ENCAPSULATION OF *LACTOBACILLUS ACIDOPHILUS* IN A PILOT-PLANT SPRAY-DRYER. EFFECT OF PROCESS PARAMETERS ON CELL VIABILITY

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ABSTRACT

Encapsulation is a practical technique for the preservation of probiotic cultures. This may be accomplished by spray-drying, even at large scales. The effect of process parameters such as feed rate and pectin concentration in the feed suspension, on cell viability and stability of encapsulated bacteria was studied. Physical characteristics of the dry powder after the drying process were determined in order to evaluate its influence on product stability and viability during 90 days. The studied parameters showed significant influence on the response variables. Process conditions that generated the highest cell viability after spray-drying are not the same as the conditions that caused the highest stability and viability after 90 days of storage. Water activity of the dry powder played a fundamental role in the stability of encapsulated bacteria. This indicates the most suitable operating conditions for the production of encapsulated lactic acid cultures in a pilot-plant spray-dryer.

PRACTICAL APPLICATIONS

The retention of viability during processing and storage of probiotic cultures is one of the most challenging problems of biotechnology and food industry. Spray-drying is a useful and economical technology to obtain encapsulated probiotic. The knowledge of the optimal encapsulating matrix and operating conditions of the pilot-plant spray-drying process allows to obtain probiotic cultures with high viability and stability. The combination of pectin and reconstituted skim milk improves bacteria protection due to the reinforcement of the structure resulting from the interaction between the components of both materials. It was found that the operational conditions that produce the highest cell viability after the drying process are not the same that conduct to the highest stability and viability after 90 days of storage. With this information, the most suitable operating conditions for a pilot-plant spray-drying can be defined.

INTRODUCTION

According to FAO/WHO (2001), probiotics are defined as live microorganisms which, when administered in adequate amounts, confer a health benefit on the host. Normally, a daily therapeutic minimum dose of 10^8 cells is proposed to ensure probiotic effects on consumer's health (Lourens-Hattingh and Viljoen 2001). These aspects are relevant and must be considered in the production and conservation of dried probiotic preparation for use as starter cultures or food supplements. A high level of viability and maintenance of the

health-related functionality during the production process and storage is required.

The functional properties of probiotic cultures in fermented foods depend on their viability after conservation procedures. Microencapsulation of probiotics is the process in which the cells are surrounded by a polymeric matrix to give small capsules. The encapsulating material provides protection from the environment, may act as nutrient source after rehydration, and allows their release under certain conditions. Techniques such as extrusion, freeze-drying, spray-drying, coacervation and emulsion have been used to encapsulate microorganisms.

To date, a number of approaches have examined the ways to improve the culture viability during spray-drying. This process costs up to six times less than freeze-drying per kg of water removed (Desmond *et al.* 2002). The loss of viability is a consequence of spray-drying technology due to the high temperature that microorganisms are exposure to ensure water evaporation. The elimination of water molecules can promote enzyme inactivation and produce irreversible damages to the bacterial membrane. These damages favor lipid oxidation that affects several biological molecules during storage, indispensable for the viability of microorganism. Preservation of these essential functions and structure is crucial for the survival of bacteria and the retention of their functionality (Crowe *et al.* 1987). Spray-drying technique is associated whit a low survival rates during drying and low stability during storage. However, several studies suggest that some strains of lactic acid bacteria can be spray-dried without a drastic loss of viability and activity (Favaro-Trindade and Grosso 2002; Silva *et al.* 2002; Medeiros *et al.* 2014).

Some studies on the evaluation of spray-drying process as cell dehydration method include the analysis of processing parameters and their optimization in order to obtain a product with high viability after a period of storage (Gardiner *et al.* 2000; Desmond *et al.* 2001; Lian *et al.* 2002). The cell survival depends on several factors such as culture media, encapsulation matrices and drying system (Fu and Chen 2011).The most common encapsulation matrices used as protecting materials, include gelatine, arabic gum, soluble starch (Lian *et al.* 2002) and milk whey protein isolate (Ananta *et al.* 2005). These substances play a key role in the drying process, since they protect bacteria from the hard operational conditions. Furthermore, encapsulation matrices promote rehydration of the final product, improving its quality and expanding its potential applications. Reconstituted skim milk (RSM) is a reputed protective carrier for improving the survival ratio of lactic acid bacteria after spray-drying (Paéz *et al.* 2012; Zheng *et al.* 2015). According Zheng *et al.* (2015), lactose is not sufficiently effective in protecting cell viability and milk fat appears associated with detrimental effects on cell viability during dehydration. The combination of proteins and polysaccharides produces improvements in the quality of the encapsulated product and this feature represents a broad field of study. Pectin is an edible and water-soluble polysaccharide which consists of linear chains of α-(1–4)-linked D-galacturonic acid. The combination of Ca^{2+} ions present in RSM and pectin could improve the resistance of the encapsulation matrix and increase the bacteria viability during the spray-drying process.

