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Freeze-Drying Versus Spray-Drying of CWBI-B1465 *Leuconostoc mesenteroides* subsp. *mesenteroides* Isolated in *kivuguto* Milk

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Abstract: The strain CWBI-B1465 *Leuconostoc mesenteroides* has been previously selected from traditional *kivuguto*, a fermented milk from Rwanda. The strain is preserved in the CWBI Collection and the sequence is deposited in GenBank under Accession number JF313445. The paper reports the production and the preservation of that strain for dairy starter culture use. The production was carried out in a 20 L fermentor with 16 L working volume in batch process. Half of this was centrifuged. The cells were harvested, cryoprotected and *freeze-dried*. The freeze-dried powders were then vacuum-sealed and stored for 90 days. The other half was also centrifuged. The cells were harvested, mixed with protectants and *spray-dried*. The spray-dried powders were then vacuum-sealed and stored for 90 days. After the two drying processes, viability in storage at 4 and 20°C was examined on six levels: membrane fatty acids, flow cytometry, soluble proteins, carbonyl proteins and enumeration on agar MRS. The production was also made in a 20 L fermentor with a 16 L working volume in a fed-batch process, only for optimal production assessment. The obtained results showed that CWBI-B1465 grows at 0.55–0.60 h^{-1} of the specific growth rate; the generation time was 1.12 and 1.25 h in batch and fed-batch, respectively. Its preservability exhibited low loss when it is stored at 4°C, and more if it is stored at 20°C. The most suitable drying process is freeze-drying, but spray-drying is also very interesting and is recommended based on the cost of the process.

Keywords: Freeze-Drying, Spray-Drying, *Leuconostoc*

1. Introduction

Spray-drying can be used to produce large amounts of dairy ingredients relatively inexpensively; the spray-dried powders can be transported at a low cost and can be stored in a stable form for prolonged periods [1]. Suitable strain selection necessitates consideration of three essential premises: encompassing general aspects (origin, identity, safety), technological aspects (growth properties during processing, survival during processing and storage) and stability of functional properties [2–4]. Spray-drying can produce stable powders of certain bacteria and yeast species; however, with the high temperatures involved in this process, the species require a certain level of thermotolerance [5]. The overall viability and subsequent oxidative damage affect primarily lipid membranes, proteins and DNA along the whole process. Polyunsaturated fatty acids and amino acid oxidation (primarily Pro, Arg, Lys and Thr), as well as protein fragmentation and protein aggregation, are the major damages for dried bacteria in storage. They are degraded into a variety of products [6–11] under many biochemical reactions. Among of them, the carbonylation is a chemical reaction that produces organic carbonyl compounds containing the C=O functional group, including aldehydes and ketones [12]. Protein carbonylation was therefore examined as a potential cause for dried bacteria's loss of viability. The reagent 2,4-dinitrophenylhydrazine (DNPH) reacts with the aldehyde or ketone carbonyl group and forms hydrazone derivatives (DNP), thus enabling spectrophotometric determination [13–14].

In this study, the strain CWBI-B1465 was investigated based on its ability to withstand the stresses associated with high temperature and acidity because it resists well to 55°C and a pH of 2.5 [15]. In this regard, our hypothesis was that it

could also withstand stresses of spray-drying. Therefore, production in the bioreactor and drying by freeze-drying in parallel to spray-drying was a suitable process allowing for understanding the level of resistance of this strain. Data of this comparative study will allow us to know the most suitable process to be used for its preservation as a dairy starter culture.

The aims of this study were therefore:

i. To examine the yields of growth during production;
ii. To evaluate viability during freeze/spray-drying and storage.

2. Material and Methods

2.1. Bacterial Strains and Cultures

From the CWBI Collection preserved at -80°C, the strain CWBI-B1465 *Leuconostoc mesenteroides* subsp. *mesenteroides* JF313445 was cultured on MRS agar and incubated for 36 hours at 30°C. Thereafter, it was inoculated in 500 mL of MRS broth as a pre-culture of a 20 L bioreactor (Biolafitte, Poissy, France).

2.2. Fermentation Processes

2.2.1. Batch Fermentation

All batch fermentations were carried out in triplicate in a 20 L bioreactor (Biolafitte, Poissy, France) with a 16 L working volume. Then, 1 L of glucose solution at 20 g. L^{-1} was added just before fermentation. The regulation of the culture parameters (pH, temperature, alkali, etc.) was ensured by a direct control system (ABB). The culture in the bioreactor was held at 27°C and at a pH of 6.5, with a constant stirring speed of 100 rpm with air flow. The foam level in the reactor was controlled by an antifoam probe placed at 10 cm from top of the vessel and by the addition of antifoam Tego KS911 (Goldschmidt, Germany). The inoculum for the bioreactor was prepared in MRS broth autoclaved in a 1 L flask filled halfway. It was prepared by inoculating some colonies of the bacterium grown on the MRS agar plate. The growth kinetic parameters were calculated following the Monod equation:

$$\mu = \mu_m \frac{S}{S+Ks}$$

where μ is the specific growth rate: $\mu = r_x / X$, with X the biomass concentration (cells dry matter). The cell yield ($Y_{X/S}$) (g cells dry matter/g glucose) was calculated from plots of $X–X_0$ vs. S–S0 [16]:

$$Y_{X/S} = \frac{X - X0}{S - S0}$$

where X and Xo are the biomass concentrations at t time and the initial time t_0 respectively; S and S0 the residual glucose ($g.L^{-1}$) at t time and t_0 time.

