

FOAM-MAT FREEZE-DRYING OF *BIFIDOBACTERIUM LONGUM* RO175: VIABILITY AND STORAGE STABILITY



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Abstract

Foaming as a pretreatment was used prior to freeze-drying of *Bifidobacterium longum* RO175 to investigate the potential acceleration of the drying rate as well as the increase in microorganism viability after the process. A study on storage of foamed and non-foamed freeze-dried products at different temperatures completed this study.

Bifidobacterium longum RO175 in foamed medium (LEST and SM) could be freeze-dried in 1/4 to 1/7 of the time employed for non-foamed solutions, respectively, and with higher viability after the process. However, reduced density of foamed materials leads to a decreased dryer load, which was only compensated for LEST medium by a shorter drying time to maintain the dryer throughput. Storage lead to a reduction in *Bifidobacterium longum* viability for all tested cryoprotectants (foamed and non-foamed). However, foamed materials had lower viability due to their increased surface area.

Introduction

Freeze-drying is a well known dehydration method widely used to preserve microorganisms. However, the expensive fixed and operation costs are the main drawback of the process.

Although freeze-drying is one of the best methods to preserve microorganisms, different probiotic cultures behave distinctively towards this process. *Bifidobacteria* with its complex shape and low oxygen tolerance are, for example, more sensitive to freeze-drying with survival rates as low as 10% (Maitrot et al., 1997).

Foaming is a pretreatment used prior to dehydration of liquids to reduce drying time or to improve product quality. In the case of vacuum drying, foaming has been shown to stabilize cultures of *Lactobacillus acidophilus* and *Lactococcus lactis* var. *cremoris* during storage having only 40% viability loss (Bronstein, 2004). The strong affinity between microorganisms and foams can be related to the fact that when foams are made from a suspension of small size hydrophobic particles (such as the cellular membranes of bacteria), these will show a tendency to be attached to the bubbles and to concentrate in the foam (Pilpel, 1985; Stratton et al., 2002).

Foaming has also been tested as a pretreatment of egg white (Muthukumar et al., 2008) and apple juice (Raharitsifa and Ratti, 2009a) freeze-drying. From these studies it was revealed that foaming reduced freeze-drying time if the comparison was done at equal sample thicknesses. However, lower density of foamed materials decreases mass load to the dryer. In the case of apple juice, foamed freeze-dried products were shown to be more thermally stable during storage than non-foamed ones (Raharitsifa and Ratti, 2009b). No information has been reported so far in the literature about foaming as a pretreatment of microorganisms freeze-drying.

Objectives

The objective of the present work is to investigate foaming as a pretreatment prior to freeze-drying of *Bifidobacterium longum* RO175 in order to increase the drying rate and the microorganism viability after the process. A study on storage of foamed and non-foamed freeze-dried products at 4°C will complete this study.

Materials and methods

Strain and culture conditions

Culture of *Bifidobacterium longum* RO 175 (Rosell Institute Inc., Montréal, QC, Canada) was carried out in modified medium 50 (Rosell Institute Inc., Montréal, QC, Canada).

The inoculum used for freeze-drying was prepared by subculturing *B. longum* RO175 twice from frozen stock (-80°C) in modified medium 50 with 1% (v/v) inoculums. The cultures were incubated at 37°C for 16 hours in anaerobic jars (BBL Microbiology System, Becton Dickinson, Mississauga, ON, Canada) without agitation. The cells were harvested by centrifugation at 4080 g for 10 min at 4°C in a Sorvall RC-5. The cell pellet was resuspended in a solution containing 0.85% NaCl in order to obtain a 20X concentration factor. Experiments were repeated four times.

Protective agents

Two protective agents, commercial LEST and skim milk (SM) based, were tested for their effect during freeze-drying of *B. longum* RO175. Concentrations of protective agents were chosen according to the preliminaries studies. LEST (Rosell Institute Inc., Montréal, QC, Canada) was mixed with the cell suspension in industrially-used proportions (0.12: 1). For the SM protector, the concentrated cell suspension was added at a 1:1 ratio to an aqueous medium composed of 24% skim milk, 4% sucrose, 0.3% ascorbic acid and 5% trehalose.

Foaming

Cell suspensions in the two protective agents were mixed with 3% (v/v) egg albumin powder in order to generate the foam. Then, the suspensions were introduced in a glass Buchner funnel where foaming was done by gently injecting pure nitrogen gas from a cylinder through the filter bottom of the funnel.