Lactobacillus acidophilus LA3 is homofermentative and produces DL-lactic acid from fermentation of carbohydrates. This probiotic culture may be applied in generic probiotic products and may be used in food supplements. To date, powder cultures of different strains of this microorganism were obtained, at laboratory scale, by spray-drying procedure under different process conditions and encapsulation matrices (Oliveira *et al.* 2007; Paéz *et al.* 2012). Nevertheless, there are no reports on larger production scales.

The objective of this work was to evaluate the effect of two process parameters, feed rate and pectin concentration in the feed suspension, on product final characteristics and its stability during storage of a dry powder culture of *Lactobacillus acidophilus* obtained with a pilot-plant spray-dryer.

MATERIALS AND METHODS

Materials

Culture medium used was MRS broth/agar (Acumedia, Brazil). Low methoxil pectin (CPKELCO, Brazil), maltodextrin (Ingredion, Brazil), milk powder (Itámbe, Brazil) were used as encapsulating materials. Sodium citrate (Dinâmica, Brazil) was utilized as dispersing media.

Culture Strain

Lactobacillus acidophilus LA3 (in freeze-dried form), which was kindly donated by Sacco Brasil (Campinas, Brazil), was activated as described below. The freeze-dried culture was activated in MRS (10 g/L) during 24 h at 37C. The cells were harvested by centrifugation at 7,000 $\times$ g for 8 min at 5C and washed twice in citrate solution (2% w/v). The pellet was resuspended in MRS (100 g/L) and the same methodology was used to obtain a suspension of 1000 g/L. The cells were harvested by centrifugation and transferred to a medium containing the encapsulation matrix. Previously, the dry weight was determinate by drying the pellet at 104C during 24 h. Each test was done by quadruplicate.

Preparation of Feed Solutions for Spray-Drying

Feed solutions were prepared following the concentrations indicated in Table 1. Pectin, maltodextrin and RSM were dilute in distiller water, and homogenized at 12,000 rpm for

TABLE 1. OPERATIONAL CONDITIONS OF SPRAY-DRYING PROCESS

Pectin (g/L)	8.00 (−1)
	12.00 (0)
	16.00 (+1)
Maltodextrin (g/L)	2.00
RSM (g/L)	30.00
Feed rate (mL/min)	30 (−1)
	40 (0)
	50 (+1)
Inlet temperature (°C)	170
Flow air (L/min)	60
Air pressure (kgf/cm^2)	9
Nozzle diameter (mm)	0.5

20 min. After that, the culture medium was sterilized at 121C for 15 min. Cells suspension was added to the medium under continuous agitation. The initial viability was 3.70×10^8 UFC/g ($\pm 1 \times 10^9$).

Spray-Drying

Feed solutions were dried by atomization in a LM-MSD model 1.0 spray-dryer (Labmac, Brazil), using the operational conditions presented in Table 1. The dry powder with the encapsulated bacteria was stored at −18C in vacuum bottles.

Cell Survival

Residual viability of the spray-dried samples was determined by the standard plate count method. The spray-dried powder (0.1 g) was rehydrated with 9.9 mL of sterile citrate solution (2%). The rehydrated samples were kept on a shaker for 30 min to get a homogeneous suspension. Diluted feed solution samples and those of rehydrated samples (1000 μL each) were plated using the spread plate method. Colony forming units were determined after incubation for 24 h at 37C. The plating was performed in triplicate. The average counts from the plates of 30–300 colonies were calculated, and the results were expressed in CFU/g sample. The survival rate (%SR) was calculated as follows (Eq. (1)):

$$\%SR = \frac{N}{N_0} \times 100 \tag{1}$$

where N_0 and N represent the number of bacteria before and after drying, respectively.