2.2.2. Fed-Batch Fermentation

The fed-batch culture conditions are the same as the batch culture. The conditions of the culture differ only to the second glucose solution of 1 L added just before seven hours. Fed-batch fermentation was conducted to increase biomass production.

2.3. Down-Stream Process

2.3.1. Concentration & Protective Agent Addition

At the end of fermentation, the cultures were harvested and centrifuged at 4°C and 4700 rpm for 40 min in a Sorvall® RC12BP™ centrifuge (Thermo Scientific Inc., Massachusetts, USA). Eight liters were concentrated by centrifugation for a further lyophilization and the other eight for atomization.

2.3.2. Freeze-Drying

The fresh paste was weighted and diluted with one quarter of the supernatant solution and was mixed for paste recovery from the centrifuge pots. Thereafter, the cream obtained was supplemented with 2% of glycerol and 5% of maltodextrin as cryoprotectants, well malaxed and stored at -20°C before the freeze-drying process (Lyophilizator Liogamma, Koeltechniek Louw B.V.B.A, Rotselaar, Belgium). The freeze-drying operation lasted 20 hours and the pressure stayed at 0.6 mbar. Next, samples of the freeze-dried cells were vacuum-sealed in metallo-plastic bags. Then, the bags were stored at 4°C and 20°C for oxidation analyses after lyophilization (1 day), and at 15, 30, 60 and 90 days. This method was complemented by enumeration on MRS agar.

2.3.3. Spray-Drying

The cream was obtained using procedures similar to the freeze-drying process. Once weighed, the supernatant at four-fold the weight of the cream was first mixed with the protective agents under agitation during one hour for feed solution preparation. As protective agents, half of the cream weight made by casein pepton and the other half by maltodextrin were prepared. The feed solution was atomized using a GEA Niro spray-dryer (Sœborg, Denmark) under the following conditions: outlet air temperature 50°C, inlet air temperature 150°C and atomizing air pressure four bars. These conditions were obtained after many assays in search of the best yield. Powder was collected in a single cyclone separator in glass bottles and was vacuum packed (Audion Elektro, Weesp, Netherlands) in metallo-plastic bags and stored at 4 and 20°C up to 90 days. The outlet temperature was maintained at 50–55°C to obtain powders with the least moisture (5%) and to control the flow rate.

Moisture content in freeze/spray-dried powders: The moisture content of dried powders was determined by oven drying at 105°C according to the International Dairy Federation Bulletin [17].

2.4. Analytical Methods

2.4.1. Determination of the Viability

After each drying process, the survival rate was calculated as follows:

$$\text{Survival rate (yield \%)} = 100 \frac{Cp}{Dp} \cdot \frac{Din}{Cin},$$

where:

- Cp is the concentration of powder (the outlet concentration in cfu.g^{-1});
- Dp is the outlet dry matter (of powder) (%);
- Cin is the inlet concentration (of cream) (in cfu.g^{-1});
- Din is the inlet dry matter (of cream) (%).

The concentration before and after freeze/spray-drying was determined as colony forming units (cfu) per g or per mL. Suitable decimal dilutions were prepared for enumeration and plated on MRS agar. After 90 days of storage, the survival rate was calculated according to the relation:

$$\text{Survival rate (\%)} = \frac{N}{No},$$

with N being the cell concentration (cfu.g^{-1}) of the freeze-dried samples at 90 days of storage and No being the cell concentration (cfu.g^{-1}) after freeze/spray-drying process.

