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Survival Ability during Freeze-Drying and Subsequent Storage of Probiotic Lactic Acid Bacteria Isolated from Traditional Fermented Cereal-Based Products

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A B S T R A C T

Freeze-drying is a commonly used drying technique for sensitive biologicals, such as lactic acid bacteria (LAB). However, The choice of an appropriate drying medium is important, so as to increase the survival rates during dehydration itself and subsequent storage. The aim of the present study was to evaluate the protective effect of different agents (skim milk, yeast extract, sucrose, trehalose and glucose) on the survival of five potential probiotic LAB originated from traditional fermented products. The strain survival during freeze-drying and after 180 days of storage at 4°C was determined. After drying, the residual water contentswere between 3.17 and 8.59%. The results evidenced that LAB resistance to freeze drying was dependent on both microorganism and lyoprotective agent used. According the obtained rate immediately after freeze-drying, the used protective agents played an important role in preserving the strains viability apart from glucose. *Lactobacillus plantarum* S32 obtained the best survival withrate greater than 85% for 4 protective agentswith an optimum value for trehalose (94,01%). *Pedioccocus acidilactici* strains also had good survival with these same protectants. Refrigerated storage made it possible to achieve dried cultures with high stability and long shelf-life.

Introduction

Lactic acid bacteria (LAB) are present in a wide range of fermented food and beverages from traditional ancestral products to these days.These products with very variable qualities are mainly obtained from cereals. During fermentation, LAB are dominant and prevent the growth of potentiel pathogenic and spoilage microorganisms, enhancing the safety and shelf-life of foods. These bacteria produce several interesting compounds, such as bacteriocins, vitamins, exopolysaccharides and

enzymes, which modify food composition and properties (Fessard and Remize, 2019). The studies of technological and safety properties of LAB from different origins evidence that such microbial group has appropriate characteristics for many applications (Berbegal *et al.,* 2016). Thus, to contribute to food quality and its bioconservation, LAB are now increasingly used for their probiotic properties, both in humans and animals (Viana de Souza and Dias, 2017).

The probiotics are live organisms that confer health benefits on the host when consumed in adequate amounts, by bringing the microbial balance in the system. The search for new strains is a crucial requirement for a successful development of the functional foods market. The industrial use of LAB as probiotics and their direct inclusion in food or dietary supplements require the delivery of stable cultures in terms of viability and functionality. Thus, the maximisation of survival of LAB cultures during drying and subsequent long-term storage is of vital importance at the technological and economical level (Selmer-Olsen *et al.,* 1999; Poddar *et al.,* 2012; Tripathi and Giri, 2014).

Freeze-drying is commonly used for the preservation and long-term storage of LAB, as it allows easy and inexpensive shipping and handling.Its also considered to be the mildest dehydration technique for preservation of cultures due to the ability to maintain a high degree of cell viability (Halim *et al.,* 2017). However, not all strains survive to the process and, among those that do, low viability rates have been reported (Abadias *et al.,* 2001). The major causes of loss cell viability in freeze-drying are probably ice crystal formation, high osmolarity due to high concentrations of internal solutes with membrane damage, macromolecule denaturation, and the removal of water, which affect properties of many hydrophilic macromolecules in cells (Zayed and Roos, 2004; Santivarangkna *et al.,* 2008). To succeed in freeze-drying lactic acid bacteria, the physiological status of the cells, the drying matrix and drying procedure need to be carefully planned (Meng *et al.,* 2008).

In addition, stability of probiotic microorganisms during freeze-drying and storage may be enhanced by addition of protective agents.The choice of an appropriate drying medium is thus very important in the case of LAB, so as to increase their survival rates during dehydration itself and subsequent storage. For most LAB cultures of commercial interest for the dairy industry, skim milk powder is selected as drying medium (Castro *et al.,* 1995; Selmer-Olsen *et al.,* 1999). Different levels of protection have also been observed with different sugars (e.g. glucose, fructose, lactose, mannose and sucrose), sugar alcohols (e.g. sorbitol and inositol) and non-reducing sugars (e.g. trehalose) (Carvalho *et al.,* 2002; Zamora *et al.,* 2006; Higl *et al.,* 2007; Miao *et al.,* 2008; Pehkonen *et al.,* 2008; Strasser *et al.,* 2009). However, the effect of protectives agents during drying and freezing was shown to be strain specific (Siaterlis and Charalampopoulos, 2009; Strasser *et al.,* 2009). There are considerable differences in the freeze-drying tolerance of closely related organisms, different strains of the same species can behave completely different. Thus, compatibility of probiotic candidates with protectors must therefore be determined on a case-by-case basis to achieve maximum viability and stability as stated by Jofré *et al.,* (2015). This work aimed to characterize the survival during freeze drying and subsequent storage of five putative probiotic LAB strains previously selected. The effect of various sugars previously reported to have protective capacity during freeze-drying and storage has been evaluated for 180 days at 4°C to find the most effective protectants for the production of cultures containing high levels of viable cells.

Materials and Methods

Origin of strains and growth medium

Strains tested in this study were LAB obtained from the culture collection of Laboratory of Biotechnology and Food Microbiology (University Nangui Abrogoua, Abidjan, Côte d'Ivoire). These strains originated from two Ivorian traditional products, *tchapalo* (sorghum beer) and *doklu*

(fermented maize dough). The study was conducted with five strains previously screened and selected according to theirtechnological and probiotic properties. The strains were identified by 16S rDNA (V1-V3 region) sequencing and submitted to the GenBank sequence database for accession numbers (Table.1).

Phylogenetic analysis showed a close association between the two *Lactobacillus plantarum* and also with the reference strain *Lactobacillus plantarum* NR113338 (Fig.S1). *Pediocccus acidilactici* strains were also related.The strains were preserved in de Man-Rogosa-Sharpe (MRS) broth with 25% (v/v) glycerol content at -80°C. For analysis, the strains were subcultured on MRS agar plate and incubated at 37°C for 48h under microaerobic conditions.