The viability of the microorganisms in the microcapsules was monitored by counting after 0, 30, 60 and 90 days of storage of the powder at temperatures of −18C in vacuum bottles. The logarithmic value of residual counts after a given storage period N_t (log$_{10}$ CFU/g) was fitted with a linear equation (Abe *et al.* 2009):

$$N_t = -k.t + N_0 \tag{2}$$

N_0 is the logarithm of initial count (log$_{10}$ CFU/g), k is the absolute value of the regression coefficient of each regression line and t is the storage period (days). The k value was defined as the inactivation rate constant (log$_{10}$ CFU/g/days) in each storage condition.

Moisture Content and Water Activity

The moisture content of the microcapsules was determined in triplicate using AOAC methodology (1995). Results were expressed in percentage (%H). Water activity (a_w) was determined in triplicate using a AQUALAB equipment (Decagon Devices, Pullman, USA).

Particle Size Analysis

The particle size distribution of the microparticles was measured using the laser-diffraction technique by Shimadzu Sald-201V (Tokyo, Japan), with ethanol as a sedimentation medium (Synth, Brazil). Sample size was 30 mg and the analysis time was 40 min. Tests were made in triplicate. Values of the Sauter mean diameter, $d_{3,2}$, were obtained as follows:

$$d_{3,2} = \sum \frac{n_i.d_i^3}{n_i.d_i^2} \tag{3}$$

where n_i is the number of particles with a diameter d_i.

Surface Particle Morphology by SEM

The morphology of the particles was observed by scanning electron microscopy. The encapsulated samples were fixed on stubs using double faced metallic tape and covered with a fine layer of gold. Observations were made using the scanning electron microscope (JEOL, JSM-T300, Tokyo, Japan) at an accelerating voltage of 10 kV.

Statistical Analysis

Response surface methodology was used to evaluate the effect of spray-drying conditions on the outlet air temperature (OT) and product characteristics such as %SR, %H, a_w and particle size distribution of the powders ($d_{3,2}$). Two independent variables with three levels were used, the food feed rate (30, 40 and 50 mL/min; X_1) and the pectin concentration (8, 12 and 16 g/L; X_2). The design included 11 experiments with tree repetition of the central point. The coded values corresponding to the natural values of each independent variable and D-optimal design of experiments are shown in Table 1. The independent variables were codified. Polynomial model (Eq. (4)), which includes linear, squared and interaction terms, was fitted to the experimental data.
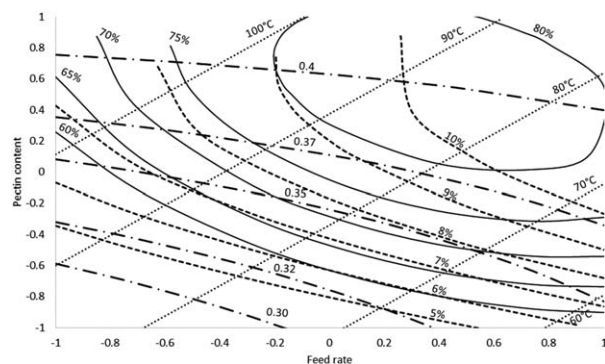
FIG. 1. LEVEL CURVES OF OT (.....), %H (- - - -), %SR (____) AND WATER ACTIVITY (_._)AS A FUNCTION OF FEED RATE AND PECTIN CONTENT

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1^2 + \beta_4 X_2^2 + \beta_5 X_1 X_2 \qquad (4)$$

where X is the coded independent variable; β_0 is the value of the constant; β_1 and β_2 are the linear; β_3 and β_4 are the quadratic coefficients; β_5 is the interaction coefficient estimated and Y is the response. The experimental results were analyzed using STATGRAFHICS Centurion XVI. An ANOVA was performed with a confidence level of 95% ($P < 0.05$) to identify the significant terms in the model for each response. The model validations were checked by R^2 and adjusted R^2.