Freeze-drying and storage

Five millilitres samples (liquid or foam) were transferred into sterilised vials, frozen in a freezer (SANYO, Medical freezer, MDF 235) for 24h at -40 °C. Freeze-drying was then carried out at 25°C in a Freeze-mobile 25 L (Virtis, Gardiner, N.Y.) model freeze-dryer, for 4h in the case of foamed media and 16h for liquid under 0.3 Torr vacuum. For the storage experiments, *B. longum* dried powders were stored at 4°C in desiccators for up to 56 days. The experiments were repeated twice.

Enumeration of survivors

After freeze-drying and at each week of storage, 0.1 g of each sample was rehydrated in 9.9 ml of peptone water (Difco, Detroit, USA) and incubated at 37°C for 35 min in a temperature-controlled bath. After serial dilutions, 1 ml aliquots of suitable were plated in modified medium 50 (Rosell Institute Inc., Montréal, QC, Canada) added to 15 g/l of agar. The plates were incubated for 48h at 37°C under anaerobiosis. Duplicate counts were conducted at each sample time. Viability was calculated as the percentage number of survivors respect to initial counts.

Determination of residual moisture

The residual moisture of the freeze-dried products was determined after freeze-drying and at each two weeks of storage (in duplicate) by difference in weight before and after drying in a vacuum oven at 55°C during 48 h in the presence of phosphorus pentoxide. Duplicate counts were conducted at each sample time.

Statistical analysis

Viability results were analysed by a General Linear Model procedure with SAS software (SAS institute, version 6.12, USA) taking 5% as level of significance.

Results and Discussion

Figure 1 shows the freeze-drying curves of *Bifidobacterium longum* RO175 in foamed LEST medium, compared to non foamed solutions. As can be seen, 12 to 14 hours freeze-drying would be required to obtain a dried product with similar moisture content as a foamed product freeze-dried for only 2 hours. Corresponding moisture contents (wet base) are 3.46 and 3.79% for non-foamed and foamed solutions, respectively. For the SM cryoprotector (results not shown), 2 and 8 hours were necessary to freeze-dry the foamed and non-foamed solutions up to moisture contents of 3.88 and 3.81% wb, respectively.