Production characteristics of strains

Cell preparation for determination of biomass production

Strains were subcultured on MRS medium. 50 ml of the medium were inoculated with 3% of the active culture incubate at 37°C by shaking (130 pm).

Bacterial growth was monitored by measuring the optical density at 600 nm (OD600) using a spectrophotometer (RAYLEIGH UV-1800, Biobase, China). The difference between the initial OD and the OD at different times was taken as an indication for the amount of growth. At the early stationary phase, the medium was divided into 2 parts; 20 and 30 ml. The cells were harvested by centrifugation (Refrigerated Centrifuge TGL-16M, China) at 7000 rpm for 10 min at 4°C. The resulting pellet of the first part (20 ml) was used for the determination of the cell dry weight. The supernatant of 30 ml was used to study the separation of biomass.

Cell dry weight

The previously obtained pellet in the centrifuge tubes was washed with saline water and dried at 105°C for 4h untila constant weight was observed.

The separation of biomass

The optical density of the previously obtained supernatant was measured at 600nm (OD_{600}) and used to express the biomass separation as described by Gerhardt (1982). Azero reading was taken as an indication for excellentseparation and the OD ranged from 0 to 0.1 indicateda good separation of biomass. While OD ranged from 0.2 to 0.3 and more than 0.3 indicated a fair and poor biomass separation, respectively.

Preparation of media and cultures for freeze-drying

Preparation of protective agents

Five lyoprotectants were used in this study: skim milk, yeast extract, sucrose, trehalose and glucose. The protective agent solutes were preparedwith distilled water into various concentrations (Table.2). The sugars were sterilized at 115°C for15 min. The other protective agents were sterilized at 108°C for 15 min.

Culture preparation and freeze-drying

Cells at the beginning of stationary phase were collected after centrifugation at 7000 rpmfor 10 min, and washed twice with sterile saline water. Cells were suspended in sterile protective agents and maintained for 1 h at room temperature to allow for equilibration between cells and added compounds. Sterile distilled water was used ascontrol. The suspensions were frozen at -80°C for 8h and then freeze dried (1 Pa, -45°C) in Biobase Biodustry freeze drier (Model BK-FD10P, China) for 24 h.

Water content determination

Freeze dryed cell water content was determined by drying at 105°C until a constant weight was obtained andcalculation was done as follow:

$$\rho = \frac{(m_1 - m_0) - (m_2 - m_0)}{m_1 - m_0} \times 100$$

ρ= water percentage in freeze dryed cell

m_0= mass of empty tube (g)

m_1= m_0+ sample (g)

m_2= m_0+ sample after drying (g)

Storage and viable counts

Dry cells were stored in closed containers at 4°C. Viable cells before and after freeze drying and during the storage were enumerated using the plate count method. Dried cells taken at random were resuspended in the originalvolume of buffered peptone water. The rehydrated cells were allowed to equilibrate for 15 min at room temperature and suitable dilutions were plated on MRS agar. Then viable cell counts were determined after 48 h of incubation at 37°C under anaerobic conditions. Viable cells enumeration during storage was carried out every 30 days during the 6 months storage at 4°C.

The change in viability after freeze-drying is expressed as "survival factor", according to the following formula:

$$Survival\ rate = \frac{Nr}{Nf} \times 100$$

Nr = Viable cells after lyophilization (CFU/ml)

Nf = Viable cells before lyophilization (CFU/ml)

Stability of lyophilized cultures

The stability of the lyophilized cultures was measured as the acidifying power determined in the period between lyophilization (zero time) and 30 days after lyophilization. The acidification activity of freeze dried and untreated cultures was followed in duplicate in maize flour extract broth at 0, 2, 4, 6, 8, 24 and 48h interval Flour extract broth was prepared with maize powder flour suspended in water (300 g in 1 L) and boiled for 15 min. The liquid phase obtained after centrifugation was sterilized by autoclaving and used for acidification test. This brothwas inoculated with 1% of fresh culture (OD = 1) and incubated at 30°C. The effect of freeze-drying on the culture stability was monitoring by measuring the pH at the various times and in comparison with the acidification of the unlyophilized strains.

Statistical analysis

The mean values and standard deviation were calculated from two independent experiments.The significance of the difference between the mean values was determined using the analysis of variance (ANOVA) with the software XLSTAT (Version 2014.5.03). The confidence interval for a difference in the means was set at 95% ($P < 0.05$) for all comparisons.

Results and Discussion

Yield after production

The criterion considered for biomass production in this study was the dry weight of strains. The results obtained are presented in Table.3. *Lactobacillus plantarum* strains had the highest cell dry weight with values of 10.61 and 10.48 g/L for S32 and S121 respectively. The strains with the lowest values were *Pediococcus acidilactici* S55 and S56.

Separation of biomass

In general, most tested strains showed a good biomass separation when collected by centrifugation (Table.3). The results revealed that all strains showed a well-formed good pellet ($OD_{600}\leq$ 0,1), indicating that these strains can be easily separated by centrifugation. The best separation value was obtained with strain*Lactobacillus plantarum* S121 for an OD_{600} of 0.03.

Water content of freeze-dryied cultures

The water contents after freeze-drying is mainly dependent onthe suspending medium during freeze-

drying. The type of lyoprotectant used is very important to the strains water content. Therefore, for all lyoprotectants used in this study, water content was determined. Indeed, with glucose as a protective agent, the water content was significantly higher compared to the other agents (Fig.1). Its value is 8.59% while with the other cryoprotectors used, the water content is between 3.17 and 4.53%.

Effect of protective agents on survival during freeze drying and storage

In this step of experiments, the aim was to investigate whether the lyoprotectants affected the ability of the cells to survive the freeze drying process and during storage. All tested strains were freeze-dried using these following protective agents: skim milk, yeast extract, sucrose, trehalose and glucose. Cells survival was determined and viability results are displayed graphically in Fig.2. This figureshows that all the protective agents have a good effect on the different strains, this because all the strains have survived after the freeze-drying and during the storage at different rates.

