



Spray drying of dairy bacteria: New opportunities to improve the viability of bacteria powders

Pierre Schuck, Anne Dolivet, Serge Mejean, C. Hervé, Romain Jeantet

► To cite this version:

Pierre Schuck, Anne Dolivet, Serge Mejean, C. Hervé, Romain Jeantet. Spray drying of dairy bacteria: New opportunities to improve the viability of bacteria powders. *International Dairy Journal*, 2013, 31, pp.12-17. 10.1016/j.idairyj.2012.01.006 . hal-01209411

HAL Id: hal-01209411

<https://hal.science/hal-01209411>

Submitted on 29 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Spray drying of dairy bacteria: New opportunities to improve the viability of bacteria powders



P. Schuck^{a,b,*}, A. Dolivet^{a,b}, S. Méjean^{a,b}, C. Hervé^c, R. Jeantet^{a,b}

^a INRA, UMR 1253, F-35000 Ovest, France

^b Agrocampus Ovest, UMR 1253, F-35000 Rennes, France

^c Laboratoire Standa, F-14000 Caen, France

ARTICLE INFO

Article history:

Received 27 October 2011

Received in revised form

14 December 2011

Accepted 25 January 2012

ABSTRACT

The most frequently used technique for dehydration of dairy bacteria is freeze drying. Of the other possible preservation techniques used in the dairy industry to produce large amounts of dairy ingredients at commercially viable processing costs, spray drying is one of the main processing tools and the cost is 10 times lower than that of freeze drying. In this work, some examples are presented for different species of dairy bacteria with respect to spray-drying processes (as an alternative approach to freeze drying) and storage conditions for production of powdered bacterial cultures with high viability (100%) and count (10^{10} g^{-1}). The advantages and disadvantages of this new approach (viability, contamination, yield during process and storage) are discussed.

© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

The propionibacteria are involved in many industrial processes: e.g., production of vitamin B12 (Bullerman & Berry, 1966; Leviton & Hargrove, 1972) and of propionic acid (Boyaval & Corre, 1987) and as dairy starter culture for Swiss-type cheese production (Hettinga & Reinbold, 1972a, 1972b, 1972c). Many other uses would open up if the cost of such bacteria were lower [e.g., as silage and high-moisture grain preservation agents (Lacey, Lord, King, & Manlove, 1978; Voelker, Casper, Lubens, & Schingoethe, 1989), probiotics (Skupin et al., 1977) and in the production of many new and improved dairy and bakery products and processes]. The high cost is due to the time needed for the growth of these microorganisms and particularly, to the preservation method used to keep the cells alive. Freezing and freeze drying are the two main methods currently employed for such cell preservation. Spray drying is a very interesting preservation method that is already used for the preservation of yeast (Beker & Rapoport, 1987; Elinzondo & Labuza, 1974) and lactic acid bacteria (Espina & Packard, 1979; Lievence, 1991). The cost of this method is more than 10 times lower than the two methods cited above (Boyaval & Schuck, 1994). Spray drying is one of the most important industrial drying systems today. The spray dryers operating in industries from aseptic pharmaceutical

processing to mining operations handle feed rates from a few kilograms per hour to well over 100 tons per hour (Masters, 1991).

The versatility of the spray-drying process and the considerable progress made through technical innovation have led to greater flexibility to meet biotechnological requirements, especially low heat treatments to avoid loss of activity. Designs are available to handle toxic materials and those which require aseptic drying conditions for cell preservation. Unfortunately, the results achieved to date for spray drying of bacterial cells are very disappointing. Reports on the spray drying of bacteria are scarce when compared with those for freeze drying, partly because of the poor results obtained to date, mainly in terms of cell viability (Johnson & Etzel, 1995). But, recently, Fu and Chen (2011) and Silva, Freixo, Gibbs, and Teixeira (2011) have realized an interesting summary of some studies on the dehydration of microorganisms by thermal process showing that the highest survival is between less than 1% and 100% dependent on the different growth media, drying media, drying systems and microorganisms. Generally, the results shown that the viability is better with *Bifidobacterium* than with *Lactobacillus* and *Lactococcus*. Moreover, laboratory results obtained using spray-drying apparatus cannot generally be applied to pilot or industrial scale processes because of the differences in the equipment used; resulting in considerable variations in operating parameters (Fu & Chen, 2011; Fu, Suen, & Etzel, 1994). Pilot spray-drying plants in which scale up is possible are very expensive.