2.4.2. Analysis of Fatty Acids Extracted from Cell Membrane

The lipids were extracted following a protocol Zelles [18] developed. The fatty acid fraction was extracted from 1 g of freeze-dried cells according to the adapted method [19]. After two hours of extraction on a fixed speed rotator SB2 of Stuart®(Chelmsford, Essex, UK) from cell wall fractions of dried cells in 15 mL of an ethanol-ether (3:1 v/v) mixture, the ethanol ether extracts were filtered on nylon membrane filters, Nylaflo™ (Pall life Sciences, Mexico, Mexico) of 0.2 µm pore size, with 47 mm of diameter and then evaporated on a Büchi Rotavapor®R-200 (Büchi Labortechnik AG, Flawil, Switzerland) coupled to a Büchi® Heating Bath B-490 (Büchi Labortechnik AG, Flawil, Switzerland) and concentrated under reduced pressure at 55–61°C. Fatty acid esters extract was then prepared from the concentrate with 14 % (w/w) solution of boron trifluoride (0.5 mL) and 0.2 mL of n-hexane. The suspension was thereafter submitted to a trans-esterification process at 70°C in a water bath for 90 min; 0.5 mL of saturated NaCl, 0.2 mL of sulfuric acid (10%) and 4 mL of n-hexane were added. The fatty acids were extracted from the upper phase after five minutes. Gas chromatographic analysis of the fatty acid methyl esters (FAME) was carried out on a HP 6890 (Hewlett Packard) gas chromatograph equipped with a flame ionization detector at 250°C. A capillary column (30×0.25 mm, film thickness 0.25 µm) was used. The conditions were as follows: the carrier gas was helium (2.4 mL/min) and the injection volume was 1 µL. Injection was done at 250°C in the splitless mode for 1 min. The oven temperature was held at 50°C for 1 min, increased by 30°C/min to 150°C and then from 150°C to 240°C at 4°C/min with a final hold of 10 min at 240°C. Fatty acids methyl esters were identified by comparing their retention times with standard mixtures FAME MIX 47885U (Supelco, Bellefonte, USA). The relative fatty acid content was estimated as a relative percentage of the total peak area using a DP 700 integrator (Spectra physics). Analyses were made in triplicate. All chemicals were analytical grade reagents.

2.4.3. Flow Cytometry Analyses

The experiments were carried out on a BD FACSCalibur system composed of a FACSCalibur two lasers flow cytometer and CellQuest software (BD Biosciences, San Jose, California, USA). For viability assessment of the freeze/spray-dried cells, the carboxyfluorescein diacetate (cFDA) and the dye exclusion DNA binding probes propidium iodide (PI) were used for live and dead discrimination. 1 mL of cell suspension from the freeze-dried cells at 10^9 cfu.g^{-1} was washed successively in 1 mL of phosphate buffer solution (PBS) and centrifuged at 12500 rpm. Then, it was stained with 10 µL of cFDA or PI and incubated at 37°C [20, 21]. Tubes were washed three times with PBS and analyzed on FACSCalibur two lasers flow cytometer. cFDA and PI were excited by a 488 nm laser; the signal was red from the cFDA in the FL-1 and that of PI in the FL-3. All experiments were repeated three times.

2.4.4. Soluble Proteins

The amounts of the cell soluble protein concentration were analyzed using Lowry *et al.'s* [22] method from 0.5 g freeze-dried cells after sonication (Bandelin Sonopuls HD 2070, Germany) with Fohlin-Ciocalteu reagent.

2.4.5. Carbonyl Proteins

The determination of carbonyl compounds was obtained spectrophotometrically by derivatization of the carbonyl group with 2,4-dinitrophenylhydrazine (DNPH) using Levine's (2002) modified method of labeling, leading to the formation of a stable 2,4-dinitrophenyl (DNP) hydrazone product. Dried cells were lysed by sonication (Bandelin Sonopuls HD 2070, Germany) and were centrifuged (1500 rpm, 10 min at 4°C). Thereafter, 0.2 ml was collected and incubated with 0.8 ml 0.2% DNPH in 2.5 M HCl for 1 h at room temperature. Then, the derivative protein contents were extracted with 10% (w/v) trichloroacetic acid, treated with ethanol/ethyl acetate 1:1 (v/v) and re-extracted with 10% trichloroacetic acid. The precipitated extract was dissolved in 6 M guanidine hydrochloride. A blank without the DNPH reagent but with 2 M HCl was treated in parallel following the same procedure. The protein carbonyl content of dried cells was calculated using a standard curve prepared from BSA standards. Carbonyl/protein contents were determined by absorption at 360 nm and expressed in nmol/mg [23, 24].

2.4.6. Statistical Analysis

Productions were done in three bioreactors. The mean values and the standard deviation were obtained from the triplicate.

3. Results and Discussion

The strain CWBI-B1465 was produced in a 20 L bioreactor in batch and fed-batch processes primarily for the

estimation of the yield of production. The working volume was 16 L. One half was then dried by freeze-drying and the other half was spray-dried. The results express a comparative study of growth parameters and conversion yield on the basis of two fermentation modes (batch and fed-batch) followed by two drying processes (freeze-drying and spray-drying). Second, the viability of the strain CWBI-B1465 in storage was examined and compared only on dried cells produced by batch mode. Here, a multiparameter analysis was applied for the viability assessment.

3.1. Production in 20 L Fermentor of CWBI-B1465 Leuconostoc Mesenteroides JF313445

The growth was performed at 27°C and at a pH of 6.5 in MRS broth. The agitation was adjusted at 100 rpm. For the batch process, the growth data were estimated with the end of the exponential phase at seven hours. For the fed-batch process, the second glucose was added after six hours and the exponential phase ended at nine hours. A half liter pre-culture allowed for direct growth in the fermentor without a significant lag phase. The cell concentrations, as illustrated in Table 1 at the end of fermentation, were 9.78 log cfu.ml^{-1} in batch, and 11.8 log cfu.ml^{-1} in the fed-batch, the figure was. This involves an increase of 20.66% only due to the action of 320 g additional glucose dissolved in 1 L of distilled water and added in the fermentor for the fed-batch process. Correspondingly, the growth was 74 times in the first phase of the fed-batch (at 6h) and 142 times at the end from the second inoculation. The specific growth rates (μ) were 0.61 and 0.55 h^{-1} in the batch and fed-batch, respectively, and the generation times (tg) were 1.12 and 1.25 h in the batch and fed-batch, respectively.

