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Improved probiotic survival to *in vitro* gastrointestinal stress in a mousse containing *Lactobacillus acidophilus* La-5 microencapsulated with inulin by spray drying

Douglas Xavier dos Santos[a,b,c], Alessandro Alberto Casazza[a], Bahar Aliakbarian[a,1], Raquel Bedani[b,c], Susana Marta Isay Saad[b,c,*], Patrizia Perego[a]

[a] Department of Civil, Chemical and Environmental Engineering, University of Genoa, Via Opera Pia 15, 16145, Genoa, Italy
[b] Department of Biochemical and Pharmaceutical Technology, School of Pharmaceutical Sciences, University of São Paulo, Av. Prof. Lineu Prestes, 580, Bl. 16, 05508-000, São Paulo, Brazil
[c] Food Research Center, University of São Paulo, São Paulo, Brazil

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ABSTRACT

This study aimed to optimize the spray drying process for the microencapsulation of *Lactobacillus acidophilus* La-5, using inulin as coating agent to increase its gastrointestinal survival. Moreover, the survival of the microencapsulated and the free microorganism incorporated or not in a synbiotic mousse to *in vitro* simulated gastrointestinal conditions was evaluated. Microencapsulation process conditions were optimized at 80 mL/min, 82%, and 10%, for feed flow, aspiration rate, and inulin concentration, respectively. Subsequently, a synbiotic diet mousse was produced with the addition both of the free and of the microencapsulated probiotic strain, and microorganism *in vitro* gastrointestinal resistance was evaluated. The lowest reduction of cell counts, after 6 h of the *in vitro* assays, occurred for mousse with microencapsulated cells (1.3 log cycles), followed by microencapsulated cells (2.0 log cycles), mousse with free cells (3.0 log cycles), and free cells (7.4 log cycles). Therefore, the spray drying process was appropriate to encapsulate the probiotic strain evaluated using inulin as coating agent and providing resistance to the microencapsulated microorganism. Moreover, the protection given by the microencapsulation process tested was further increased by the food product.

1. Introduction

Microencapsulation of probiotic bacteria leads to cell protection against unfavorable conditions in the food matrix, as well as along the gastrointestinal tract (Würth et al., 2015). Microencapsulation technologies enable development of more stable probiotic products with the preservation of the viability of the microorganisms during processing, distribution, storage, and especially during the digestive process (Amine et al., 2014).

The microencapsulation technique through spray drying consists of forming a suspension containing microorganism and coating agents, which is nebulized with hot air or nitrogen (Encina, Vergara, Giménez, Oyarzún-Ampuero, & Robert, 2016). This process is convenient in terms of energy requirements, operating costs, and leads to high process yield and is often used for probiotic encapsulation (Pinto et al., 2015).

The capsule should be able to provide good protection against hydrochloric acid, which leads to damage of the probiotic cells. The coating agent should not present cytotoxicity and anti-microbial activities, and therefore compromise the viability of the probiotic culture. (Cook, Tzortzis, Charalampopoulos, & Khutoryanskiy, 2012). These materials promote conditions suitable for the microorganisms' survival, increasing their stability during storage (Salar-Behzadi et al., 2013).

Interaction between microorganisms and the polymer is an important factor during the choice of the coating agent (Anekella & Orsat, 2013). In addition, the microencapsulated probiotic ought to maintain viability in the product and its release in the gut has to occur in a controlled manner (Corona-Hernandez et al., 2013).

It is noteworthy that the high temperatures employed in the spray drying process might be stressful to probiotic cells, decreasing their viability. Among the promising coating agents, prebiotic compounds,

* Corresponding author. Department of Biochemical and Pharmaceutical Technology, School of Pharmaceutical Sciences, University of São Paulo, Av. Prof. Lineu Prestes, 580, Bl. 16, 05508-000, São Paulo, Brazil.
E-mail address: susaad@usp.br (S.M.I. Saad).
[1] Present address: The Axia Institute - Michigan State University, 715 E. Main Street, Suite 115, Midland, MI, 48640, USA.

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such as inulin, have been used widely to protect the probiotic cells and improve their survival during spray drying (Bustamante, Villarroel, Rubilar, & Shene, 2015). Inulin is thermally stable and poorly soluble due to its high polymerization degree (Wada, Sugatani, Terada, Ohguchi, & Miwa, 2005). Nunes et al. (2018) observed that inulin and hi-maize used as encapsulating agents for *L. acidophilus* La-5 showed the greatest encapsulating efficiency compared to arabic gum and trehalose. Besides the technological advantage of using inulin, it can be selectively used by the host microorganisms, conferring a health benefit (Gibson et al., 2017). Therefore, inulin may serve as coating agent as well as a prebiotic ingredient.

Although other studies have already investigated the use of prebiotic compounds as microencapsulating agents for *L. acidophilus* strains in the optimization of the spray drying process, to the best our knowledge, there is no report regarding the comparison between the survival of free and microencapsulated *L. acidophilus* La-5 in a synbiotic diet mousse submitted to simulated gastrointestinal stress, as well as with the survival of the microorganism's free and microencapsulated cells not incorporated in a food product. Therefore, the objective of this study was the optimization of the spray drying conditions for the microencapsulation of *L. acidophilus* La-5, using inulin as coating agent. Moreover, the survival of the microencapsulated microorganism incorporated in a synbiotic mousse to *in vitro* simulated gastrointestinal conditions was evaluated and compared with the survival of the free cells in mousse and of the free and the encapsulated cells as pure cultures.

