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ORIGINAL PAPER

# Influence of Microencapsulation and Spray Drying on the Viability of *Lactobacillus* and *Bifidobacterium* Strains

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Abstract

Improved production methods of starter cultures, which constitute the most important element of probiotic preparations, were investigated. The aim of the presented research was to analyse changes in the viability of *Lactobacillus. acidophilus* and *Bifidobacterium bifidum* after stabilization (spray drying, liophilization, fluidization drying) and storage in refrigerated conditions for 4 months. The highest numbers of live cells, up to the fourth month of storage in refrigerated conditions, of the order of $10^7$ cfu/g preparation were recorded for the *B. bifidum* DSM 20239 bacteria in which the N-Tack starch for spray drying was applied. Fluidization drying of encapsulated bacteria allowed obtaining a preparation of the comparable number of live bacterial cells up to the fourth month of storage with those encapsulated bacteria, which were subjected to freeze-drying but the former process was much shorter. The highest survivability of the encapsulated *L. acidophilus* DSM 20079 and *B. bifidum* DSM 20239 cells subjected to freeze-drying was obtained using skimmed milk as the cryoprotective substance. Stabilization of bacteria by microencapsulation can give a product easy to store and apply to produce dried food composition.

Key words: *Bifidobacterium*, *Lactobacillus,* freeze drying, fluidization, microencapsulation, spray drying, probiotics

## Introduction

Majority of vegetative forms of microorganisms are characterized by poor thermostability. They exhibit considerably high rates of dying and loss of activity as a result of thermal inactivation at the range of temperatures from 40 to 60°C. With regard to microbial biomass, there is certain critical water content (depending on the object property) which, when exceeded, results in dehydration inactivation. This can be attributed to the fact that in the case of vegetative forms of microorganisms water does not only provide environment for their life but it also acts as a substrate for biochemical reactions and its removal below a certain level prevents maintenance of metabolic functions and, consequently, leads to the death of cells. Among dehydration methods which allow maintaining viability of microbial biomass are: freeze-drying, sublimation drying, including fluidization drying using inert materials (carriers) and spray drying.

The aim of this study was to assess the impact of the microencapsulation process with a liquid-core and different methods of drying on the bacterial survivability and preparation stability during storage at the temperature of 4°C. Both free and encapsulated *Lactobacillus acidophilus* and *Bifidobacterium bifidum* bacteria were fixed in the course of the performed experiments.

Since the age of bacteria subjected to the concentration process is essential to obtain their active concentrate, the drying process was carried out on bacterial biomass obtained by centrifugation of the liquid culture at logarithmic growth phase. Literature data indicate that it is exactly at the end of the logarithmic growth phase that lactic acid bacteria achieve their highest activity (Champagne *et al*., 1996).

## Experimental

### Materials and Methods

**Microorganisms.** The following two bacterial strains were used in the test: *Lactobacillus acidophilus* DSM 20079 and *Bifidobacterium bifidum* DSM 20239

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which were obtained DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen).

**Immobilisation of bacteria by encapsulation with a liquid-core.** Bacteria encapsulation was conducted using the method of extrusion as described by Yoo *et al.* (1996) and Dembczynski and Jankowski (2000; 2002).

A sterile syringe with 0.8 mm diameter needle was filled with 4% (w/v) cation starch solution containing 100 mM calcium chloride and bacterial suspension. The applied starch was soluble in water at 20°C and was characterized by the substitution degree of 0.04 (Central Laboratory of Potato Industry in Luboń, Poland). The starch solution containing $10^8$ cfu/ml of bacteria was added drop by drop to 0.6% (w/v) solution of sodium alginate type MV (Sigma-Aldrich, St. Louis, USA) from the height of about 40 mm and mixed with a magnetic mixer for 15 minutes with the speed of approximately 700 rpm. The developed capsules were filtered off, washed with sterile bi-distilled water and placed for 15 min in hardening solution of calcium chloride of the concentration of 1.22% (w/v) stirring it all the time. Next, the capsules were filtered off again and washed with bi-distilled water.