Table 1. *Production in 20 L fermentor of CWBI-B1465 Leuconostoc mesenteroides subsp. mesenteroides JF313445.*

Processes	Fermentation time(h)	End of fermentation cells concentration (log cfu.mL^{-1})	[1]μ (h^{-1})	[2]tg (h)	[3]$Y_{x/s}$ (g.g^{-1})	
Batch	7.33	9.78±0.17	0.61±0.01	1.12±0.02	0.15 ± 0.00	[4]FD
					0.15 ± 0.02	[5]SD
Fed-batch	9.50	11.08±0.05	0.55 ±0.07	1.25±0.02	0.16 ± 0.02	FD

[1]μ: the specific growth rate; [2]tg: the generation time; [3]$Y_{x/s}$: conversion yield coefficient; [4]FD: freeze-drying; [5]SD: spray- drying.

Ziadi *et al.* [25] found about 0.9 h^{-1} of μmax and 0.7 of tg for two lactococci strains produced in fed-batch in M17 medium. These data are in agreement with the growth behavior of the two genera, as it's well known that generally *Lactococcus* grow more quickly than *Leuconostoc*.

3.2. Viability of CWBI-B1465 Leuconostoc Mesenteroides JF313445 to Freeze-Drying Vs Spray-Drying

Cells grown in a 20 L bioreactor (in batch and fed-batch) were harvested, concentrated by centrifugation and thereafter protected by addition of protective agents.

A half was freeze-dried, whilst another half was spray-dried. Data of viable cells are presented in table 2. The batch process followed by freeze-drying showed a viable count of 11.90±1.04 log cfu.g^{-1}, while viable population resulted from the batch followed by spray-drying was 10.74±0.11 log cfu.g-1. The two values represented respectively the survival rates (%) of 27.8 and 21.2 which showed clearly how the freeze-drying mode [26, 27] proved effective in achieving high viable cells. However, the value obtained by spray-drying is very interesting in terms of the cost of the processes.

Table 2. *Effect of drying process on the viability of CWBI-B1465 Leuconostoc mesenteroides JF313445.*

Process	Before drying		After drying		Survival rate (%)	
	[1]Concentration (log cfu.mL^{-1})	Dry matter (%)	[2]Concentration (log cfu.g^{-1})	Dry matter (%)		
Batch	11.90±1.04	24.58±0.52	11.43±0.31	91.88±1.29	27.85	[3]FD
	10.74±0.11	19.71±0.46	10.68±0.10	93.35±1.00	21.23	[4]SD
Fed-batch	11.44±0.25	22.42±0.86	11.08±0.05	91.65±0.45	23.89	FD

[1]biomass before drying; [2]biomass after drying; [3]FD: freeze-drying; [4]SD: spray-drying

And comparing the batch and the fed-batch processes followed by the freeze-drying, it was also proven that the batch process seems to be the best as the fed-batch had only 23.89 % of viable free-dried cells. In all cases, the cells recovery after the drying process (FD or SD) was more than 96 % and the dry matters were >91 %. Even if there was a decrease in viability after the drying process [28], the survival rates after drying processes were somehow high.

Coulibaly *et al.* [19] found 18 % in the same conditions on another freeze-dried strain of *Leuconostoc mesenteroides*, meaning that our *Leuconostoc* was very resistant.

3.3. Viability of Freeze/Spray-Dried Cells in Storage

During storage on 90 days, the enumeration showed a decrease in time with freeze- spray-dried cells produced by batch process. Powders stored at 4°C were reduced of 1.8 % for freeze-dried cells and 2.3 % for spray-dried cells. At 20°C, the decrease was very high with 46.0 % and 42.1 % respectively. These data showed again that freeze-drying is the best preservation technique, but the difference is very low. Viability assessment was also evaluated through the fatty acids extracted from cell membrane and the physiological state of cells by flow cytometry analyses. Analyses of the oxidation of cellular constituents were also carried out for cells viability estimation. For that issue, carbonyl proteins and soluble proteins contents were analyzed after sonication of dried powders stored for 90 days.

3.3.1. Fatty Acids Extracted from Cell Membrane

The cellular fatty acids (CFAs) composition in this study has been examined for linking cells viability to conditions of storage during 90 days at two temperatures: 4°C and 20°C. Freeze-dried cells (FD) and spray-dried (SD) cells were compared with regard to the fatty acids oxidation. As well known, the lipid oxidation of membrane fatty acids is deemed responsible for cell death during storage [29-32]. Each CFA relative percentage has been calculated from the total fatty acid methyl esters. The data are means of three independent experiments and the coefficients of variability were lower than 5% ($p<0.05$).