Pediococcus pentosaceus A6

Yeast extract and sucrose were found to be the best protective agents during drying processof *Pediococcus pentosaceus*A6 (78.8 ± 3.4 % and 76.47 ± 1.7 % repectively). However, skim milkand trehalose have also shown a good protective effect. Glucose obtained the lowest result with 33.2 ± 2.3%. During storage, no considerable decrease in the viability of the A6 strain was observed regardless of the protector used. The values obtained with glucose remained almost constant during the 6 months of storage, however a decrease of approximately 1 Log CFU/mL of viable cells was observed between the 3rd and 6th months while using yeast extract as a lyoprotector.

Lactobacillus plantarum S32

The different protective agents used in the drying process of *Lactobacillus plantarum* S32 have shown very good survival rates. The values obtained were greater than 85% except for the glucose which gave a level of 60±4.31%. Very good values were also recorded during storage. Apart from a drop in survival of approximately 1.6 Log CFU / mL between the 5th and 6th month of the trehalose-based lyophilisates, very good survival was obtained with the other protectors throughout the storage period.

Lactobacillus plantarum S121

Among the five (5) lyoprotectors used during the drying process of *Lactobacillus plantarum* S121, four (4) have given satisfactory results. The best survival rate was obtained with skim milk (88.46±3.45%) while the lowest ratewas obtained with glucose (17.77 ± 2.12%). During storage, the best survivals were observed with skim milk. The greatest loss observed is about 2 Log CFU / mL of viable cells for lyophilisates with trehalose. Storage with other protectors has given satisfactory results.

Pediococcus acidilactici S55

Of the five (5) protective agents used in the study, four (4) have given very good yields during the drying process of strain *Pediococcus acidilactici* S55. Only freeze-drying done with glucose has given a low survival rate with 36.9±4.3%. During storage, the greatest loss of viability was observed with trehalose (approximately 2 Log CFU/mL over 180 days), mainly during the last month. The other protective agents ensured a good preservation of which the best with skim milk which registers a loss of only 0.4 Log CFU/mL during all the storage.

Pediococcus acidilactici S56

Pediococcus acidilactici S56 withstood the drying process with survival rates higher than 65% regardless of the protective agent used. The best rates were 95.24±2,24% and 91.30±3,24% respectively with skim milk and trehalose.Glucose once again gave the lowest rateafter drying. The strains preserved in the presence of sucrose suffered

the biggest loss during storage. This loss has been gradual throughout the shelf life with a total loss of approximately 1.7 Log CFU/mL. The best survival rate was obtained with glucose which cell losses were less than 0,2 Log CFU/mL.

Stability after freeze drying

To evaluate the ability of the freeze-dried strains to recover their acidification activity after deshydratation, the pH were measured during fermentation in maizeflour extract broth.

According to the difference between the activity of freeze drying and unfrozen cultures in relation to ΔpH, all strains regained their acidification capacity regardless of the cryoprotectant used. Thus, the pH differences for all strains remained sufficiently close to those obtained with undried strains (Fig.3).

Inclusion of beneficial LAB strains in food products requires a methodology to preserve high cell viability and beneficial properties during product elaboration and subsequent storage.

Maximization of survival of LAB cultures during drying and subsequent storage for long periods is of vital importance, both technologically and economically. In this work, lyophilization ability of fives LAB strains and the efficacy of five lyoprotectants during drying process and the later storage was evaluated.

The effect of these protectors on bacterial viability and maintenance of the beneficial properties of these potential probiotic candidates was studied. In order for probiotics to have a beneficial effect in the gut, the recommended concentration of viable cells is ~10^8–10^{10} CFU/day, which corresponds to ~10^6–10^8 CFU/g in the product at the time of consumption (Champagne *et al.,* 2011; Dianawati *et al.,* 2013). These values demonstrate the importance of good biomass production before drying. In this study, the dry weight of strains were used to reflect the amount of growth. Compared with other strains, *Lactobacillus plantarum* obtained the highest cell dry weightwith values of 10.61 and 10.48 g/L. These results are in agreement with those of Ayad *et al.*, (2004) who found that *Lactobacillus* strains had generally the highest biomass production.

Concerning the biomass separation, the tested strains obtained a good separation when collected by centrifugation. The results revealed that all strains showed a well-formed pellet, indicating that these strains can be easily separated by centrifugation (OD_{600} < 0,1).

After freeze drying, the residual water content is an important parameter for the stability of dried cells (Clementi and Rossi, 1984). Several studies showed that for freeze-dried bacterial solutions, a high residual water content negatively affects viability during storage (Zayed et Roos 2004; Santivarangkna *et al.,* 2011).

The results in this study showedwater contents between 3.17 and 8.59%, mainly dependent on the used lyoprotectant. Similar observations were reported by Zayed and Roos (2004) in their study. These authors reported a water content of 5 - 8% for active dry yeasts.

Overall, the water content of the dried samples was within the recommended values for long-term storage of dried free probiotic cells, less than 10% w/w (Santivarangkna *et al.,* 2007; Vesterlund *et al.,* 2012).

The obtained suvival rates after drying could give us a better visibility on the most appropriate cryoprotectant for these strains. The results showed thatsuspensions of LABin water without any protective agent before freeze-drying gave a very low survival rates immediately after freeze-drying.

Considering these values obtained, the addition of protective agents were found to significantly improve the survival rate of the strain with different degrees of protection.

