach wodnych emulsyjnych układach dwufazowych: PVP/dekstran, PEG4000/dekstran i PEG8000/dekstran. Emulsje te składały się z różnych typów polimerów zmieszanych w różnych proporcjach faz rozproszonych (dekstran) i rozpraszającej (PEG i PVP).

**Wyniki.** Badania wykazały, że przeżywalność bakterii bezpośrednio po suszeniu zależy w większym stopniu od zastosowanego podczas suszenia czynnika ochronnego niż od metody suszenia. Po 30 dniach przechowywania wysuszonych preparatów bakterii największą przeżywalność zaobserwowano u komórek zawieszonych w mleku. W suszeniu rozpyłowym największą przeżywalność wykazały bakterie zawieszone w emulsji PVP3,6%/dekstran2,4%.

**Wnioski.** Stwierdzono, że stopień przeżywalności bakterii nie jest ściśle zależny od temperatury przechowywania, lecz od substancji ochronnej stosowanej podczas suszenia.

**Słowa kluczowe:** ATPS, liofilizacja, bakterie probiotyczne, suszenie rozpyłowe

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