We describe in this study a new method of spray drying of two dairy propionibacteria strains (*Propionibacterium acidipropionici*) in

* Corresponding author. Tel.: +33 2 23 48 53 22; fax: +33 2 23 48 53 50.
E-mail address: pierre.schuck@rennes.inra.fr (P. Schuck).

a pilot spray-drying plant. The microbiological characteristics and physico-chemical properties (wettability, dispersibility, solubility, water activity, glass transition, particle size distribution) of the different powders obtained were monitored for 3 years at two temperatures (4 °C and laboratory room temperature, 21 ± 2 °C). To the best of our knowledge, this is the first report describing the feasibility of the spray drying of cells of *Propionibacterium* spp.

2. Materials and methods

2.1. Strains

Two strains of *P. acidipropionici* (Laboratoire Standa, Caen, France), named A and B, were used throughout this study. The strains (A and B) were selected for possible applications in the environment and animal feed. The strains (A and B) were maintained in a Yeast Extract-Lactate medium (YEL) with glycerol (10%, v/v) at -80 °C (Malik, Reinbold, & Vedamuthu, 1968). Three successive inoculations were carried out in the fermentation medium before inoculation of the bioreactor.

2.2. Medium

The medium was prepared as follows: 10% (w/v) acid whey permeate powder (Lactalis, Mayenne, France), 2.5% (w/v) corn steep powder (Roquette, Lestrem, France) and 1% (w/v) yeast extract (Fould Springer, France) were dissolved in tap water. The pH was adjusted to 6.5 with 10 M NaOH (Ecolab, Issy-lès-Moulineaux, France) before heat treatment (1 min at 128 °C) (Actijoule 32 kW, Actini, France).

2.3. Fermentation

A three thousand L stainless steel tank was used as bioreactor (TL 988, Guerin, France). Four hundred litres of inoculum (A and B) were prepared in a 750 L bioreactor. The temperature was maintained at 30 °C and the pH at 6.0 ± 0.1 by addition of 10 M NaOH (Ecolab, Issy-lès-Moulineaux, France). After 70 h, the cells were collected and concentrated using an ultrafiltration device (mineral membranes, 7 kg mol^{-1}) from Tami, Nyons, France, surface area of 6.65 m^2) equipped with positive pumps. The concentrated cell suspension (300 L) was stored at 4 °C until spray drying.

2.4. Cell viability

Total cell number and *Propionibacterium* cells were evaluated before and just after spray drying. *Propionibacterium* cells were enumerated on a selective medium (6 d at 30 °C) according to Madec, Rouault, and Maubois (1993). The cell suspension was prepared as follows: 10 g of powder were mixed with 90 mL of sterile water containing 1 g peptone (Difco, USA) and 8.5 g NaCl per litre of demineralized water and adjusted to pH 7.0. This suspension was maintained at 37 °C for 30 min then homogenized in a stomacher over 3 min (Labo. Blender 400, AJ Seward, UK). Total thermophilic and mesophilic flora were evaluated on Agar plate count medium (Difco, USA) by plating 1 mL of the cell suspension and incubating the Petri dishes 3 days for 55 °C and 30 °C, respectively, according to ISO (2003). The coliforms were enumerated according to ISO (1998). Sulphite-reducing clostridia were enumerated according to the NF standard (NF, 1982).

2.5. Spray drying

For the experiments, 30 kg of concentrated cells (A and B) were mixed with 125 kg of sweet whey permeate powder (Lactalis,

Mayenne, France) and 125 kg of water. The concentrated microbial suspensions were spray dried in a Niro Atomizer (GEA-PE, Saint Quentin en Yvelines, France) pilot Bionov spray dryer (Rennes, France) equipped with a pressure nozzle (orifice diameter 0.73 mm with 4 slot core of a nominal slot width of 0.51 mm, leading to a sprayer angle of 60°). The evaporation capacity was $70\text{--}120 \text{ kg h}^{-1}$. All spray-drying parameters were constant ($3200 \pm 100 \text{ kg h}^{-1}$). The inlet air temperature was at 130 ± 5 °C, and the outlet air temperature 60 ± 2 °C, the air temperature of both sections of the vibro-fluidizer (VF) (or external fluid bed) were 82 ± 2 °C, the concentrate flow rate was at $88 \pm 2 \text{ L h}^{-1}$ and the air flow rate was at $3200 \pm 100 \text{ kg h}^{-1}$. A new crystallizer (GEA-PE, Saint Quentin en Yvelines, France) was used before the VF, in place of the internal fluid bed, which was removed during the experiments. This device was equipped with a rotating disc (0.2 rpm) and the air temperature in the crystallizer was approximately 25 °C. Continuous withdrawal was achieved by a collecting screw located on a radius. The powder was collected at the entrance to the vibro-fluidizer.