Table.1 LAB strains tested in this study and their origins

Strains designation	Species	Origin	GenBank accession number
A6	*Pediococcus pentosaceus*	Sorghum beer	OK415419
S32	*Lactobacillus plantarum*	Fermented maize dough	OK415420
S121	*Lactobacillus plantarum*	Fermented maize dough	OK415421
S55	*Pediococcus acidilactici*	Fermented maize dough	OK415422
S56	*Pediococcus acidilactici*	Fermented maize dough	OK415423

Table.2 Protectives agents and their concentrations used in this study

Protective agents	Concentrations (w/v)
Skim milk	25%
Yeast extract	6%
Sucrose	25%
Trehalose	8%
Glucose	12%

Table.3 Cells dry weight and biomass separation values of fives LAB strains isolated from fermented cereal-based products

Strains	Dry weight (g/L)	Separation (OD_{600})
***Pediococcus pentosaceus* A6**	7.51 ± 0.03^{a}	0.046 ± 0.001^{a}
***Lactobacillus plantarum* S32**	10.61 ± 0.05^{b}	0.052 ± 0.001^{a}
***Lactobacillus plantarum* S121**	10.48 ± 0.04^{b}	0.03 ± 0.001^{b}
***Pediococcus acidilactici* S55**	6.88 ± 0.001^{a}	0.064 ± 0.002^{c}
***Pediococcus acidilactici* S56**	6.73 ± 0.05^{a}	0.076 ± 0.002^{c}

Fig.1 Water content values of freeze dried LAB strains with different lyoprotectants

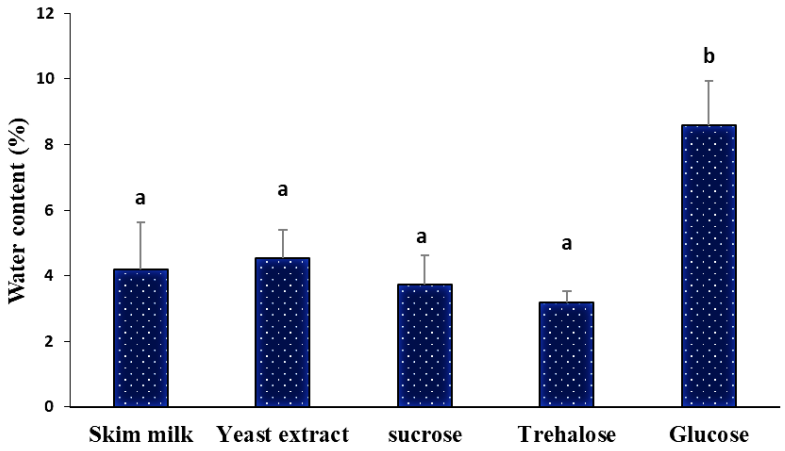


Fig.2 Process survival and survival curve during storage of dried LAB strains

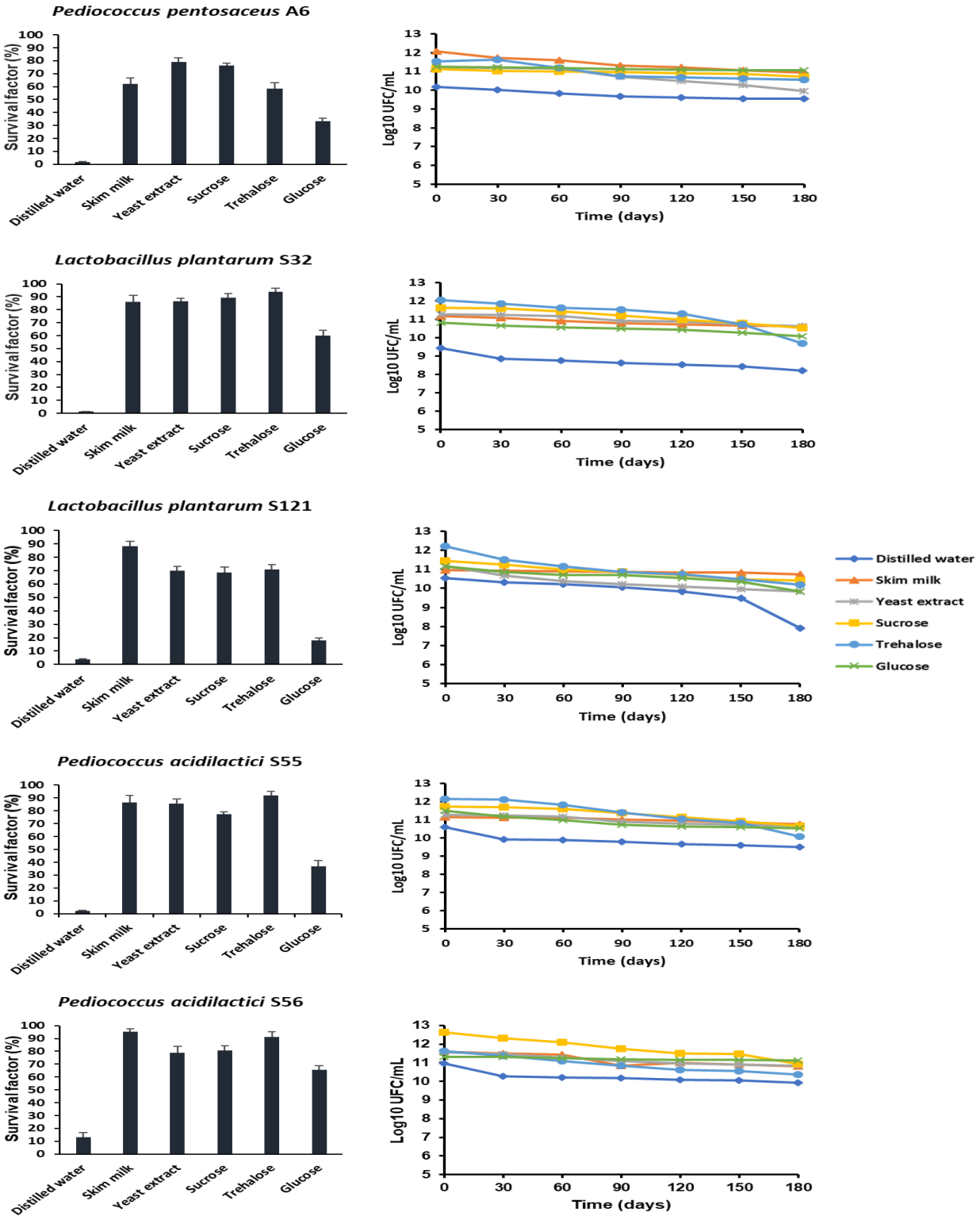


Fig.3 Acidification ability of LAB strains before and after freeze drying using different cryoprotectors