**Spray-drying of bacteria.** The centrifuged bacterial biomass was quantitatively transferred to 30% (w/v) solutions of three types of modified starch: N-Tack, N-Lok and Hylon VII (National Starch & Chemical). The number of live bacteria in the solution subjected to drying amounted to $10^8$ cfu/ml. The starch solution with the suspension of bacteria was dried in a Niro type Atomizer (Genea) at the air temperature of 185°C at the inlet and 85°C at the outlet. The amount of evaporated water was 5 kg/h.

**Fluidization drying of encapsulated bacteria.** The encapsulated bacteria were dried in a pulse-fluidized drier with the air flow velocity of 4 $m^3$/s, at 40°C for 45 minutes. The total of 100 g of capsules was dried with the number of bacteria in 1 g of capsules amounting to $2 \times 10^8$ cfu.

**Freeze-drying of bacteria in alginate capsules.** Alginate capsules were placed for 30 min in one of the following solutions of the cryoprotective substances: skimmed milk (SM "Mlekpol" – Grajewo, Poland), skimmed milk (SM "Mlekpol" – Grajewo, Poland) with an addition of 5% (w/v) saccharose (POCh, Poland) and 0.35% (w/v) ascorbic acid (Sigma-Aldrich) or 20% (w/v) saccharose solution (POCh S.A., Poland). After filtration, 100 g of capsules containing $2 \times 10^8$ cfu/g were placed on trays of a lyophilization drier. All the operations were carried out in sterile conditions in a laminar-flow cabinet. Next, the capsules were initially frozen for 24 hours at the temperature of –70°C. The freeze-drying process was carried out in a drier consisting of an oil-vacuum pump Vacuumbrand type RZ 2 (Germany) of 2.2 $m^3$/h output and a freezing device of Heto – Holten A/S (Denmark) type FD 3. The drying process was conducted for 48 hours at the pressure of 37 Pa.

**Determination of the number of live bacteria using the Koch's plate method.** The number of live (cfu/g) bacteria in the preparations was determined using the plate method (Burbianka *et al.*, 1983) on the Medium 58 for *B. bifidum* and MRS for *L. acidophilus* (Merck) at 37°C. Cultures of the *L. acidophilus* were carried out in the atmosphere modified by 10% proportion of $CO_2$, while the *B. bifidum* cultures were conducted in special anaerobic generators also modified by 10% $CO_2$ (Schlegel, 2000).

In order to estimate the number of live bacteria in 1 g of capsules, they were placed in 10 ml of 3% (w/v) sodium citrate solution and mixed until a homogenous mixture was obtained. In order to determine the number of live bacteria in 1 g of spray-dried capsules, they were placed in 10 ml of Ringer's solution and mixed until complete solution. The result was given in cfu/g of dry substance. The numbers of live bacteria in capsules following fluidization drying and lyophilization were determined respectively at 10 and 30-day intervals up to the fourth month of storage.

**Statistical analysis.** Each experiment of determination of the number of live bacteria using the plate method was done in triplicate. In the course of the performed statistical analysis of results with the assistance of the Excel 2000 software, all the experimental designs were analysed employing mean descriptive statistics and single-factorial analysis of variance for $P<0.05$.

## Results and Discussion

**Impact of spray-drying on bacterial survivability.** Hylon VII turned out to be the best carrier for the drying process of the *L. acidophilus* DSM 20079 bacteria in which $7.32 \times 10^9$ cfu/g of the carrier were determined after drying (Fig. 1). The highest number of live *B. bifidum* DSM 20239 bacteria was obtained after drying with the N-Tack starch – $1.24 \times 10^{10}$ cfu/g of the dried carrier (Fig. 2). However, these high numbers of bacteria decreased significantly during 10 days of storage falling down to $4.42 \times 10^7$ cfu/g of the carrier in the case of the *Lactobacillus* strain, and to $5.16 \times 10^7$ cfu/g of the carrier for the *Bifidobacterium* strain. In the case of the *B. bifidum* DSM 20239 strain with the N-Tack starch, the number of live cells during further storage did not change and remained at the level of $10^7$ cfu/g of the carrier. A different situation occurred in the case of the *L. acidophilus* DSM 20079 strain with the Hylon VII starch where after 30 days of storage, only $1.98 \times 10^6$ cfu/g were recorded in the preparation. The number of live cells decreased and