2.6. Chemical analysis

Several analyses were performed on water, support, microbial concentrates, mix and microbial powders (pH at 20 °C, with a pH meter (Radiometer, Copenhagen, Denmark) with a combined electrode; total solid (TS) content by oven drying at 105 °C for 7 h for liquids and concentrates and for 5 h for powders and total nitrogen matter (TNM: total nitrogen $\times 6.38$) according to the Kjeldahl method using the Tecator apparatus (Humeau, France)).

2.7. Physical properties

2.7.1. Wettability index

The wettability index (WI) was determined according to Haugaard Sorensen, Krag, Pisecky, and Westergaard (1978, chaps. 3 and 5). This index is the time necessary for a given weight of powder to penetrate the still surface of water. Ten grams of powder were dropped in a funnel below the surface of 100 mL of distilled water at 20 °C. When all the powder was wetted, the time was measured (in s) between the start and the end of the analysis. Values were determined at ± 1 s. In the dairy sector, a powder is said to be wettable if its WI is lower than 60 s.

2.7.2. Dispersibility index

The dispersibility index (DI) was determined according to Haugaard Sorensen et al. (1978, chaps. 3 and 5). This index represents the quantity of powder (in %) able to pass through a sieve of 210 μm . Ten grams of powder were added to 100 mL of distilled water at 20 °C. After stirring with a spoon for 15 s, the mixture was passed through a 210 μm sieve and 1.5 mL of the filtrate was dehydrated by oven drying at 105 °C for 7 h. Expressed as a %, DI is equal to:

$$DI = \frac{(100 + w) \cdot X_{DM}}{w \cdot \frac{100 - X_{RW}}{100}} \quad (1)$$

where w is the weight of the powder used, X_{RW} the residual free water content of the powder (% w/w) and X_{DM} the dry matter of the filtrate after sieving (% w/w).

With the result expressed to one decimal place, the standard deviation (SD) was 2%. In the dairy sector, a powder is said to be dispersible if its DI is higher than 95%.

2.7.3. Solubility index

The solubility index (SI) and insolubility index (II) were determined according to ISO (2005). This is an index (in %) to determine

the ability of a powder to be dissolved in water. Ten grams of powder were added to 100 mL distilled water at 24 °C and blended in a glass mixing container for 90 s. The mixed sample was poured into conical centrifuge tubes to the 50 mL mark and centrifuged (5 min, 160 × g). The supernatant was siphoned off to within 5 mL of the surface of the sediment level. The residues were dispersed in distilled water and centrifuged again (5 min, 160 × g), the supernatant again being siphoned off. The insolubility index is equal to the volume of sediment (mL) remaining after the second centrifugation from 50 mL of reconstituted liquid.

Expressed as a %, SI is equal to:

$$SI = 100 - [2 \times II] \quad (2)$$

Expressed to one decimal place, the standard deviation (SD) was 2%. In the dairy sector, a powder is considered soluble if its SI is higher than 99%.

2.7.4. Water activity

Water activity (a_w) was measured in triplicate with a Novasina water activity meter (RTD-33 TH-2, Pfäffikon, Switzerland) at a constant temperature of 25 °C.

2.7.5. Particle size distribution

Particle size distributions were determined by static light scattering (Mastersizer S, Malvern Instruments Ltd., Malvern, UK) with a 5 mW He–Ne laser operating at a wavelength of 632.8 nm with a 300F lens. Size distributions were determined using a dry powder feeder attachment and the standard optical model for presentation of particles dispersed in air was used. The results obtained were average diameters calculated from Mie theory (mean of three analyses). The criterion selected was $d(0.50)$, meaning that 50% of the particles had diameters smaller than this criterion. The method of Gaiani et al. (2010) was used.