Seven major fatty acids (CFAs) were found as illustrated on Figs. 1 & 2. They are meristic acid (C14); palmitic acid (C16); palmitoleic acid (C16:1); stearic acid (C18); oleic acid (C18:1); linoleic acid (C18:2t); linolenic acid (C18:3t). Among the 7 CFAs, palmitic acid and palmitoleic acid represented more than 70 %. We have calculated the decrease of unsaturated fatty acids due to oxidation based on the ratio between each polyunsaturated fatty acid and the palmitic acid, since saturated fatty acids are not altered by oxidation. The ratio C14:0/C16:0; C18:0/C16:0; C18:2t/C16:0 stays unchanged for 90 days at 4°C and 20°C for freeze/spray-dried powders. The changes occurred on freeze-dried cells as following: at 4°C, the ratio C18:1/C16:0 decreases for 2.2 %, whilst it decreases by 4.8 % at 20°C. At the two temperatures, the changes are very low so that the oxidation phenomenon does not affect the viability.

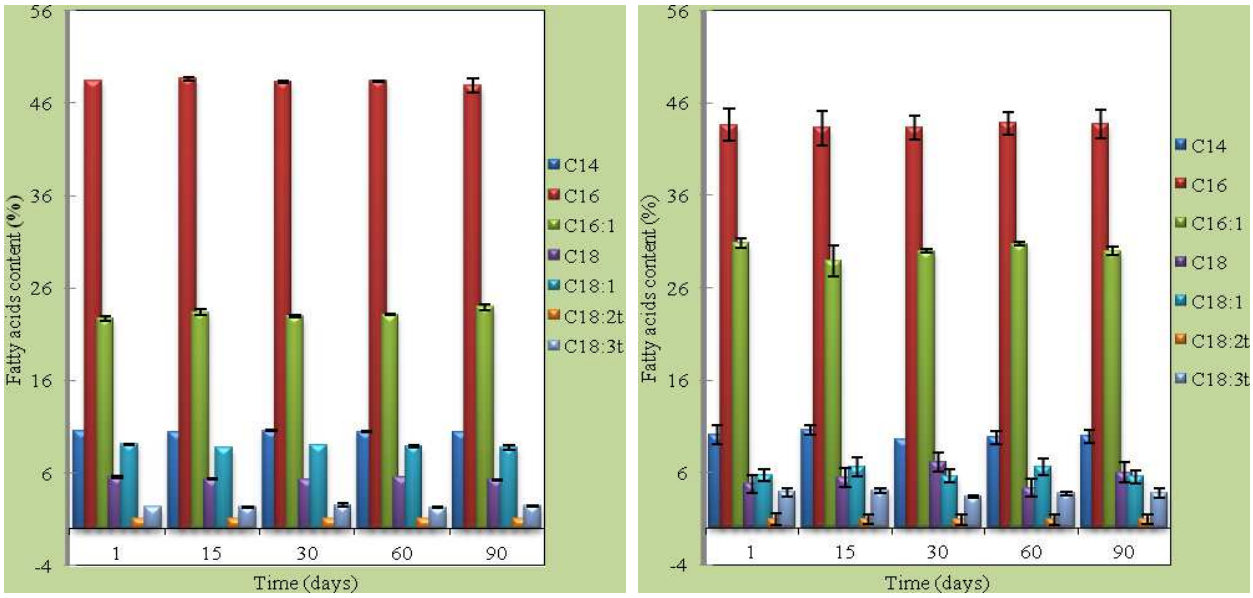
On spray-dried cells, decreases were observed on the ratio C16:1/C16:0. At 4°C, the ratio C18:1/C16:0 decreased by 3.0 % and by 4.1 at 20°C. Slight decrease was also present on the ratio C18:3t/C16:0 for 6.9 % at 20°C. Globally, it seems that the phenomenon of oxidation is very low and its value may be beared on the palmitoleic acid, the oleic acid and the linolenic acid in storage.

Our results disagreed with data obtained by Coulibaly *et al.* [19] on another strain of *Leuconostoc mesenteroides*. He found decreases on the ratio C18:2/C16:0 and C18:3/C16:0. In general, CWBI-B1465 *Leuconostoc mesenteroides* stayed stable at 4°C for freeze-dried cells and spray-dried cells. But our results were consistent with our hypothesis that the strain CWBI-B1465 *Leuconostoc mesenteroides* is highly resistant.