RESULTS AND DISCUSSION

The outlet temperature of the spray-drying equipment and the product characteristics (%H, a_w, %SR and $d_{3.2}$) obtained for all the experiments are presented in Table 2. The response variables were fitted with a polynomial model (Eq. (4)). Regression analysis and ANOVA were conducted on the obtained data. Statistical significance of effects ($P < 0.05$) was examined. Response surface was used to determine the effect of each independent variable, as well as the interactions between variables, on the responses. Regression equations for each dependent variable, regression coefficient and P value are shown in Table 3. To better rationalization of

results, parametric curves for different response variables were obtained through regression equations (Fig. 1). More details of regression analysis are given in the following sections.

Outlet Temperature

In the first stage of water evaporation from the droplet, the rate of evaporation is essentially independent of the droplet composition. The evaporation rate depends on the vapor pressure at that temperature, the surface area/volume ratio and the rate of airflow over the surface. This first stage is the longest and lasts until the solid mater has reached approximately 60–70% volume fraction (Steward *et al.* 2000). The drying process in controlled by the water evaporation rate. During this period the particle temperature does not exceed the "wet-bulb" temperature. As water evaporates, viscosity increases, glass transition temperature increases, free volume decreases and the rate of loss of the solvent becomes dependent on how rapidly water molecules can diffuse to the surface of a particle so that they can evaporate. The remaining water leaves the particle initially via any intraparticle channels and then by diffusion through the dry outer shell, but the rate of evaporation eventually slows down to the asymptotically approach that of diffusion alone. The process is controlled by the water diffusion through the particle.

Table 2 shows that the increase in OT of spray-drying process was significantly related to % SR of the probiotic bacteria and final characteristics of the powder. The outlet air temperature was influenced by feed rate and pectin content of the feed suspension. An increase in feed flow rate produced a decrease in OT because the higher energy consumption required to evaporate more water. Conversely, increasing the pectin content decreases OT because the lower relative amount of water to be evaporated (Table 3). Ananta *et al.* (2005), indicate that outlet temperature is directly related with %SR of bacteria. According to Izadi *et al.* (2014), the adiabatic saturation effect reduces air temperature as it passes through the chamber. The high evaporation enthalpy of water, and therefore, the high energy consumption of droplet evaporation, leads to the powder product

TABLE 2. EXPERIMENTAL DESIGN AND RESULTS*

Experiment code	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
Feed rate (mL/min)	−1	0	+1	−1	0	+1	−1	0	+1	0	0
Pectin (g/L)	−1	−1	−1	0	0	0	+1	+1	+1	0	0
Outlet temperature (°C)	81[a]	81.5[a]	68[b]	87[c]	87[c]	81[a]	98[d]	98[d]	76[a]	86[c]	90[c]
%H	5.41[a]	6.21[b]	6.82[b]	6.92[b]	8.86[c]	9.41[c]	7.23[b]	9.11[c]	10.35[d]	8.13[c]	9.35[c]
Log$_{10}$ CFU/g	8.22[a]	8.35[b]	8.39[bc]	8.40[c]	8.46[d]	8.48[d]	8.41[c]	8.47[d]	8.45[d]	8.46[d]	8.45[d]
%SR	45.26	61.04	67.18	67.44	77.52	76.04	70.00	79.07	76.04	73.64	75.63
d$_{3.2}$ (μm)	21.41[a]	24.83[a]	26.62[ab]	21.76[ac]	23.71[ac]	23.67[ac]	25.61[ad]	27.64[ad]	29.66[ae]	24.00[ac]	24.62[ac]
a_w	0.309[a]	0.336[b]	0.352[c]	0.361[cd]	0.368[d]	0.370[d]	0.385[e]	0.391[e]	0.394[e]	0.368[d]	0.367[d]

*Similar letters refer equality between means.

TABLE 3. REGRESSION EQUATIONS OF DEPENDENT PARAMETER AS A FUNCTION OF FEED RATE (X_1) AND PECTIN CONTENT (X_2)

Parameter	Regression equation	R^2	P value
Tout	$84.7273 - 13.6667\ X_1 + 14\ X_2$	0.7285	0.0049
%SR	$75.9312 + 12.1915 \cdot X_1 + 17.2126 \cdot X_2 - 9.3638 \cdot X_1^2 - 12.752 \cdot X_2^2 - 7.93848 \cdot X_1 X_2$	0.9800	0.0000
%H	$8.5343 + 2.34 \cdot X_1 + 2.75 \cdot X_2 - 2.0247 \cdot X_2^2$	0.9167	0.0000
a_w	$0.3638 + 0.02003 \cdot X_1 + 0.0577 \cdot X_2 - 0.017 \cdot X_1 \cdot X_2$	0.9633	0.0000
$d_{3,2}$	$24.87 + 1.8592 \cdot X_1 + 1.6714 \cdot X_2$	0.7949	0.0018

temperature never overtakes the outlet temperature, regardless of air inlet temperature exceeds 100C.