2.7.6. Glass transition temperatures (T_g)

Glass transition temperatures (T_g) and heat capacity changes (ΔC_p) were determined by differential scanning calorimetry (DSC) (Q-1000, TA Instruments, Saint Quentin en Yvelines, France) calibrated with indium (melting point 156.6 °C). This method was described by Schuck et al. (2005) with the following modifications. The samples were scanned twice to eliminate the hysteresis effect of thermal relaxation that is typical of glass transition. The initial scanning rate was 5 °C min⁻¹ from –20 °C to +70 °C. Samples were cooled at 10 °C min⁻¹ to –20 °C, and the T_g range was determined

from the DSC curve of the second scan at 5 °C min⁻¹ from –20 °C to +140 °C, giving three values: T_g onset (T_{go}), T_g inflection (T_{gi}) and T_g endset (T_{ge}). ΔC_p values were established according to the change in heat capacity between T_{go} and T_{ge} .

2.7.7. Powder preservation

Samples of each powder (500 g) were analyzed immediately after spray-drying day (D + 0) and over storage time (D + 4, +12, +18, +25, +51, +106, +176, +385, +1121) in order to study the keeping properties of these powders. Storage conditions were either 4 ± 0.5 °C with a relative humidity of 75 ± 3% or room temperature (21 ± 3 °C) with a relative humidity of 45 ± 10%.

3. Results and discussion

3.1. Physico-chemical properties

Table 1 shows the results of the physico-chemical analysis of each sample during the process. The TS (total solid) content of the 2 concentrates with cell suspensions A and B was around 44%. The total nitrogen matter (TNM) of the 2 types of final bacteria powder (A and B), was low (between 5.8% for B and 6.8% for A); corresponding to the low TNM in the UF permeate powder. The moisture content and a_w of the 2 types of bacterial powder (A and B) were 2.2% (A) and 3.3% (B), and 0.17 (A) and 0.19 (B), respectively. The glass transition temperature (T_g) and heat specific capacity (ΔC_p) were 43 °C (A), 41 °C (B) and 0.27 J g⁻¹ °C⁻¹ (A), 0.24 J g⁻¹ °C⁻¹ (B). The moisture content, a_w , T_g and ΔC_p were compatible with optimal storage conditions (T_g > storage temperature and a_w between 0.18 and 0.22) (Efsthathiou, Feuadent, Mejean, & Schuck, 2002; Labuza, 1975; Schuck et al., 2005; Schuck, Méjean, Dolivet, Jeantet, & Bhandari, 2007).

Table 1 shows that the particle size distribution was around 100 µm for both types of final bacteria powder, which is a classical size for dairy powders (Pisecky, 1997, chaps. 10 and 12). According to the dairy sector, the rehydration behaviour showed that the 2 types of bacteria powder were soluble (SI > 99.0%) and wettable (WI < 60 s) but not that much dispersible (72% for A and 64% for B). The most important result was that the powders were soluble and wettable for rehydration. The dispersibility index (DI) is often considered as an important characteristic in order to decide whether a product is instant or not on the basis of a single property, whatever the SI and WI values (Pisecky, 1997, chaps. 10 and 12). This was not the most important characteristic for this study.

Table 1
Physico-chemical analyses of each sample throughout the process, from the liquid to the powder.^a

Sample	pH	TNM (%)	TS (%)	a_w	$d(0.5)$ (µm)	SI (%)	DI (%)	WI (%)	T_g (°C)	ΔC_p (J g ⁻¹ °C ⁻¹)
Water	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
UF permeate powder	nd	6.1 ± 0.1	98.1 ± 0.1	0.20 ± 0.02	120 ± 4	>99.0 ± 0.0	77.0 ± 2.2	>60 ± 0	20 ± 2	0.11 ± 0.01
UF permeate powder + water	5.95 ± 0.03	3.0 ± 0.0	48.8 ± 0.0	nd	nd	nd	nd	nd	nd	nd
Bacteria A	6.45 ± 0.01	2.2 ± 0.0	3.4 ± 0.0	nd	nd	nd	nd	nd	nd	nd
UF permeate powder + water + bacteria	5.88 ± 0.02	3.0 ± 0.0	44.0 ± 0.0	nd	nd	nd	nd	nd	nd	nd
Powder on crystallizer (A)	nd	nd	88.1 ± 0.1	0.43 ± 0.02	nd	nd	nd	nd	15 ± 3	0.51 ± 0.03
Powder before VF (A)	nd	6.6 ± 0.1	97.1 ± 0.1	0.19 ± 0.01	81 ± 3	>99.0 ± 0.0	67.0 ± 1.5	29 ± 1	42 ± 2	0.19 ± 0.01
Powder after VF (Final) (A)	nd	6.8 ± 0.1	97.8 ± 0.1	0.17 ± 0.02	101 ± 3	>99.0 ± 0.0	72.0 ± 1.6	14 ± 1	43 ± 1	0.27 ± 0.02
Bacteria B	6.31 ± 0.03	2.4 ± 0.1	3.8 ± 0.0	nd	nd	nd	nd	nd	nd	nd
UF permeate powder + water + bacteria	5.89 ± 0.03	2.9 ± 0.0	43.6 ± 0.0	nd	nd	nd	nd	nd	nd	nd
Powder on crystallizer (B)	nd	nd	87.1 ± 0.1	0.48 ± 0.02	nd	nd	nd	nd	16 ± 3	0.53 ± 0.03
Powder before VF (B)	nd	5.9 ± 0.0	96.0 ± 0.1	0.21 ± 0.02	83 ± 4	>99.0 ± 0.0	56.0 ± 2.8	24 ± 1	37 ± 2	0.24 ± 0.02
Powder after VF (final) (B)	nd	5.8 ± 0.1	96.7 ± 0.1	0.19 ± 0.01	100 ± 3	>99.0 ± 0.0	64.0 ± 1.7	17 ± 1	41 ± 2	0.24 ± 0.01