3.3.2. Flow Cytometry Analyses

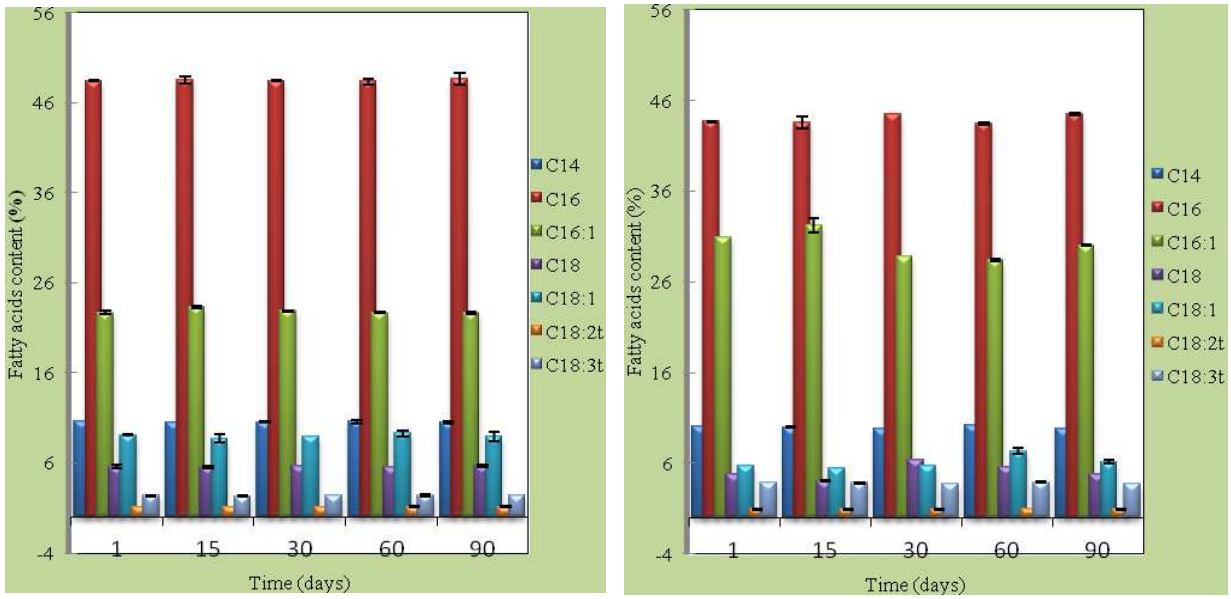
The flow cytometry analyses were applied for the assessment of the impact of the drying process on cells viability in storage. Prior to flow cytometry analyses on dried cells, cFDA (carboxyfluorescein diacetate) and PI (propidium iodide) were tested for control on CWBI-B1465 *Leuconostoc mesenteroides* fresh cells supposed to be at 100 % alive and on 100% fully heat-killed cells, as well as a multiparameter dot plot obtained after PI/cFDA double staining of their mixture (at a ratio of 1:1 vol/vol). These cells were not submitted to any drying process and were collected in exponential phase for 100 % living cells and a fraction was heat-killed for dead cells. Living cells and dead cells subpopulations were spatially separated in dot plots of FL1 and FL2; and relative percentages obtained on living cells stained with cFDA showed 99.11 % of viable cells, 0.01 % of dead cells and 0.74 % of intermediate cells (viable but non cultivable); whereas living cells stained by PI gave 0.08 % alive cells, 9.49 % of dead cells, 0.11 % of intermediate cells and 90.32% of non stained cells (LL). Data obtained (Fig. 3) with fully dead cells were 0.0 % of dead cells, 0.0 % of intermediate cells; 99.99% of non-stained cells (LL) and 0.01 % living cells for cells stained with cFDA; whilst 99.98 % of dead cells, 0.0 % of intermediate cells and 0.0 % alive cells for cells stained with PI.

These findings enabled us the use of the double staining cFDA/PI for a live/dead discrimination, as live/dead assays with two differentially staining probes are attractive because detection is easier when all cells are labeled [20].



(FD) (SD)

Figure 1. *Cellular fatty acids contents of freeze-dried (FD) vs spray-dried (SD) CWBI-B1465 Leuconostoc mesenteroides during 90 days at 4°C. Meristic acid (C14); palmitic acid (C16); palmitoleic acid (C16:1); stearic acid (C18); oleic acid (C18:1); linoleic acid (C18:2t); linolenic acid (C18:3t); SFA (Saturated Fatty Acids): C14:0; C16:0; C18:0; UFA (unsaturated fatty acids): C16:1; C18:1; C18:2t; C18:3t. Values are means of 3 experiments. Content in %.*



(FD) (SD)

Figure 2. *Cellular fatty acids contents of freeze-dried (FD) vs spray-dried (SD) CWBI-B1465 Leuconostoc mesenteroides during 90 days at 20°C. Meristic acid (C14); palmitic acid (C16); palmitoleic acid (C16:1); stearic acid (C18); oleic acid (C18:1); linoleic acid (C18:2t); linolenic acid (C18:3t); SFA (saturated fatty acids): C14:0; C16: 0; C18:0; UFA (unsaturated fatty acids): C16:1; C18:1; C18:2t; C18:3t. Values are means of 3 experiments. Content in %.*