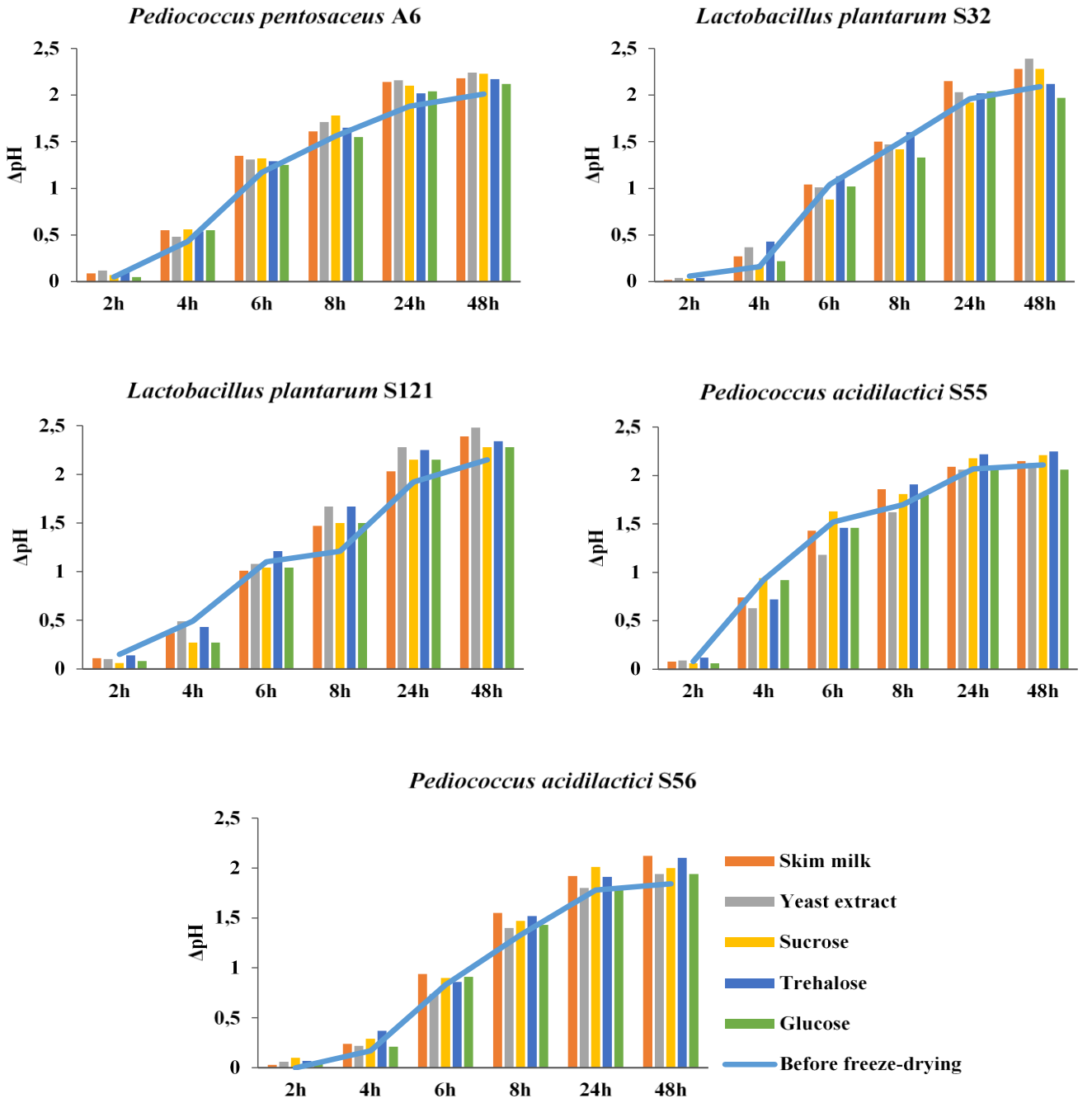
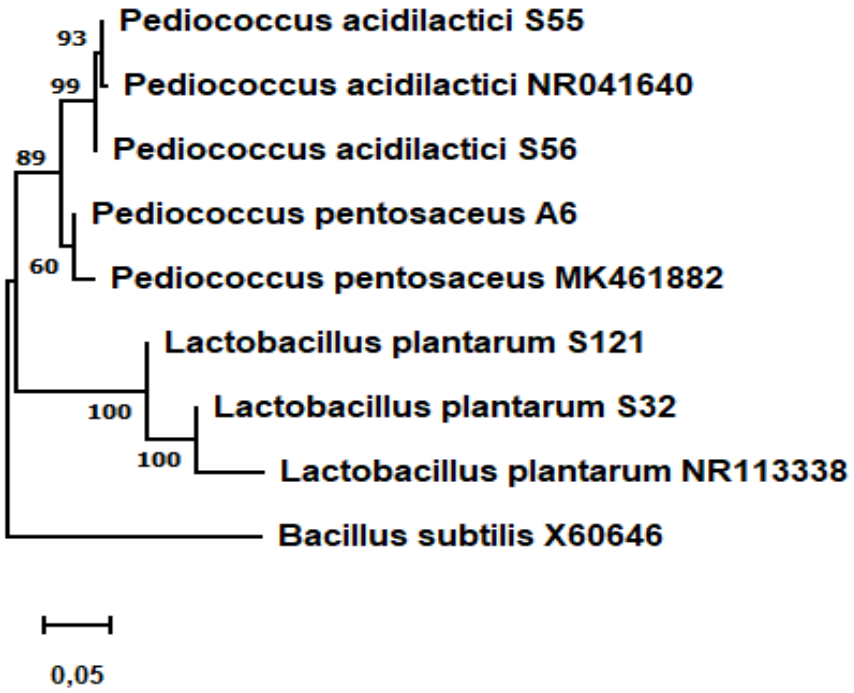


Fig.4 Neighbour joining phylogenetic tree based on 16S rDNA sequences. The relative positions of LAB isolates (A6, S32, S121, S55 and S56) in comparison to reference strains conducted in Mega X software. The percentage of replicate trees in which the associated taxa are clustered together in the bootstrap test of 500 replicates is shown next to the branches. *Bacillus subtilis* is the out group used for tree construction.