The survival rate of the culture was strongly affected by the process parameters and was related to the final characteristics of the product. An increase in pectin content had a positive effect on %SR. Instead, the feed rate initially displays a positive effect on %SR, becoming negative for high flow values (Fig. 1).

Water Content and Water Activity

Water content can be used as indicative for the stability of dried cultures during storage. This should be high enough to maintain stable and viable microorganisms and sufficiently low to avoid clumping and increased degradation rates. The moisture content of powder after spray-drying was ranged between 5.41 and 10.35% (Table 2). Increasing feed flow rate rose the moisture content of powders because the greater mass of water that must be evaporated from the system. The % H increased with increasing solid concentration because water molecules were retained by the pectin matrix (Table 3). The reduction in moisture content of the powder was related to an increase in OT. In general, microorganisms survive better at low water activity. Increasing feed rate and pectin content produced a linear increase in a_w. However, the influence of solids content on a_w was more important than the effect of flow rate (Fig. 1). The hygroscopic character of pectin and its capacity to retain water could explain that behavior.

SEM Images and Particle Size of Powder

Typically, microcapsules size may vary from 10 to 100 μm when obtained by spray-dryer (Fang and Bhandari 2010). Figure 2 shows the scanning electron photomicrographs of spray-dried bacterial powders (samples I–IX). The particles were spherical and displayed a broad range of diameters. They showed concavities typical of materials produced by spray-drying. Small differences in particle structure between samples obtained using different spray-drying conditions were detected (Table 2). The high rate of evaporation of water molecules and volatile molecules, and changes in the surface tension of the droplets could be the explanation for the concavities on the surface particles. Saénz et al. (2009) indicated that this phenomenon is attributable to the shrinkage of the particles caused by the rapid loss of water molecules.

During microencapsulation, capsule size distribution is a function of process parameters and characteristics of cell suspension. The increases in feed rate and solid content broadened the particle size distribution (Table 3). All samples exhibited Gaussian distribution with a Sauter mean diameter between 21.41 and 29.66 μm. Changes in pectin content significantly altered viscosity of culture suspension. It was observed that the increase in viscosity resulted in larger particles. The same observation was reported by Lievense et al. (1993). According to Ré (1998), the more concentrated the feed suspension, the larger the dry particles. The larger particles require longer drying times because the longer diffusion path of water molecules within the particle prior to evaporation. As a consequence, the increase in particle size leads to higher %H of the final powder (Table 2). On the other hand, the retention of bacterial viability is closely related to the increase in water content of the particles and particle size, because the protecting effect of both parameters on the encapsulated cell. However, a very long contact time of the particles with hot air may cause damage to bacterial viability because protein denaturalization and enzyme inactivation inside the cells.