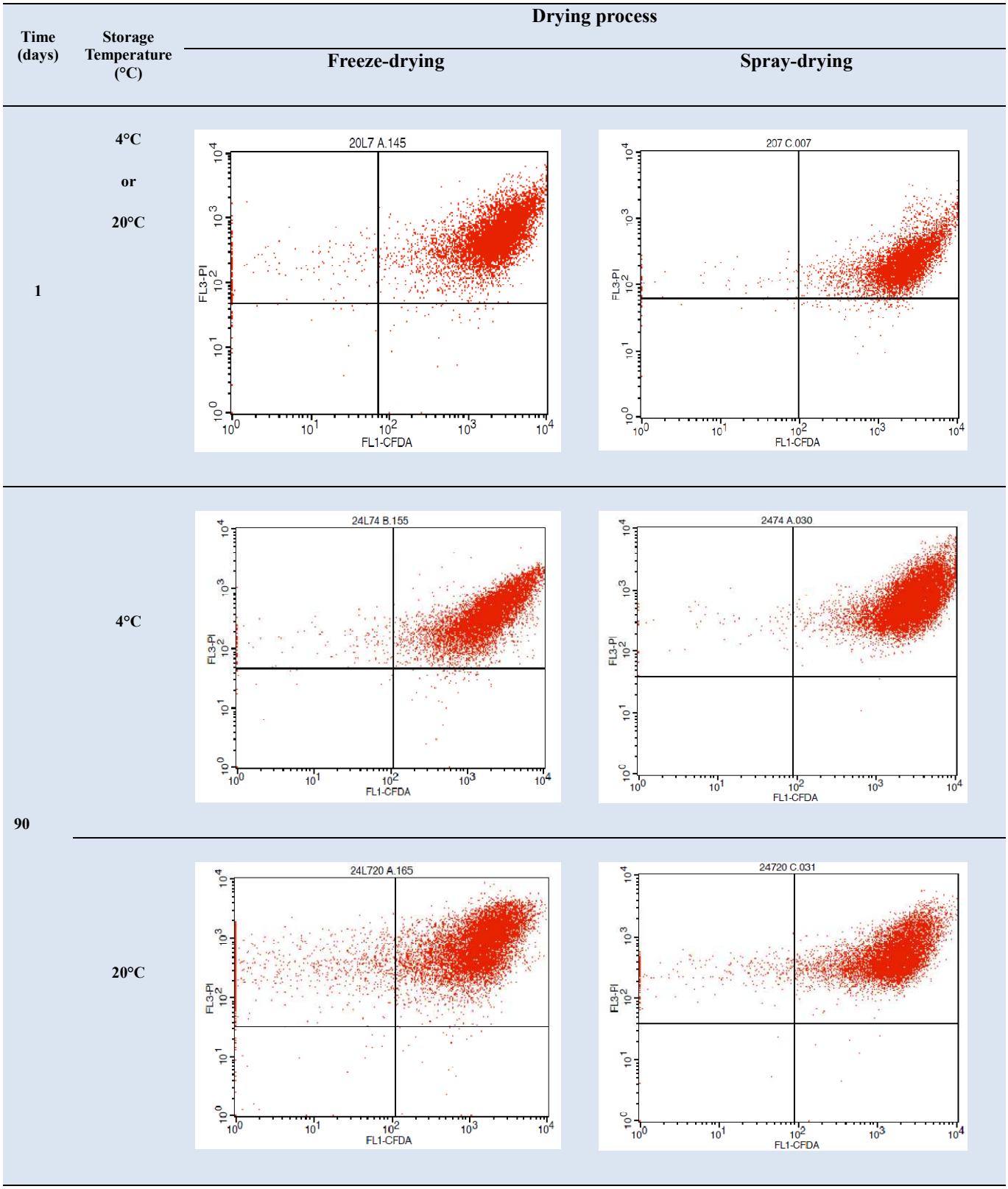


Figure 3. *Flow cytograms of CWBI-B1465 Leuconostoc mesenteroides in storage. Cells were freeze-dried and spray-dried, stored at two temperatures 4 and 20°C during 90 days. Flow cytograms shown here were analyzed at the initial time (day 1) and the end of storage time (day 90)3.3.3. Soluble proteins (SP).*

Analyses of protein contents in freeze/spray-dried powders stored at 4°C and 20°Cwas also carried out on 90 days. Measurements were made at day 1 (after the drying process), day 15, day 30, day 60 and day 90. Results of dried cells (fig. 4) stored at 4°C showed that there is a slight decrease for freeze-dried cells 88 % to 87 % and from 88 % to 85% for spray-dried cells which is not significant in terms of the process yield.

However, for cells stored at 20°C (figure 5), the decrease of freeze-dried cells was in the range of 88 % to 80 %, whereas the range was very high from 88 % to 64.19 % for spray-dried cells. In all cases, the decrease is an indication of protein oxidation, which is expressed by protein insolubilization. And the slope of this phenomenon showed that the insolubilization is higher in freeze-dried cells than in spray-dried cells

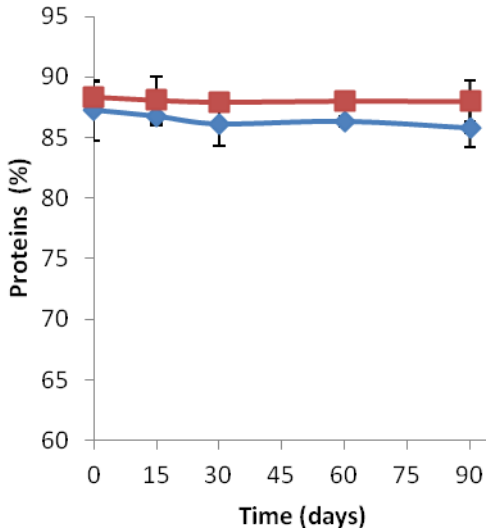


Figure 4. *Soluble proteins content (%) during 90 days storage at 4°C of CWBI-B1465 Leuconostoc mesenteroides (■ freeze-dried and ♦ spray-dried).*