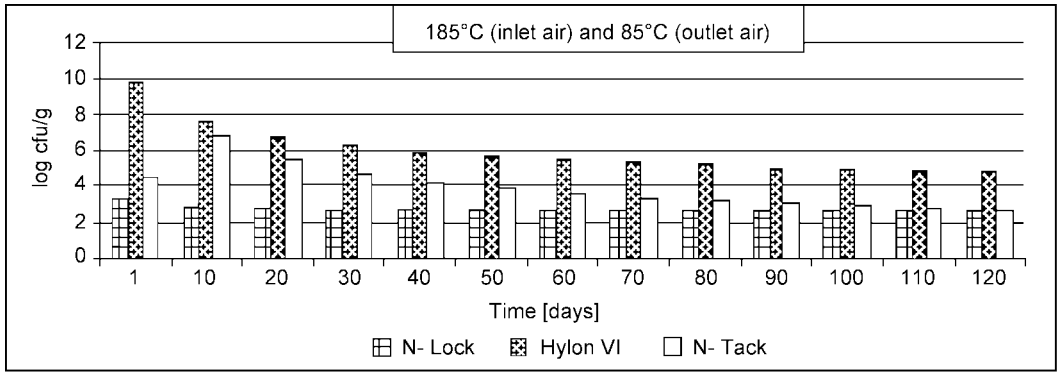


Fig. 1. Change in the number of live *L. acidophilus* DSM 20079 bacteria spray-dried on N-Lock, Hylon Vll and N-Tack carriers during storage at 4°C.

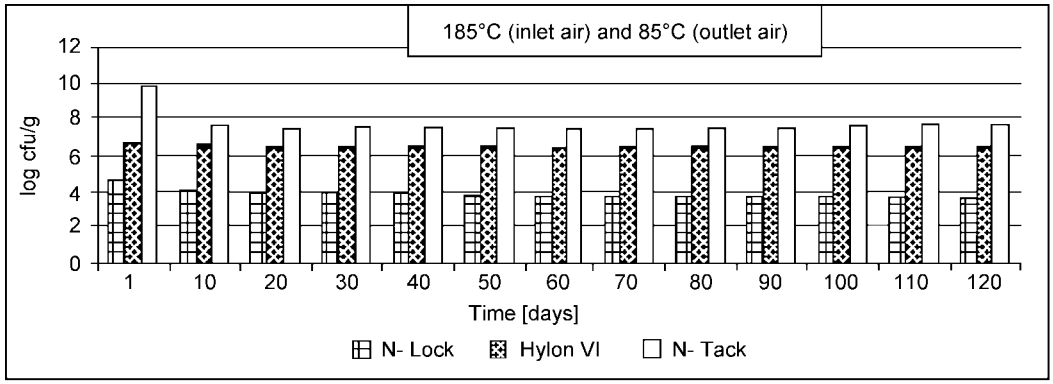


Fig. 2. Change in the number of live *B. bifidum* DSM 20239 bacteria spray-dried on N-Lock, Hylon Vll and N-Tack carriers during storage at 4°C.

after 4 months of storage amounted to $6.5 \times 10^4$ cfu/g of the carrier. The numbers of live *L. acidophilus* DSM 20079 bacteria in the preparations with the N-Tack and N-Lock carriers were unstable during storage. Although in the case of the former, a higher number of cells were recorded after drying, it decreased to the value of $5.25 \times 10^2$ cfu/g of the carrier on day 120 of storage and was similar to the value of $4.52 \times 10^2$ cfu/g of the N-Lock carrier on the last day of storage. Dried *B. bifidum* DSM 20239 bacteria on the Hylon VII carrier turned out stable during storage since in the case of this preparation the number of live cells remained at the level of $10^6$ cfu/g of the carrier during the entire storage period. The N-Lock starch turned out to be the worst carrier for this strain, as after 10 days of storage the number of live cells was only at the order of $10^3$ cfu/g of the carrier which remained relatively stable up to the 4th month of storage. The application of these carriers during the process of bacterial drying yields a product which is stable during storage but the number of live cells in the preparation itself is too low in many cases.