Survival Rate

Figure 1 exhibits the level curves of %SR as a function of feed rate and pectin content. The response variable (%SR) was ranged from 45.26 to 81.40% (Table 2). Statistical analysis showed that both independent variables have significant influence on cell viability (Table 3). The %SR exhibited a maximum in the evaluated range of pectin concentration and feed speed. Moreover, Fig. 1 evinces the relations between %SR, OT, %H and aw. Parametric curves are useful for optimization of industrial processes. The study of the combination of some independent variables enables to optimize the process to obtain a dry product with the best characteristics. However, this analysis must be complemented with curves of cell inactivation as a function of time, since; microorganism viability during storage time depends on several factors, both environmental and operational. Anekella and Orsat (2013) obtained a similar range of %SR using a combination of raspberry juice and maltodextrin as feed
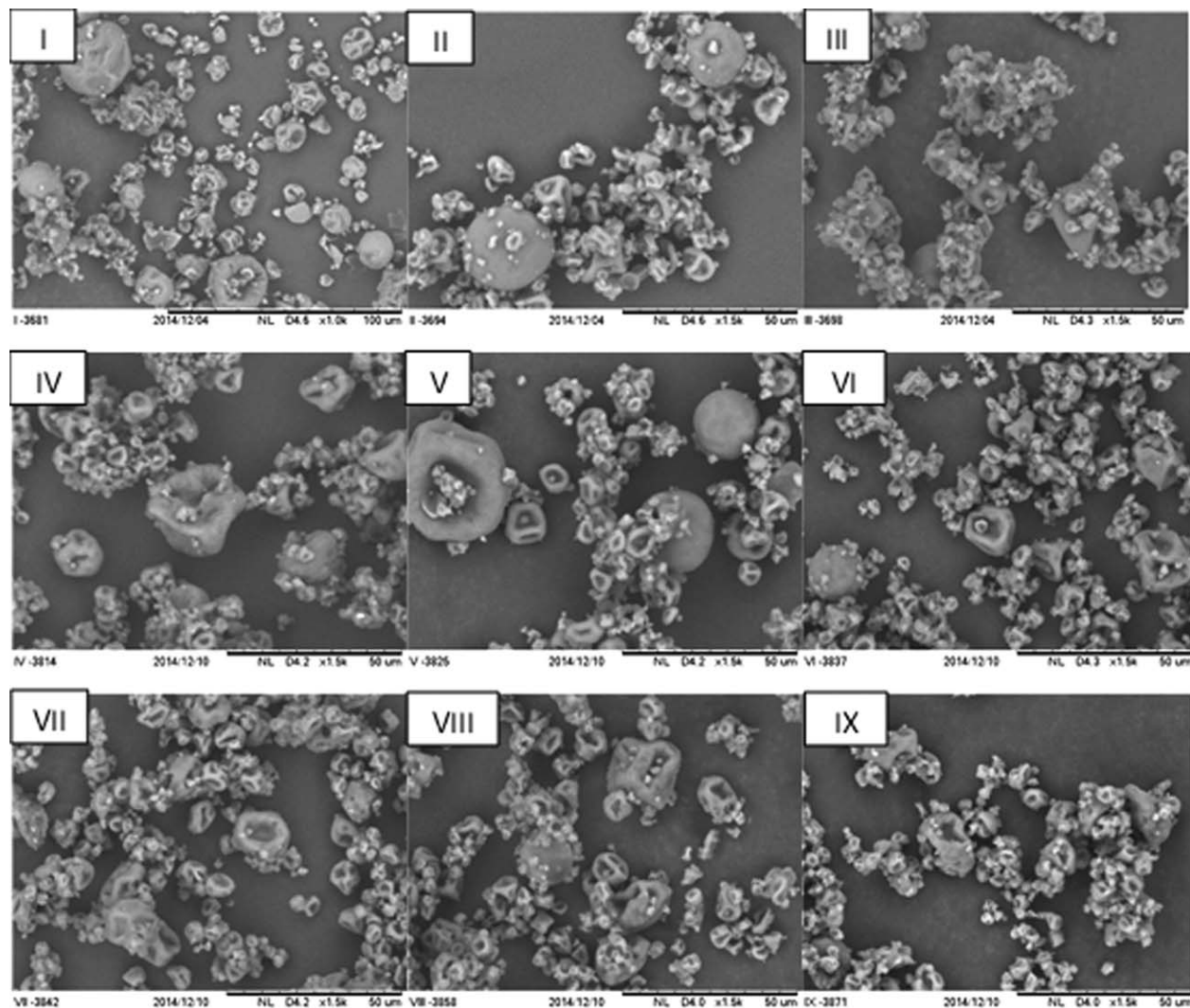
FIG. 2. SEM IMAGES OF PARTICLES OBTAINED BY SPRAY DRYING FOR EACH TREATMENT

solution. Paéz *et al.* (2012) attained up to 95% SR after spray-drying. RSM was used as feed solution and a heat treatment was applied prior to drying. In both cases, lactic acid cultures were dried in a laboratory scale spray-dryer. Several factors, which act in favor and against the cell viability, are interrelated during the spray-drying process. As mentioned in Outlet Temperature, in the first stage of the drying, evaporation rate controls the process and particle temperature is limited to wet bulb temperature, protecting the cell from the heat damage. However, when the diffusion through the dry outer shell controls the process, the temperature of the particle begins to rise and the damage on the bacteria increases significantly. In this stage, the evaporative cooling is no longer available and the thermal inactivation increases (Elizondo and Labuza 1974; Boza *et al.* 2004). Thus, the increase in feed rate, as well as the decrease in pectin concen-

tration, extended the duration of the first stage and caused a diminution in OT. Besides, it is also recognized that bacteria are less sensitive to the effect of heat in the intermediate moisture range (Karel 1995). Table 2 shows that higher %SR were obtained at high values of %H and a_w.