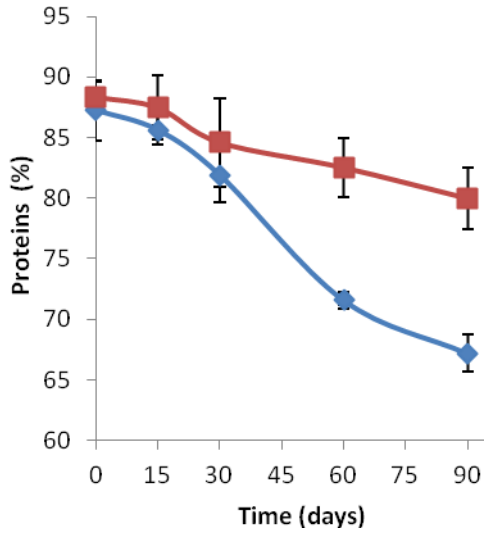


Figure 5. *Soluble proteins content (%) during 90 days storage at 20°C of CWBI-B1465 Leuconostoc mesenteroides (■freeze-dried and ♦ spray-dried).*

3.3.4. Carbonyl Proteins Contents (CP)

The carbonyl contents in freeze/spray-dried cells were compared as for soluble proteins. Freeze-dried cells have more carbonyl contents than spray-dried cells (figs. 6 & 7), about 3 folds. And we observed a slow growth during the storage at 4°C in the two cases. The growth was very high at 20°C storage also in both cases. An increase of carbonyl contents is an indication of the oxidation phenomenon.

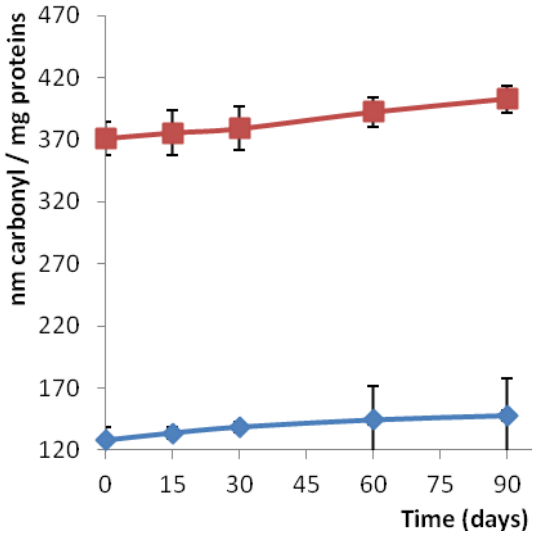


Figure 6. *Protein carbonyl content (nmol/mg) during 90 days storage at 4°C of CWBI-B1465 Leuconostoc mesenteroides (♦freeze-dried and ■ spray-dried).*

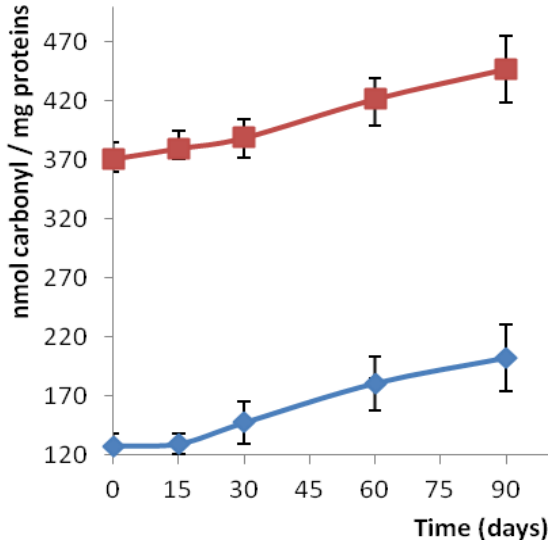


Figure 7. *Protein carbonyl content (nmol/mg) during 90 days storage at 20°C of CWBI-B1465 Leuconostoc mesenteroides (♦ freeze-dried and ■ spray-dried).*

4. Conclusion

The dried bacteria are subject to relative high viability loss from the concentration, and through the drying process as well as during storage. The strain CWBI-B1465 *Leuconostoc mesenteroides* can be produced in bioreactor in biomass concentration of 10^{10} cfu.g^{-1} by batch mode. In order to increase this biomass, the application of fed-batch mode is required. The generation time is therefore near 1.2 h, which stays low than *Lactococcus* or *Lactobacillus* strains. It is

however in the range of growth characteristic of *Leuconostoc* strains. Cells produced can be either freeze-dried or spray-dried, as this strain showed good yields in both cases.

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