The presence of maize starch with high amylose content is believed to increase the survivability of *Bifidobacteria* at low pH at the presence of bile salts and during the passage through the gastrointestinal tract in mice (Crittenden *et al*., 2001). Presumably, adhesion of bacterial cells to starch is responsible for their increased survivability. That is why the Hylon VII preparation was used as a carrier in the spray-drying of bacteria.

Both the inlet and outlet air temperatures exert a significant influence on the survivability of bacteria subjected to the process of spray-drying. Gardiner *et al.* (2002) reported a significantly higher survivability of the bacterial cells of *Lactobacillus paracasei* NFBC 338 when the outlet temperature was 68°C than when the outlet temperature was 72°C. It should be emphasized that the above-mentioned researchers used powdered milk as the thickening substance which, until now, appears to be the most effective carrier used in the drying of lactic fermentation bacteria.

The survivability of bacteria subjected to spray-drying was influenced significantly by their initial adaptation to high temperatures applied by Desmond *et al.* (2001) for *L. paracasei* NFBC 338 bacteria. Moreover, the supplementation of the medium with betaine or trehalose was reported to enhance the survivability of bacteria subjected to spray-drying (Champagne and Gardner, 2001).

The concentration of live bacterial cells obtained on N-Tack, N-Lock and Hylon VII carriers was not as high as that reported by other researchers who used powdered milk as a protective agent. In the application recommendations, the starch manufacturer does not mention utilization of tested starch sorts for spray-drying. Since literature data report increased survivability of probiotic bacteria in unfavourable environmental conditions when maize starch characterized by high amylose content is used as a carrier, we decided to use in our experiments the Hylon VII preparation. The N-Lock and N-Tack preparations are obtained from maize when it is at its dough stage and are carbohydrate polymers. N-Tack is treated as glucose syrup and perhaps it is its composition that affects the high concentration of *B. bifidum* DSM 20239 cells during the entire period of storage of the spray-dried preparation.

Effect of fluidization drying on the survivability of encapsulated bacteria. Encapsulated bacteria whose number in 1 g of capsules reached $2\times10^8$ cfu were subjected to drying at 40°C for 45 minutes. Directly after drying, the higher number of live cells in 1 g of capsules ($1.7\times10^9$ cfu) was determined in the *L. acidophilus* strain than in the *B. bifidum* strain ($5.2\times10^8$ cfu) (Fig. 3). In both cases, the number of live bacteria in 1 g of dry material was higher than in the capsules subjected to drying, which is quite understandable as 1 g of dry material contains more capsules than 1 g of capsules before drying. During storage, the number of live *B. bifidum* DSM 20239 bacteria in the dry material dropped slightly and amounted to $4.31\times10^7$ cfu/g of capsules in the 4th months of storage. A more dynamic decrease in the number of live bacterial cells in the dry material was observed in the case of *L. acidophilus* DSM 20079 reaching $2.67\times10^7$ cfu/g of capsules already on day 30 of the storage. However, this number remained almost unchanged up to the 4th month of storage when $1.66\times10^7$ cfu in 1 g of dried material was determined. The stability of encapsulated bacteria dried using the fluidization method is greater than that of spray-dried bacteria with the application of carriers.

Reports published in literature indicate that also in the case of the fluidization drying, the type of the applied carrier influences the degree of bacterial survivability. The comparative studies in which the solution of lactic acid bacteria was sprayed onto lactose and saccharose showed clearly that the former carrier was more This happens because saccharose with its smooth crystals increases the speed of drying and therefore, the solution sprayed over its surface evaporates faster. Moreover, in the case of fluidization drying, it was observed that the lower the temperature and shorter the time, the higher the survivability of the treated bacteria (Witrowa-Rajchert and Samborska, 2002).