The results evidenced that LAB resistance to freeze drying was dependent on both microorganism and lyoprotective agent used. Several studies were conducted to test the efficacy of skim milk, trehalose, yeast extract, sucrose and glucose as protective agents during freeze drying of lactic acid bacteria (Leslie *et al.,* 1995; Giulio *et al.,* 2005; Schoug *et al.,* 2006; Strasser *et al.,* 2009; Li *et al.,* 2011; Jalali *et al.,* 2012; Jofré *et al.,* 2015; Shu *et al.,* 2015).

If sugars are generally the most used during the drying of LAB, the effectiveness of other substances such as skim milk has been reported (Zamora *et al.,* 2006; Mendoza *et al.,* 2013; Shu *et al.,* 2015). Skim milk has been selected as drying medium for most lactic acid bacteria of commercial interest because it prevents cell damage by stabilizing cell membrane constituents and provides a protein coating that protects cells (Castro *et al.,* 1995; Selmer-Olsen *et al.,* 1999; Tan *et al.,* 2007). In this study, four of the five strains achieved a survival rate above 80% with skim milk. Much higher survival values with skim milk as a protective agent were recorded by Mendoza *et al.,* (2013) after freeze-drying of *Lactococcus lactis, Lactococcus garvieae* and *Lactobacillus plantarum*.

These authors reported survival rates between 91 and 97%. Similar interesting rates were obtained using yeast extract as a lyoprotectant.Although few studies have been performed on the use of yeast extract as a protectant, the results obtained in our case are promising.

The use of yeast extract as a protective agent was done by Zhao and Zhang (2005) during the lyophilization of *Lactobacillus brevis* and *Oenococcus oeni.* These authors reported better survival for *Lactobacillus brevis* using yeast extract as a protective agent. Yeast extract contains many amino acids, mainly glutamate. This protection by

amino acids would be the result of a reaction between the carboxyl groups of the bacterial proteins and the amino group of the protectant, stabilizing the proteins structure (Moriche, 1970). With sugars, the lowest protection values were obtained with glucose. Sucrose and trehalose provided very good survival rates with most strains. These results are in agreement with those of several authors, who have indicated that disaccharides are more effective than monosaccharides and polyols (Carpenter, 1993; Lerbert, 2005; Vincourt-Vitse, 2012).

Monosaccharides have the disadvantage of not being able to interact sufficiently due to their small size. Although skim milk is the most used industrially and the one for which most of the information is available, sucrose and trehalose are considered the best cryoprotectants for various types of bacteria (Leslie *et al.,* 1995; Conrad *et al.,* 2000; Zayed et Roos 2004; Meng *et al.,* 2008).

The protective capability of these sugars could be due to the stabilization of cell membranes by replacing the water between lipid headgroups and the prevention of unfolding and aggregation of protein by hydrogen bonding with polar group of protein (Crowe *et al.,* 2001). In addition, the flexibility of their relatively large cycle allows them to interact more with the polar heads of phospholipid molecules (Wang *et al.,* 2006).

In general, *Lactobacillus plantarum* strainsachieved good survival rates with 4 protective agents: skim milk, yeast extract, sucrose and trehalose. These results are in agreement with the ones reported by Gisela *et al.,* (2014) who stated thatskim milk powder, sucrose and trehalose play an important role in preserving the viability of *Lactobacillus plantarum* when it is subjected to a freeze drying process and storage. *Pedioccocus acidilactici* strains also had good survival with these same protectants, in contrast to *Pedioccocus pentosaceus* which had a slightly lower rate with skim milk and trehalose. The survival stability of freeze-dried strains in various protective agents was evaluated during storage temperature of 4°C for 180 days.For most of the strains, no considerable decrease of the viability was observed regardless of the protector usedapart from a slight decrease not exceeding 2 Log UFC/mL observed for some strainsduring the last weeks of storage. This survival stability could be explained by the storage temperature used in our study. Previous studies showed that temperature is a critical parameter in microbial survival during storage (Teixeira *et al.,* 1995; Gardiner *et al.,* 2000; Abadias *et al.,* 2001). Savini *et al.,* (2010) observed in their study a decrease in viability over time when freeze-dried LAB in the presence of severale protectors were stored for 5 months at room temperature but not at 4 °C. Other studies on*Lactobacillus bulgaricus* (40 days at 5 and 20 °C), *Lactobacillus murinus* (2 months at 5 and 20 °C) and *Lactobacillus plantarum* (6 months at 4, 22 and 35 °C) also showed that storage under refrigeration better preserved the viability of freeze-dried cultures (Castro *et al.,* 1995; Zamora *et al.,* 2006; Strasser *et al.,* 2009). Low temperatures maintain chemical and biochemical reaction rates at a low level and increase storage stability. This is why 0 - 5°C is usually the temperature range chosen for storage (Selmer-Olsen *et al.,* 1999). Furthermore, increased survival of freeze-dried cultures at low temperatures may be due to a reduction in the rate of unsaturated fatty acid oxidation (Castro *et al.,* 1995).

Freeze-drying and exposure to low water content impose environmental stresses determining survival but also physiological stability of the bacterial cells. The development of stable freeze-dried lactic acid bacteria requires maintaining the cells biologicalactivity. In this study the comparison of the acidification activity before and after freeze drying was used to evaluate the ability of the strains to recover their benefical properties after deshydration. The obtained ΔpH for all strains showed that lyophilization had no significant effect on their acidification capacity. The study of Ayad *et al.,* (2004) on many species of LAB had also revealed a maintaining at a high level of technological characteristics including the acidification capacity after freeze-drying.

This study contributes to the knowledge on the effect of freeze drying, long term storage and cryoprotectant used in freeze drying to the viability and properties of LAB strains. Apart from glucose, the other protective agents used play an important role in preserving the strains viability. Refrigeration is necessary to achieve dried cultures with high stability and long shelf-life. Freeze drying or storage after freeze drying do not affect the properties of the tested strains. These results will be useful in the production of commercial probiotic cultures and thereby it will aid to the development of new probiotic food products which are important in disease prevention.