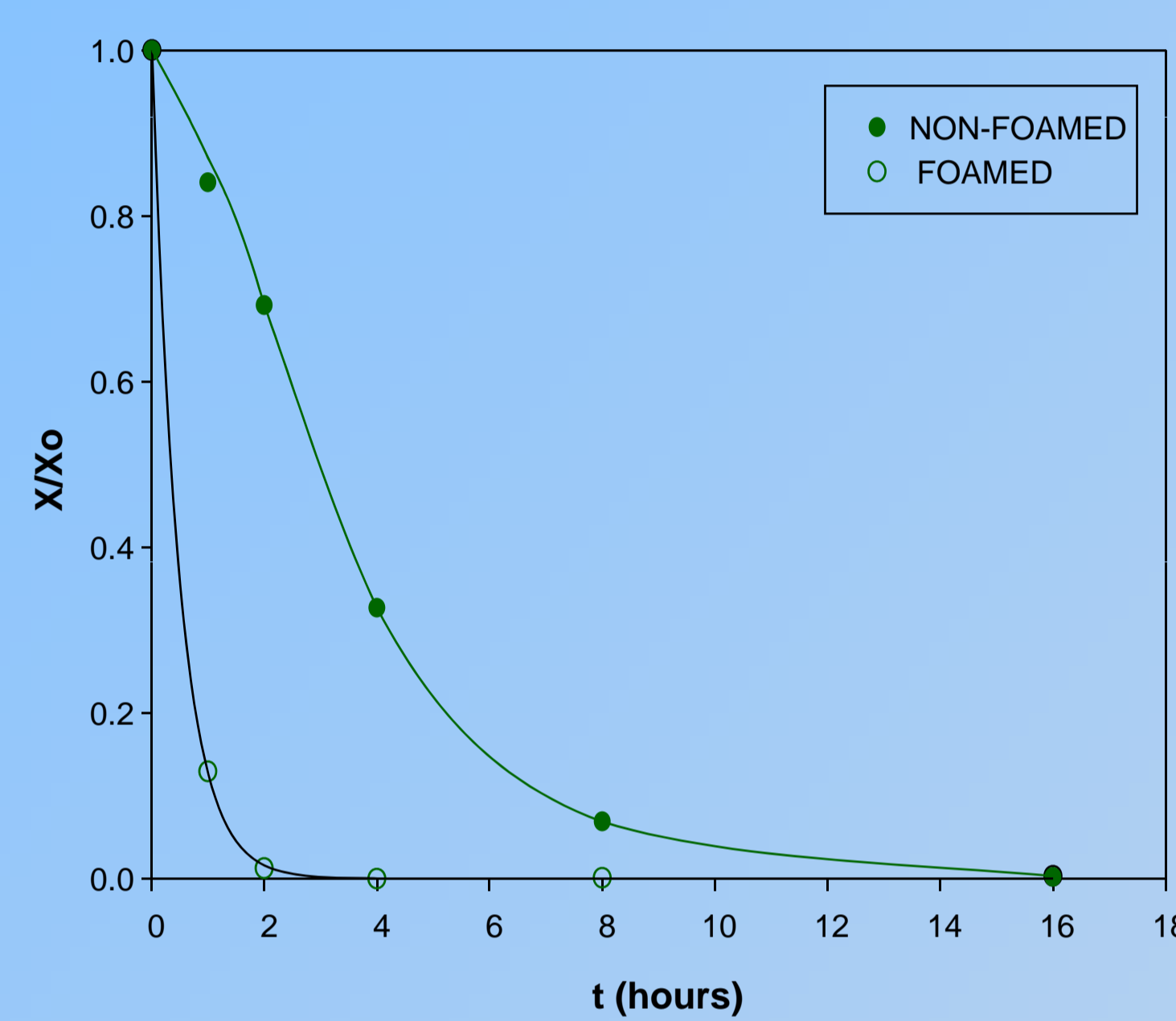


Figure 1: Freeze-drying curves of foamed and non-foamed cultures of *Bifidobacterium longum* RO175 in LEST medium.

Thus, the foaming pretreatment can reduce the time for freeze-drying *Bifidobacterium longum* RO175 in LEST medium approximately by a factor of 7. In the case of the SM cryoprotector, the time reduction factor was lower, approximately 4. Being the foam overrun (density of foam/density of solution) about 700%, the foaming pretreatment could be beneficial to reduce freeze-drying time in industrial applications only when applied to LEST medium.

Figure 2 shows the viability of *Bifidobacterium longum* RO175 after freeze-drying in LEST and SM media. Foamed suspensions presented a higher viability after the process, particularly for the SM medium. It should be taken into account that 1% higher viability percentage in foamed LEST medium correspond to a number of microorganisms of 109, which is not negligible. This better survival of *Bifidobacterium longum* RO175 could be related to the affinity of bacteria to be attached to foamed media.