**Impact of cryoprotective additives on the freeze-drying of encapsulated bacteria**. There are numerous reports about the highest survivability of freeze-dried bacteria. Since the application of cryoprotective substances increases the survivability of bacteria, the following media were employed in the performed experiments: skimmed milk, skimmed milk with the addition of 5% (w/v) saccharose and 0.35% (w/v) ascorbic acid and 20% (w/v) solution of saccharose. The last of the factors was recommended by Champagne *et al.* (1992; 1996). The experiments revealed that out of the applied cryoprotective substances, skimmed milk turned out to be the most effective for *L. acidophilus* DSM 20079 and *B. bifidum* DSM 20239 bacteria (Fig. 4 and 5). The number of live cells of both bacterial strains in the 4th month of storage, when milk was used as a cryoprotector, was significantly higher ($P<0.05$) than the number of live cells in the capsules without the cryoprotective substance – control sample and amounted to $1.64\times10^8$ cfu/$g_{caps}$. and $6.76\times10^8$ cfu/g of capsules in the case of *B. bifi-*

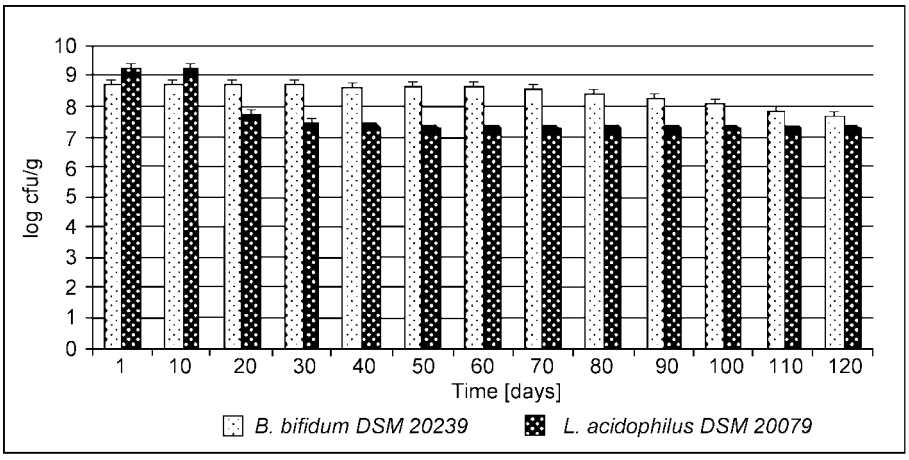


Fig. 3. Change in the number of live *L. acidophilus* DSM 20079 and *B. bifidum* DSM 20239 bacteria in capsules dried with the aid of fluidization during storage at 4°C.

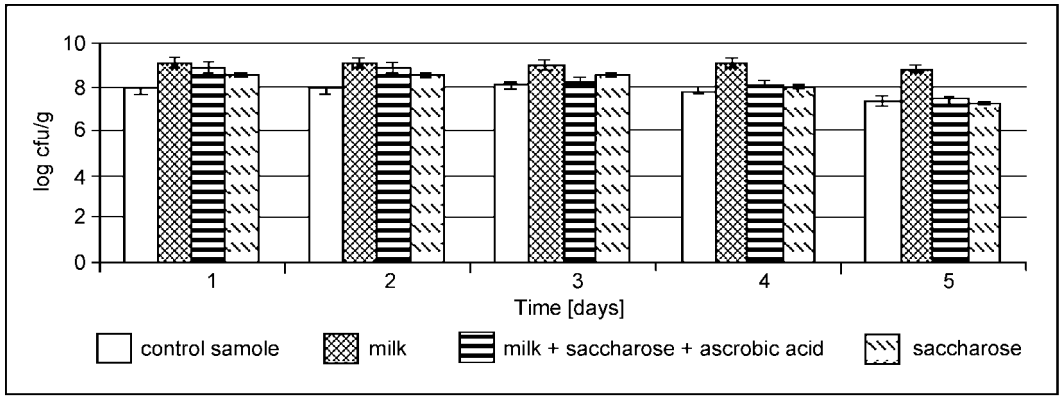


Fig. 4. Change in the number of live *L. acidophilus* DSM 20079 bacteria in capsules freeze-dried with the addition of cryoprotective substances during storage at 4°C.