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References

Abadias, M., Benabarre, A., Teixido, N., Usall, J.andVi, I., 2001. Effect of freeze drying and protectants on viability of the biocontrol yeast Candida sake. Int. J. Food Microbiol. 65, 173-82.

Ayad, E. H. E., Nashat, S., El-Sadek, N., Metwaly, H.andEl-Soda, M., 2004. Selection of wild lactic acid bacteria isolated from traditional Egyptian dairy products according to production and technological criteria. Food Microbiol.21,715-725.

Berbegal, C., Peña, N., Russo, P., Grieco, F., Pardo, I., Ferrer, S., Spano, G.and Capozzi, V., 2016. Technological properties of *Lactobacillus plantarum* strains isolated from grape must fermentation. Food Microbiol. 27, 187-194.

Carpenter, J. F., Prestrelski, S. J. and Arakawa, T., 1993. Separation of freezing- and drying-induced denaturation of lyophilized proteins using stress-specific stabilization. I. Enzyme activity and calorimetric studies. Arch. Biochem. Biophys. 303, 456-464.

Carvalho, A. S., Silva, J., Ho, P., Teixeira, P., Malcata, F. X. and Gibbs, P., 2002. Survival of freeze-dried *Lactobacillus plantarum* and *Lactobacillus rhamnosus* during storage in the presence of protectants. Biotechnol. Lett. 24, 1587-1591.

Castro, H. P., Teixeira, P. and Kirby, R., 1995. Storage of lyophilized cultures of *Lactobacillus bulgaricus* under different relative humidities and atmospheres. Appl. Microbiol. Biotechnol. 44(1),172-176.

Champagne, C. P., Ross, R. P., Saarela, M., Hansen, K. F. and Charalampopoulos, D., 2011. Recommendations for the viability assessment of probiotics as concentrated cultures and in food matrices. Int. J. Food Microbiol.149(3), 185–193.

Clementi, F. and Rossi, J., 1984. Effect of drying and storage conditions on survival of *Leuconostoc oenos*. Am. J. Enol. Vitic. 35 : 183-186.

Conrad, P. B., Miller, D. P., Cielenski, P. R. and Pablo, J. J., 2000. Stabilization and preservation of *Lactobacillus acidophilus* in saccharide matrices. Cryobiology.41,17-24.

Crowe, J. H., Crowe, L. M., Oliver, A. E., Tsvetkova, N., Wolkers, W.and Tablin, F., 2001. The trehalose myth revisited: Introduction to a symposium on stabilization of cells in the dry state. Cryobiology. 43, 89-105.

Dianawati, D., Mishra, V.and Shah, N. P., 2013. Stability of microencapsulated *Lactobacillus acidophilus* and *Lactococcus lactis* ssp. *cremoris* during storage at room temperature at low aw. Food Res. Int. 50(1), 259-265.

Fessard, A.and Remize, F., 2019. Genetic and technological characterization of lactic acid bacteria isolated from tropically grown fruits and vegetables. Int. J. Food Microbiol.301, 61-72.

Gardiner, G. E., O'Sullivan, E., Kelly, J., Auty, M. A. E., Fitzgerald, G. F., Collins, J. K., Ross, R. P. andStanton, C., 2000. Comparative survival rates of human-derived probiotic *Lactobacillus paracasei* and *L. salivarius* strains during heat treatment and spray drying. Appl Environ Microbiol. 66, 2605–2612.

Gerhardt, P., 1982. Diluents and biomass measurment. In: Gerhardt, P., Murry, R. G., Castlow, R. H., Nester, E. W., Wood, W. A., Kriec, N. R., Briggs, P. G. (Eds.), Manual of Methods for General Bacteriology. American Society for Microbiology, Washington, pp. 504–50.

Gisela, G., Leonardo, A. E., Lucia, P., Rodrigo, V., Eduard, G.andAngeles, C. M., 2014.

Enhacement of the Viability of *Lactobacillus plantarum* during the Preservation and Storage Process Based on the Response Surface Methodology. Food Nutr Sci. 5, 1746-1755.

Giulio, B. D., Orlando, P., Barba, G., Coppola, R., Rosa, M. D. and Sada, A., 2005. Use of alginate and cryoprotective sugars to improve the viability of lactic acid bacteria after freezing and freeze-drying. World J. Microbiol. Biotechnol.21, 739-746.

Halim, M., Mohd, M. N., Othman, M., Wasoh, H., Kapri, M. and Ariff, A. B., 2017. Effect of encapsulant and cryoprotectant on the viability of probiotic *Pediococcus acidilactici* ATCC 8042 during freeze-drying and exposure to high acidity, bile salts and heat. Food Sci. Technol. 81, 210 - 216.

Higl, B., Kurtmann, L., Carlsen, C. U., Ratjen, J., Först, P., Skibsted, L. H., Kulozik, U. and Risbo, J., 2007. Impact of water activity, temperature, and physical state on the storage stability of *Lactobacillus paracasei* ssp. *paracasei*freeze-dried in a lactose matrix. Biotechnol. Prog. 23, 794-800.

Jalali, M., Abedi, D., Varshosaz, J., Najjarzadeh, M., Mirlohi, M. and Tavakoli, N., 2012. Stability evaluation of freeze-dried Lactobacillus paracasei subsp. tolerance and Lactobacillus delbrueckii subsp. bulgaricus in oral capsules. Res Pharm Sci.7(1), 31-36.

Jofré, A., Aymerich, T. and Garriga, M., 2015. Impact of different cryoprotectants on the survival of freeze-dried Lactobacillus rhamnosus and Lactobacillus casei/paracasei during long-term storage. Benef. Microbes. 6(3), 381-386.

Lerbret, A., 2005. Etude de l'action bioprotectrice des sucres : une investigation par dynamique moléculaire et spectroscopie Raman. Thèse de Doctorat. Université des Sciences et Technologies de Lille, Lille. pp 116.