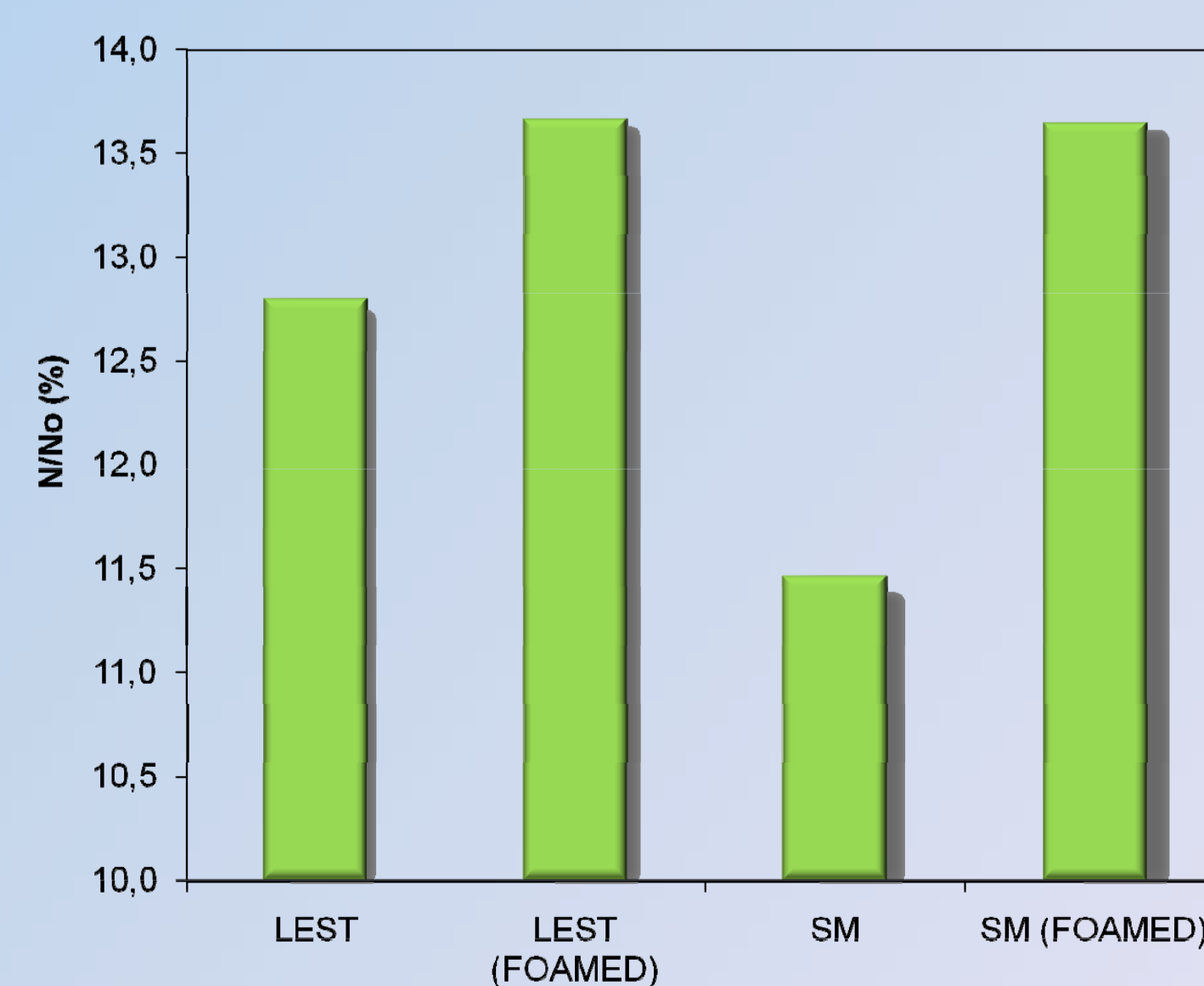


Figure 2: Viability of *Bifidobacterium longum* RO175 after freeze-drying in LEST and SM media (foamed and non-foamed)

B. longum survival during storage was calculated as the ratio of viable cells by their initial number (storage day 0), and represented as a function of storage time as shown in Figure 3. A marked decrease in survival was found in the first 30 days of storage for all solutions tested, with a plateau reached after 40 days. The number of viable microorganisms at 56 days was (in average) 1.88×10^9 , which is in the number range of viable microorganisms offered in commercial probiotic supplements. Foamed freeze-dried materials showed a lower viability than non-foamed ones during storage, probably due to their increased surface exposed to oxygen.