On the other hand, Lian *et al.* (2002) postulated that higher solid content in the feed solution would result in larger particles, which are subjected to greater heat damage than smaller ones, and thus, in a decreased survival of microorganisms. It is provable that those findings refer to long residence times, where the particle reaches very low moisture contents, even in the centre. On the contrary, in our study a protective effect of increased flow rate and content of the encapsulating matrix was observed. It could be due to the range of the parameters evaluated in this study. At constant solid content, %SR increased with the increment

TABLE 4. EVOLUTION OF CULTURE VIABILITY WITH STORAGE TIME

Experimental code	$-\text{Log}(N/No)^{a,b}$			k (log UFC/g/day)[b]	R^2
	30 days	60 days	90 days		
I	0.516 (0.097)[a]	1.233 (0.085)[a]	1.816 (0.075)[a]	0.02055[a]	0.9888
II	0.435 (0.087)[a]	1.040 (0.079)[ab]	1.531 (0.081)[b]	0.01733[b]	0.9866
III	0.449 (0.065)[a]	0.876 (0.091)[bc]	1.317 (0.071)[bc]	0.01459[c]	0.9879
IV	0.468 (0.076)[a]	0.722 (0.080)[c]	1.119 (0.079)[d]	0.01204[d]	0.9704
V	0.541 (0.062)[a]	0.796 (0.074)[cd]	1.241 (0.092)[cde]	0.01326[e]	0.9688
VI	0.497 (0.066)[a]	0.783 (0.087)[ce]	1.210 (0.081)[de]	0.01305[e]	0.9749
VII	0.512 (0.081)[a]	0.830 (0.073)[bc]	1.277 (0.071)[cef]	0.01383[e]	0.9796
VIII	0.478 (0.067)[a]	0.892 (0.061)[bc]	1.349 (0.081)[befg]	0.01487[c]	0.9895
IX	0.484 (0.059)[a]	1.007 (0.074)[bcde]	1.504 (0.084)[bg]	0.01678[b]	0.9915
X	0.536 (0.069)[a]	0.805 (0.061)[c]	1.208 (0.073)[cdf]	0.01298[e]	0.9722
XI	0.542 (0.730)[a]	0.835 (0.079)[bc]	1.246 (0.063)[cdf]	0.01344[e]	0.9744

Standard deviation between brackets.
Similar letters refer equality between means.

of feed rate. The increase in feed rate is related to an increment of %H and a_w.

One of the most important aspects that should be studied when cultures powder are obtained by spray-drying is the preservation of the viability during the storage period (Ananta *et al*. 2005). It is known that the water content and a_w has a high influence in the viability of probiotic powder. Table 4 shows the viability loss and inactivation rate constant (k) determined during storage at $-18C$ for 90 days. The decline in bacterial load was represented by logarithmic values of the survival fractions after different storage periods. Sample IV showed the best %SR and the lowest k value after a storage period of 90 days. Nevertheless, at initial time, the higher cell survival was obtained for experimental conditions corresponding to sample VIII (Table 2). Corcoran *et al*. (2004) noted that the operating conditions that allow a good storage survival may not represent the selection of the best conditions for survival immediately after spray-drying, as was observed in the present study. Simpson *et al*. (2005) indicated that a sublethal damage is produced during of spray-drying process that leads to a lethal form during storage, and then producing a considerable decrease of cell viability. The stability of bacterial viability is dependent on several factors like temperature, ambient humidity and physical characteristics of the particles. Oliveira *et al*. (2007) detected that the higher survival rate of *L. acidophilus* at elevated storage temperature, suggests that the casein/pectin complex may offer protection against oxidative stress and other injuries produced by the dehydration process. Amongst the major components of RSM, it is considered that calcium improves the intrinsic heat resistance of bacteria, whereas milk proteins might lead to a mild temperature variation rate at the later stage of drying (Zheng *et al*. 2015). Noppakundilograt *et al*. (2015) indicate that the incorporation of Ca^{2+} ions promotes the cross-linking and enhances the protection offered by the microcapsules. The combina-

tion of RSM, maltodextrin and pectin in different concentrations evinced an additional improvement in the performance of encapsulated bacteria. Pectin has carboxyl groups which make it an acidic polysaccharide (polyanion). The abundant carboxyl groups of this acidic polysaccharide can be ionically crosslinked by calcium ions (Fang *et al*. 2007, 2008). Thus, both components could contribute to the protective effects of RSM on cell against a rapid viability loss.