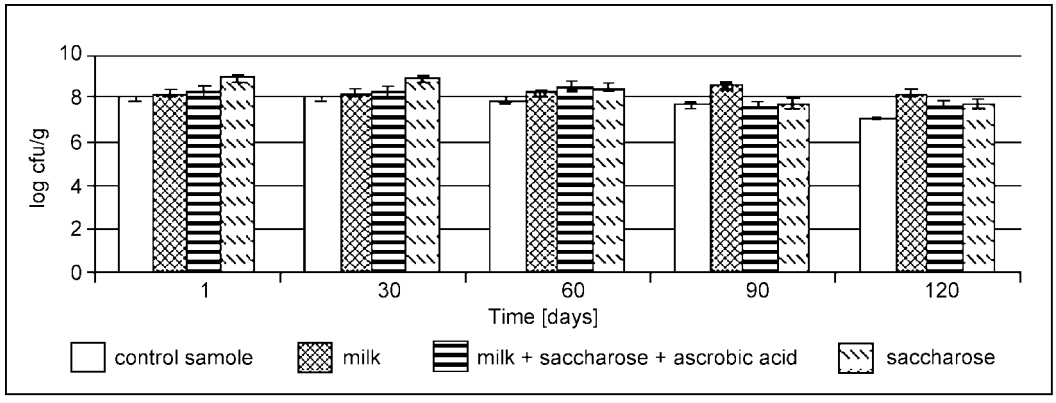


Fig. 5. Change in the number of live *B. bifidum* DSM 20239 bacteria in capsules freeze-dried with the addition of cryoprotective substances during storage at 4°C.

*dum* DSM 20239 and *L. acidophilus* DSM 20079, respectively. When saccharose was applied as the protective agent for the *B. bifidum* DSM 20239 capsules, in comparison with the control, the concentration of live bacteria in the 4th month of storage was higher and amounted to $5.55 \times 10^7$ cfu/g of capsules. Saccharose employed as a cryoprotective agent for the capsules of the *L. acidophilus* DSM 20079 strain failed to give such good results and the number of live cells in the 4th month of storage was slightly lower than in the control sample ($1.72 \times 10^7$ cfu/g of capsules). The number of live cells in capsules fixed in the solutions of milk, saccharose and ascorbic acid, even though significantly higher than in the control sample ($P<0.05$), on the last day of storage was of the same order as that of the control sample and amounted to $2.45 \times 10^7$ cfu/g of capsules for the *L. acidophilus* DSM 20079 strain and $4.78 \times 10^7$ cfu/g of capsules for the *B. bifidum* DSM 20239 strain. The least effective were: the mixture of milk, saccharose and ascorbic acid for the *B. bifidum* DSM 20239 strain and the saccharose mixture for the *L. acidophilus* DSM 20079 strain. Up to the 4th month of storage, in the case of capsules dried using the freeze-drying method without the addition of cryoprotective substances, the numbers of live cells of *L. acidophilus* DSM 20079 and *B. bifidum* DSM 20239 remained at the level of $10^7$ cfu/g and amounted to: $2.07 \times 10^7$ cfu/g and $1.26 \times 10^7$ cfu/g of capsules respectively.

Experiments carried out by Dembczynski and Jankowski (2002) showed that in comparison with free culture bacteria, they obtained higher productivity of the biomass when batch cultures of *Lactobacillus rhamnosus* bacteria was carried out in capsules of calcium alginate with a liquid-core. Lee and Heo (1999) conducted experiments on the survivability in the stomach juice and in the presence of bile acid salts of *B. longum* bacteria immobilized in sodium alginate. The published data confirm that also this technique of microencapsulation improves bacterial survivability in unfavourable environmental conditions. However, it depends on the content of alginate in the capsule, size of capsules and the initial content of bacteria in the capsule as well as on the bacterial strain (Chandramouli *et al*., 2004). The advantages of the technique of microencapsulation in sodium alginate presented in literature encouraged the authors to adopt it in the presented experiments.

From among all the drying methods employed in our experiments, fluidization drying of encapsulated bacteria proved to be the most effective from technological point of view. The observed high survivability of bacteria during the process, the stability of preparations up to the 4th month of storage as well as low energy expenditure confirm that this method can be offered as an alternative in the production of concentrated bacterial preparations. The employed spray-drying of *B. bifidum* DSM 20239 bacteria using N-Tack starch allows obtaining the preparation of the highest number of live cells up to the 4th month of storage in refrigerated conditions of the order of $10^7$ cfu/g of preparation.

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