^a Abbreviations are: UF, ultrafiltered; VF, vibro-fluidizer; TNM, total nitrogen matter; TS, total solid content; a_w , water activity; $d(0.5)$, 50% of particle sizes below the indicated value; SI, solubility index; DI, dispersibility index; WI, wettability index; T_g , glass transition temperature; ΔC_p , heat specific capacity; nd, not determined.

Table 2

Cell enumeration of contaminating flora (thermophilic, mesophilic, coliform and sulphate reducing flora) of each sample throughout the process, from the liquid to the powder.^a

Sample	Thermophilic flora (log cfu g ⁻¹)	Mesophilic flora (log cfu g ⁻¹)	Coliform flora (log cfu g ⁻¹)	Sulphate reducing flora (log cfu 10 g ⁻¹)
Water	nd	nd	nd	nd
UF permeate powder	3.6	2.2	nd	0.0
UF permeate powder + water	3.2	2.2	nd	0.6
Bacteria A	1.1	0.0	nd	0.0
UF permeate powder + water + bacteria	2.8	1.4	nd	0.3
Powder before VF (A)	3.1	2.0	nd	0.3
Powder after VF (final) (A)	3.5	2.0	nd	0.9
Bacteria B	0.0	0.7	nd	0.0
UF permeate powder + water + bacteria	3.1	1.8	nd	0.0
Powder before VF (B)	3.2	2.1	nd	0.6
Powder after VF (final) (B)	3.2	2.2	nd	0.8

^a Abbreviations are: UF, ultrafiltered; VF, vibro-fluidizer; cfu, colony-forming unit; nd, not detected.

3.2. Microbial results

Table 2 shows the values of the enumeration of contaminating microflora (except *Propionibacterium*). The values (expressed in log cfu g⁻¹) were 3.5 (A) and 3.2 (B) for thermophilic flora, 2.0 (A) and 2.2 (B) for mesophilic flora, and 0.9 (A) and 0.8 (B) for sulphate reducing flora. No coliforms were detected in the bacteria powder. Table 2 shows that the major source of contamination was due to the UF permeate powder. We therefore propose that for further experiments, it would be interesting to perform a heat treatment or microfiltration (1.4 µm) on the UF permeate powder + water mixture to remove bacterial contamination.

Table 3 shows the enumeration of *Propionibacterium*, expressed in log cfu g⁻¹, in log cfu g⁻¹ total solid (TS) content and in % of survival. The *Propionibacterium* population in the feed suspension was between 10.4 log cfu g⁻¹ (A) and 10.9 log cfu g⁻¹ (B), representing a high cell concentration. These values were decreased in the UF permeate + water + bacteria mixture partly due to the dilution (1.1 log) and partly due to the osmotic pressure stress of the cell in the UF powder + water mixture to reach 10.1 log cfu g⁻¹ TS (A) and 9.5 log cfu g⁻¹ TS (B); these cell concentrations were further considered as reference values for the determination of the percentage of survival after spray drying. Thus, after the 1st step of spray drying including the crystallizer, before drying on vibro-fluidizer (VF) and after drying on VF, no decimal reductions were measured in powder A or B, achieving a survival level of 100% for both strains with a high *Propionibacterium* concentration at 10.4 log cfu g⁻¹ (A) and 10.7 log cfu g⁻¹ (B) (Table 3). These excellent results were due to the specific spray-drying process achieved, which included the crystallizer step between the bottom of the spray dryer and the VF. This device made it possible to spray dry at a low outlet air temperature (60 °C) to produce a powder with a high-moisture content (12–13%) and high a_w (0.43–0.48 at 25 °C)