Meng *et al*. (2008) indicate that the viability of the microorganisms can be higher when the water activity is low, even when the water content of the powder is low. Figure 3 displays k values versus a_w for different spray-drying conditions. The lower rate of viability loss was related to samples with low water activity. It is well known that at low a_w, chemical reaction rates are slowed down. Particularly, lipid oxidation presents a minimum at a_w between 0.2 and 0.4. Therefore, reactions that cause cell death are limited and reduced during storage time. The fall in viability observed at initial time, just after the drying process, can be due to the damage produced by the spray-drying in DNA, proteins and cellular membrane of bacteria (Wirjantorot and Phianmongkhol 2009). As can
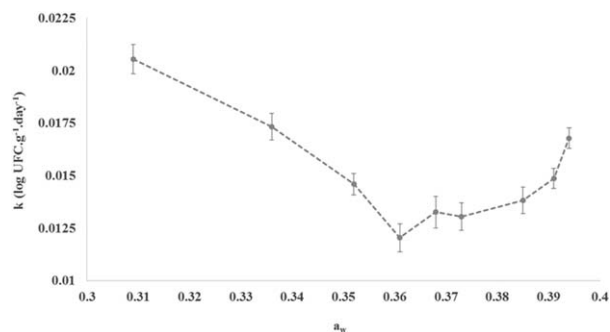
FIG. 3. INACTIVATION RATE CONSTANT VERUS WATER ACTIVITY

be observed in Table 2, in the case of sample IV, OT was significantly lower than in sample VIII, thus the thermal injury on cells can be assumed lower. Nevertheless, as the storage period progresses, free radicals, produced by lipid oxidation during spray-drying, can result in a greater damage on viable cells. Thereby, the statistical analysis indicated not significant differences on viability rate between samples at 30 days of storage. That is, the samples with high viability after spray-drying at initial time (samples V–IX) have higher rate of viability loss than the other samples. On the other hand, considering that sample IV has a lower a_w than sample VIII, it is possible to assume that the rate of deterioration reactions is lower, and therefore this sample presents the lowest value of k parameter after 60 and 90 days of storage. Furthermore, sample IV has a low value of %H which favors preservation of the dry powder culture. According Alzamora et al. (2003), a_w values near to 0.3 are sufficient to control the microbial growth, biochemical reactions and physicochemical reactions that can reduce the stability of dehydrated foods. Is noteworthy that inactivation rate depends on a combination of factors, which should not be evaluated individually in order to ensure a complete and adequate analysis of the results.

CONCLUSION

Lactobacillus acidophilus LA3 culture was dehydrated to a dry powder by spray-drying. RSM, maltodextrin and three different concentrations of pectin were used as encapsulating matrix. Cell viability and physical characteristics of the dry powder were determined after the drying process. Also, bacteria survival was checked during 90 days in order to evaluate stability and viability of the dry culture. Flow rate and pectin content of feed suspension showed significant influence on the response variables. It was found that the process conditions for the greater cell viability after spray-drying are not the same as the conditions that generate the greater stability and viability after 90 days of storage. Water activity of the dry powder played a fundamental role in the stability and viability of encapsulated bacteria. The highest cell survival, after 90 days, was obtained at 0.36 of water activity and moisture content less than 8%. Culture viability of the drying product is dependent on final characteristics of the powder (a_w and moisture content) and operational parameters (feed rate, OT and pectin concentration) of the drying process. Analysis of parametric curves and inactivation rate constants shows a maximum in cell viability after 90 days for a flow rate of 35 mL/min and a pectin concentration of 14 g/L. These results lead to the most suitable operating conditions for the production of dried lactic acid cultures in a pilot-plant spray-dryer and constitute relevant information for scaling up.

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