Li, B., Tian, F., Liu, X., Zhao, J., Zhang, H. and Chen, W., 2011. Effects of cryoprotectants on viability of *Lactobacillus reuteri* CICC6226. Appl. Microbiol. Biotechnol. 92, 609–16.

Mendoza, G. M., Pasteris, S. E., Otero M. C.and Fatima N-M, M. E., 2013. Survival and beneficial properties of lactic acid bacteria from raniculture subjected to freeze-drying and storage. J. Appl. Microbiol. 116, 157-166.

Meng, X. C., Stanton, C., Fitzgerald, G. F., Daly, C. and Ross, R. P., 2008. Anhydrobiotics: the challenges of drying probiotic cultures. Food Chem. 106, 1406–1416.

Miao, S., Mills, S., Stanton, C., Fitzgerald, G. F., Roos, Y.and Ross, R. P., 2008. Effect of disaccharides on survival during storage of freeze dried probiotics. Dairy Sci. Technol. 88, 19-30.

Morichi, T., 1970. Nature and action of protective solutes in freezedrying of bacteria. In Proceedings of the First International Conference on Culture Collections (ed). Tokyo, Japan: pp. 121–125.

Pehkonen, K. S., Roos, Y. H., Miao, S., Ross, R. P. and Stanton, C., 2008. State transitions and physicochemical aspects of cryoprotection and stabilization in freeze-drying of L*actobacillus rhamnosus* GG (LGG). J. Appl. Microbiol. 104, 1732-1743.

Poddar, D., Nag, A., Das, S.andSingh, H., 2012. Stabilization of probiotics for industrial application. In: Ghosh, D., Das, S., Bagchi, D. and Smarta, R. B. (eds.) Innovation in healthy and functional foods. *CRC Press*, *Boca-Raton*, *FL*, *USA*, pp. 269-304.

Santivarangkna, C., Aschenbrenner, M., Kulozik, U.and Foerst, P., 2011. Role of glassy state on stabilities of freeze-dried probiotics. J. Food Sci. 76(8), 152-156.

Santivarangkna, C., Kulozik, U.and Foerst, P., 2008. Inactivation mechanisms of lactic acid starter cultures preserved by drying processes. J. Appl. Microbiol. 105, 1-13.

Santivarangkna, C., Kulozik, U.andFoerst, P., (2007). Alternative drying processes for the industrial preservation of lactic acid starter cultures. Biotechnol. Prog. 23(2), 302-315.

Savini, M., Cecchini, C., Verdenelli, M. C., Silvi, S., Orpianesi, C.and Cresci, A., 2010. Pilot-scale production and viability analysis of freeze-dried probiotic bacteria using different protective agents. Nutrients. 2, 330-339.

Schoug, A., Olsson, J., Carlfors, J., Schnürer, J. and Hakansson, S., 2006. Freeze-drying of *Lactobacillus coryniformis* Si3-effects of sucrose concentration, cell density, and freezing rate on cell survival and thermophysical properties. Cryobiology. 53, 119-127.

Selmer-Olsen, E., Birkeland, S. E. and Sørhaug, T., 1999. Effect of protective solutes on leakage from and survival of immobilized *Lactobacillus* subjected to drying, storage and rehydration. J. Appl. Microbiol. 87, 429-437.

Shu, G., Hui, Y., Chen, S., Wan, H.andChen, H., 2015.Application of plackett-burman design in screening freeze drying cryoprotectants for *Lactobacillus bulgaricus*. Food Technol.39(1), 70-80.

Siaterlis, C. and Charalampopoulos, D. D., 2009. Effect of Culture Medium and Cryoprotectants on the Growth and Survival of Probiotic Lactobacilli during Freeze Drying. Lett. Appl. Microbiol.48, 295-301.

Strasser, S., Neureiter, M., Geppl, M., Braun, R.and Danner, H., 2009. Influence of Lyophilization, Fluidized Bed Drying, Addition of Protectants, and Storage on the Viability of Lactic Acid Bacteria. J. Appl. Microbiol.107, 67-177.

Tan, C. S., van Ingen, C. W. and Stalpers, J. A., 2007. Freezedrying fungi using a shelf freeze-drier. In Methods in Molecular Biology : Cryopreservation and Freeze-Drying Protocols. Humana Press Incorporated. Totowa.

Tripathi, M. K. and Giri, S. K., 2014. Probiotic functional foods: survival of probiotics during processing and storage. J. Funct. Foods. 9, 225-241.

Vesterlund, S., Salminen, K.and Salminen, S., 2012. Water activity in dry foods containing live probiotic bacteria should be carefully considered: A case study with Lactobacillus rhamnosus GG in flaxseed. Int. J. Food Microbiol. 157(2), 319-321.

Viana de Souza, Jand Dias, F. S., 2017. Protective, technological, and functional properties of select autochthonous lactic acid bacteria from goat dairy products. Curr. Opin. Food Sci. 13, 1-9.

Vincourt-Vitse, V., 2012. Contribution à la formulation et à l'évaluation de liposomes d'ATP. Thèse de Doctorat, Universite Paris Descartes. Paris. pp 105.

Wang, H., Du, S., Zhai, F.and Popkin, B. M., 2006. Trends in the distribution of body mass index among Chinese adults, aged 20–45 years (1989-2000). Int. J. Obes. 31, 272-278.

Zamora, L. M., Carretero, C.andParés, D., 2006. Comparative survival rates of lactic acid bacteria isolated from blood, following spray-drying and freeze-drying. Food Sci. Technol. Int. 12, 77-84.

Zayed, G. and Roos, Y. H., 2004. Influence of trehalose and moisture content on survival of *Lactobacillus salivarius* subjected to freeze-drying and storage. Process Biochem.39, 1081-1086.

Zhao, G.and Zhang, G., 2005. Effect of protective agents, freezing temperature, rehydration media on viability of malolactic bacteria subjected to freeze-drying. J. Appl. Microbiol. 99 : 333-338.

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