and a low T_g (–15 to –16 °C) (Table 1). According to Roos (1997, 2002), the lactose can crystallize in around 2 min in β -form when the difference between powder temperature and product T_g is about 40 °C; in our experiments, the difference between the air temperature around the powder (25 °C) and the T_g (–15 °C for powder A) combined with the residence time of the powder in the crystallizer (fixed at 5 min) was in accordance with this hypothesis. Thus, after one rotation of the crystallizer, the lactose crystallized, thereby improving the flowability of the bacteria powder, which is compatible with the next step, i.e., post drying in a VF, and led to a limitation of an excessive moisture content thus limiting the destruction of bacteria in the powder (Vuataz, 2002). The same strain was tested with the same process parameters but without the crystallizer to obtain a decimal reduction between 3 and 5 (not shown and not published). Other strains (*Bifidobacterium*) were tested with the same process and with the crystallizer to obtain also the same decimal reduction (3–5) (not shown and not published).

3.2.1. Storage conditions

Table 4 presents the cell viability of the powders for up to 1121 days at 4 °C and room temperature. The level of viability of the *Propionibacterium* powder remained not significantly changed after 25 d storage, whatever the type of bacteria and the temperature of storage, before and after the VF.

3.2.2. Powder after crystallizer and before vibro-fluidizer

After 25 d, cell viability decreased more quickly in the powder stored at room temperature before the VF, probably due to a slightly higher moisture content and a_w (Table 1) than in the powder after the VF, hence the need to finish drying the excess moisture of the powder issuing from the crystallizer on a VF (or external fluid bed). At 4 °C, there was no decimal reduction for these powders until

Table 3

Cell enumeration of *Propionibacterium* and % of survival of each sample throughout the process, from the liquid to the powder.^a

Sample	<i>Propionibacterium</i> flora (log cfu g ⁻¹)	<i>Propionibacterium</i> flora (log cfu g ⁻¹ TS)	Survival (%)
Bacteria A	10.4	11.9	–
UF permeate powder + water + bacteria	9.7	10.1	–
Powder before VF (A)	10.5	10.5	100
Powder after VF (final) (A)	10.4	10.4	100
Bacteria B	10.9	12.4	–
UF permeate powder + water + bacteria	9.1	9.5	–
Powder before VF (B)	10.6	10.7	100
Powder after VF (final) (B)	10.7	10.7	100

^a Abbreviations are: UF, ultrafiltered; VF, vibro-fluidizer; cfu, colony-forming unit; TS, total solids.

Table 4

Cell viability of bacteria powders according to storage conditions (room temperature and 4 °C) on 2 species of bacteria (A and B) before and after vibro-fluidizer (VF).

Days storage	A				B			
	Powder before VF		Powder after VF (final)		Powder before VF		Powder after VF (final)	
	Room temp.	4 °C	Room temp.	4 °C	Room temp.	4 °C	Room temp.	4 °C
4	10.5	10.4	10.4	10.4	10.6	10.6	10.6	10.6
12	10.4	10.4	10.4	10.5	10.7	10.7	nd ^a	10.7
18	10.4	10.6	nd	10.5	nd	10.8	nd	nd
25	9.7	10.5	10.2	10.5	10.6	10.7	10.6	10.7
51	8.1	10.6	10.0	10.5	10.5	10.8	10.5	10.6
106	nd	10.1	8.5	10.1	9.6	10.3	10.8	10.3
176	5.9	10.2	nd	10.2	nd	10.4	9.4	10.3
385	nd	9.7	nd	9.8	nd	10.2	nd	nd
1121	nd	8.1	nd	7.1	nd	9.6	nd	9.7

^a nd, Not determined.

176 d for A and 385 d for B, despite the slightly higher a_w and moisture content than the final powder. It was due to the low storage temperature limiting the loss of bacteria.