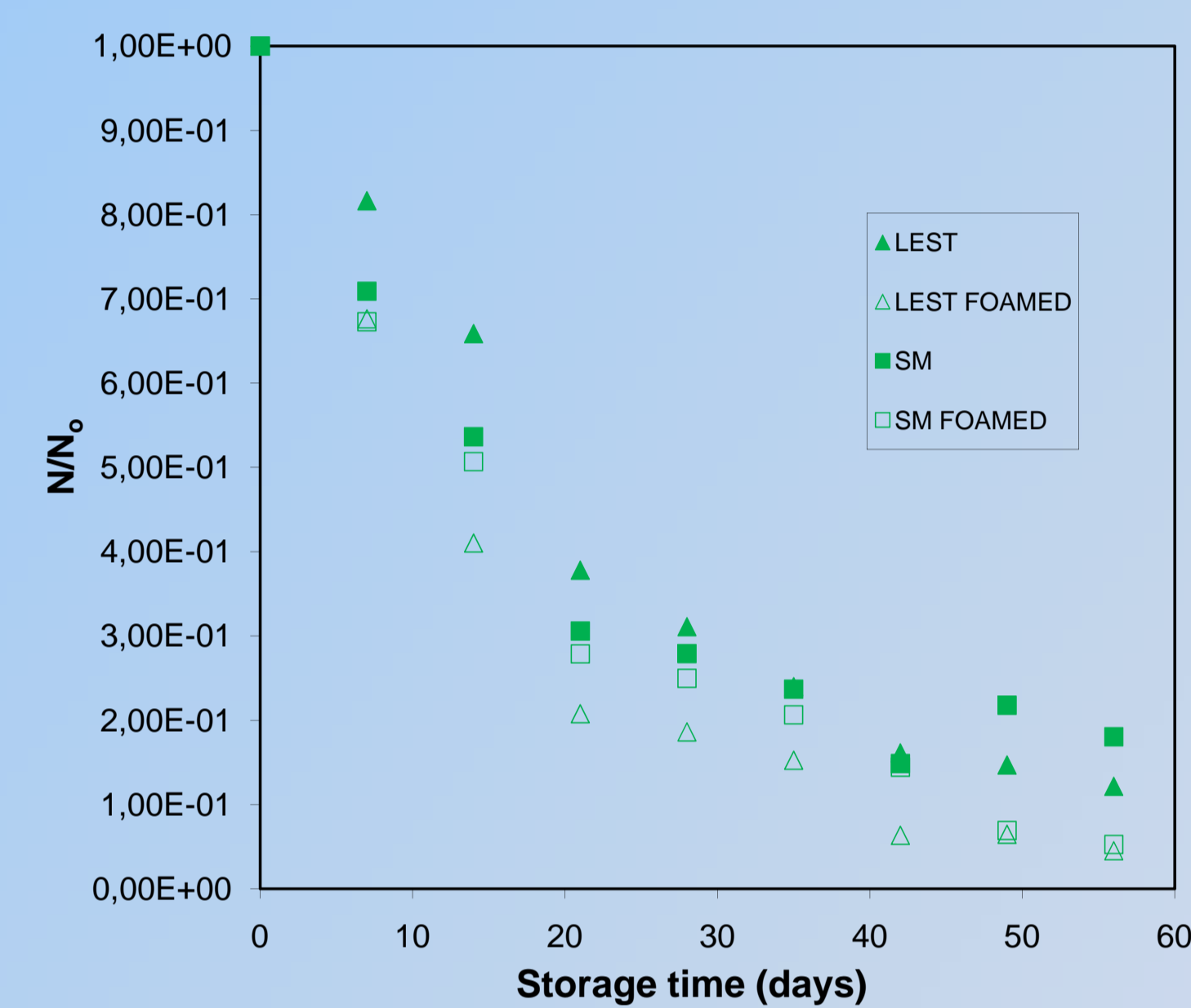


Figure 3: Viability of freeze-dried *Bifidobacterium longum* RO175 during storage at 4°C in LEST and SM media

Conclusion

Bifidobacterium longum RO175 in foamed medium (LEST and SM) could be freeze-dried in 1/4 to 1/7 of the time employed for non-foamed solutions, respectively, and with higher viability after the process. However, reduced density of foamed materials leads to a decreased dryer load, which was only compensated for LEST medium by a shorter drying time to maintain the dryer throughput.

Storage lead to a reduction in *Bifidobacterium longum* viability for all tested cryoprotectants (foamed and non-foamed). However, foamed materials had lower viability due to their increased surface area.

References

- Bronstein, V. (2004). Preservation by foam formation, *Pharmaceutical Technology*, 28, 86–92.
- Maitrot, H., C. Paquin, C. Lacroix and C.P. Champagne (1997). Production of concentrated freeze-dried cultures of *Bifidobacterium longum* in K-carrageenan-locust bean gum gel. *Biotechnol. Technol.* 11, 527–531.
- Muthukumar, A., C. Ratti, V.G.S. Raghavan (2008). Foam-Mat Freeze Drying of Egg White-Mathematical Modeling Part II: Freeze Drying and Modeling. *Drying Technology*, 26(4), 513 – 518.
- Pilpel, N. (1985). Foams in pharmacy, *Endeavour*, New Series, 9 (2), 87-91.
- Raharitsifa, N. and C. Ratti (2009a). Foam-mat freeze-drying of apple juice: experimental data and ANN simulations. *Journal of Food Process Engineering* (in press).
- Raharitsifa, N. and C. Ratti (2009b). Foam-mat freeze-drying of apple juice. Part 2: Stability of dry products during storage. *Journal of Food Process Engineering* (in press).
- Stratton, H. M., P.R. Brooks, P.C. Griffiths and R.J. Seviour (2002). Cell surface hydrophobicity and mycolic acid composition of *Rhodococcus* strains isolated from activated sludge foam. *Journal of Industrial Microbiology and Biotechnology*, 28, 264-267.