3.2.3. Powder after vibro-fluidizer (final powder)

There was no decimal reduction for the storage of the final powder, after 51 d and 106 d at room temperature for bacteria A or B, respectively, or after 176 d at 4 °C for bacteria A and B. After 1121 d at 4 °C, the decimal reduction was 3 for bacteria A but only 1 for bacteria B. The results therefore showed that depending on the type of bacteria (A or B), if the moisture content and a_w are optimal, it is possible to store bacteria powder at room temperature and even better at 4 °C in good conditions to avoid decimal reduction for a long period. Laroche, Fine, and Gervais (2005) have shown that the optimal a_w regarding to the viability is between 0.3 and 0.5 and the lowest viability occurs when the a_w is lower than 0.1. The a_w of bacteria powders A and B are close to 0.2, avoiding in parallel the Maillard reaction and caking that start at 0.3 of a_w (Labuza, 1975). Thus the good results of viability during storage could be explained by the storage temperature and by an a_w close to the optimal value.

4. Conclusions

We showed in this study that it is possible to produce a spray dried bacteria powder using a less expensive process (about 10 times less) than freeze drying with a high level of cells ($>10^{10}$ cfu g⁻¹) and high viability during the process and during storage. The process is not yet optimal, because the use of low air temperatures during spray-drying results in high stickability of the powder, thus decreasing the powder production yield ($<50\%$ against 99% on skim milk powder). However, new equipment will be tested in further study to replace the crystallizer by a belt before the VF to avoid stickiness in the chamber, without changing the spray-drying parameters. The process that we tested also depends on the type of bacteria and probably also on stress generated during the process. Nevertheless, we showed that spray drying may be an alternative to freezing or freeze drying for producing powders with viable varying levels of bacteria (viability $>95\%$) over a long time (several months) at room temperature and longer at 4 °C. Improvements in the bacteria (type, resistance to stress, temperature, etc.), and in the process (air treatment, sterilization of the support and introduction of a belt in the place of the crystallizer, before the VF) should improve the effectiveness of the drying of dairy bacteria.

References

Beker, M. J., & Rapoport, A. (1987). Conservation of yeasts by dehydration. *Advances in Biochemical Engineering/Biotechnology*, 32, 127–171.

- Boyaval, P., & Corre, C. (1987). Continuous fermentation of sweet whey permeate for propionic acid production in a CSTR with UF recycle. *Biotechnology Letters*, 9, 801–806.
- Boyaval, P., & Schuck, P. (1994). Le séchage des microorganismes par atomisation. *Industries Alimentaires et Agricoles*, 111, 807–818.
- Bullerman, L. B., & Berry, E. C. (1966). Use of cheese whey for vitamin B12 production. *Applied Microbiology*, 14, 553–557.
- Efstathiou, T., Feuadent, C., Mejean, S., & Schuck, P. (2002). The use of carbonyl analysis to follow the main reactions involved in the process of deterioration of dehydrated dairy products: prediction of most favourable degree of dehydration. *Lait*, 82, 423–439.
- Elinzondo, H., & Labuza, T. P. (1974). Death kinetics of yeast in spray-drying. *Biotechnology and Bioengineering*, 26, 1245–1259.
- Espina, F., & Packard, V. S. (1979). Survival of *Lactobacillus acidophilus* in a spray-drying process. *Journal of Food Protection*, 42, 149–152.
- Fu, N., & Chen, X. D. (2011). Towards a maximal cell survival in convective thermal drying process. *Food Research International*, 44, 1127–1149.
- Fu, W. Y., Suen, S. Y., & Etzel, M. R. (1994). Injury to *Lactococcus lactis* subsp. *lactis* C2 during spray-drying. In V. Rudolph, & R. B. Keey (Eds.), *Drying 94 vol. B, proceedings international drying symposium* (pp. 785–792). Gold Coast, Australia.
- Gaiani, C., Arab Therany, E., Morand, M., Jacquot, M., Schuck, P., Jeantet, R., et al. (2010). How surface composition of high milk proteins powders is influenced by spray-drying temperature. *Colloids and Surfaces B: Biointerfaces*, 75, 377–384.
- Haugaard Sorensen, I., Krag, J., Pisecky, J., & Westergaard, V. (1978). *Méthodes d'analyses des produits laitiers déshydratés*. Copenhagen, Denmark: Niro A/S.
- Hettinga, D. H., & Reinbold, G. W. (1972a). The propionic acid bacteria. A review. I. Growth. *Journal of Milk and Food Technology*, 35, 295–301.
- Hettinga, D. H., & Reinbold, G. W. (1972b). The propionic acid bacteria. A review. II. Metabolism. *Journal of Milk and Food Technology*, 35, 358–372.
- Hettinga, D. H., & Reinbold, G. W. (1972c). The propionic acid bacteria. A review. III. Miscellaneous metabolic activities. *Journal of Milk and Food Technology*, 35, 436–447.
- ISO. (1998). *Milk and milk products – Enumeration of coliforms*. ISO standards 4831 and 4832. Geneva, Switzerland: ISO.
- ISO. (2003). *Milk and milk products – Enumeration of microorganisms*. ISO standard 4833. Geneva, Switzerland: ISO.
- ISO. (2005). *Dried milk and dried milk products – Determination of insolubility index*. ISO standard 8156. Geneva, Switzerland: ISO.
- Johnson, J. A. C., & Etzel, M. R. (1995). Properties of *Lactobacillus helveticus* CNRZ 32 attenuated by spray-drying, freeze-drying or freezing. *Journal of Dairy Science*, 78, 761–768.
- Labuza, T. P. (1975). Sorption phenomena in foods: theoretical and practical aspects. In C. K. Rha (Ed.), *Theory, determination and control of physical properties of food materials*. Dordrecht, Germany: Reidel Hahn.
- Lacey, J., Lord, K. A., King, H. G. C., & Manlove, R. (1978). Preservation of baled hay with propionic and formic acids and a proprietary additive. *Annals of Applied Biology*, 88, 65–73.
- Laroche, C., Fine, F., & Gervais, P. (2005). Water activity affects heat resistance of microorganisms in food powders. *International Journal of Food Microbiology*, 97, 307–315.
- Leviton, A., & Hargrove, R. E. (1972). Microbiological synthesis of vitamin B12 by propionic acid bacteria. *Industrial and Engineering Chemistry*, 44, 2651–2655.
- Lievens, L. C. (1991). *The inactivation of Lactobacillus plantarum during drying*. Ph.D. thesis, University of Wageningen, The Netherlands.
- Madec, M. N., Rouault, A., & Maubois, J. L. (1993). Milieu sélectif et procédé pour le dénombrement des bactéries propioniques. French patent no. 9300823.
- Malik, A. C., Reinbold, G. W., & Vedamuthu, E. R. (1968). An evaluation of the taxonomy of *Propionibacterium*. *Canadian Journal of Microbiology*, 14, 1185–1191.
- Masters, K. (1991). *Spray-drying handbook* (5th ed.). London, UK: Longman Scientific and Technical Press.
- NF. (1982). *Edible gelatine – Enumeration of sulfite-reducing anaerobic micro-organism spores – Anaerobic colony count technique at 37 °C*. NF V59–106.

- Pisecky, J. (1997). *Handbook of milk powder manufacture*. Copenhagen, Denmark: Niro A/S.
- Roos, Y. H. (1997). Water in milk products. In P. F. Fox (Ed.), *Advanced dairy chemistry*, Vol. 3 (pp. 303–346). London, UK: Chapman & Hall.
- Roos, Y. H. (2002). Importance of glass transition and water activity to spray drying and stability of dairy powders. *Lait*, 82, 478–484.
- Schuck, P., Blanchard, E., Dolivet, A., Méjean, S., Onillon, E., & Jeantet, R. (2005). Water activity and glass transition in dairy ingredients. *Lait*, 85, 295–304.
- Schuck, P., Méjean, S., Dolivet, A., Jeantet, R., & Bhandari, B. (2007). Keeping quality of dairy ingredients. *Lait*, 87, 481–488.
- Silva, J., Freixo, R., Gibbs, P., & Teixeira, P. (2011). Spray-drying for the production of dried cultures. *International Journal of Dairy Technology*, 64, 321–335.
- Skupin, J., Pedziwilk, F., Giec, A., Nowakowska, K., Trajanowska, K., & Jaszewski, B. (1977). Nutritive value of Propionibacteria and lactose-fermenting yeast grown in whey. *Journal of Food Processing and Preservation*, 1, 207–216.
- Voelker, H. H., Casper, D. P., Lubens, F. C., & Schingoethe, D. J. (1989). High moisture corn preserved with esters of propionic acid for lactating cows. *Journal of Dairy Science*, 72, 89–92.
- Vuataz, G. (2002). The phase diagram of milk: a new tool for optimising the drying process. *Lait*, 82, 485–500.