2. Material and methods

2.1. Microencapsulation of Lactobacillus acidophilus La-5 through spray drying

2.1.1. Preparation of the encapsulant solution

Inulin HP (High Performance) (Beneo-Orafti, Oreye, Belgium) was used as coating agent. Inulin is a polysaccharide with a degree of polymerization (DP) above 23, with purity levels of 99.5%. The commercial freeze-dried probiotic culture of *L. acidophilus* La-5 (Christian Hansen, Hoersholm, Denmark), DVS type (direct vat set), was added to a sterilized pre-mixture containing 6% (w/v) of fructooligosaccharides (FOS) (Beneo-Orafti, Oreye, Belgium) dissolved in UHT (ultra-high temperature) skimmed milk (COOP, Casalecchio di Reno, Italy). The suspension was then mixed for 120 min at 37 °C (Komatsu et al., 2013). The activated probiotic strain was inoculated into the suspension containing the coating agent in a ratio of 1:9 (v/v).

2.1.2. The spray drying process

The spray drying process was performed according to Fritzen-Freire, Prudêncio, Pinto, Muñoz, and Amboni (2013) with some modifications in the process parameters. Since higher process temperatures lead to an increase in cell mortality (Bustamante et al., 2015), for the experiments, a Buchi B-290 Mini spray dryer (Buchi, Flawil, Switzerland) was used with air inlet temperature of 120 °C. Encapsulating agent solution containing *L. acidophilus* La-5 was maintained under magnetic stirring at room temperature before being used. A drying air flow rate of 55 m^3/h and the compressor air pressure of 0.4 MPa were used.

2.2. Optimization of spray drying process parameters through Box-Behnken experimental design

The response surface methodology and the Box-Behnken experimental design with three factors and three levels were chosen to optimize and investigate the influence of the process variables feed flow (X_1), aspiration rate (X_2), and inulin concentration (X_3) in terms of the survival rate after the spray drying process (Y_1), probiotic cell counts (Y_2), and the survival rate in acidic conditions (Y_3). The complete design consisted of 15 experiments with three replicates (used to estimate experimental error) of the central point (Table 1).

Table 1
Box–Behnken experimental design matrix employed.

Factors		Levels		
		−1	0	+1
X_1	Feed flow (mL/min)	4	7	10
X_2	Aspiration rate (%)	70	80	90
X_3	Inulin concentration (%)	10	15	20

Response surface methodology was applied to further optimize probiotic microencapsulation. A quadratic polynomial model was fitted to each response following the equation given below:

$$Y_i = \beta_0\beta_i \sum X_i + \beta_{ii} \sum X_i^2 + \beta_{ij} \sum_i \sum_j X_i X_j \quad (1)$$

where Y is the response, β_0 the constant, β_i the linear coefficient, β_{ii} the quadratic coefficient, and β_{ij} the interaction coefficient, whereas X_i and X_j are the independent variables (Ismail & Nampoothiri, 2010).

2.3. Microcapsules powder analysis

2.3.1. Count of microencapsulated and free cells of Lactobacillus acidophilus La-5

Probiotic cells counts were determined according to Gomez-Mascaraque, Morfin, Perez-Masi, Sanchez, and Lopez-Rubio (2016) with some modifications for the microencapsulated cells. The microcapsules obtained by spray drying (1.0 g) were re-suspended in a ratio of 1:9 with sterile peptone water (0.1% w/v) and kept under mixing for 3 min to release the cells. Samples were serially diluted in peptone water solution and seeded in MRS agar modified by the addition of maltose (50% w/v) using the pour plate method with incubation at 37 °C for 48 h (Buriti, Castro, & Saad, 2010). The survival rate (%) compared to the initial count before the spray drying was calculated according to Equation (2) (Pinto et al., 2015):

$$\%\ \text{survival} = (N/N_0)x\ 100 \quad (2)$$

Where N is the number of probiotic cells (log CFU/g) after the spray drying process and N_0 is the number of probiotic cells (log CFU/g) before the process start.

2.3.2. Survival of the encapsulated probiotic strain in acidic conditions

The survival rate in acidic conditions was evaluated according to Costa, Ooki, Vieira, Bedani, and Saad (2017). Other adjustments were necessary and proceeded as follows: microencapsulated probiotic cells were added to a saline solution (0.5% w/v) with pH adjusted to 2.0–2.5 through the addition of a solution containing 1 N HCl (Merck, Darmstadt, Germany), without pepsin and lipase. The samples were incubated at 37 °C for 2 h, for the microencapsulated probiotic cells to be released, and their viability was measured in triplicate. The survival rate to acidic conditions (%) compared to the initial count before the spray drying was calculated according to Equation (3) (Guo et al., 2009):

$$\text{Survival rate to acidic conditions}\ (\%) = (N/N_0)x\ 100 \quad (3)$$

Where N is the number of probiotic cells (log CFU/g) after exposure to acidic conditions (2 h) and N_0 is the number of probiotic cells (log CFU/g) before exposure to acidic conditions (0 h).

2.3.3. Moisture content

The moisture content (% wet basis) of samples after the spray drying process was determined gravimetrically, according to Paini et al. (2015), in triplicates.

2.3.4. Microencapsulation yield

The microencapsulation yield was determined according to Gomez-Mascaraque et al. (2016), using Equation (3):

$$\text{Microencapsulation yield (\%)} = \frac{\text{Mass of spray drying products recovered from collector}}{\text{Mass of solids in the processed suspension}} \times 100 \quad (4)$$

2.3.5. Water solubility index, water absorption index and swelling capacity

The water solubility index (WSI) and the water absorption index (WAI) were determined according to Ahmed, Akter, Lee, and Eun (2010), by dissolving 1 g of product in 12 mL of water. The WSI and WAI were calculated according to Equations (5) and (6), respectively.

$$WSI = DW_{sup}/DW_{part} \text{ x } 100 \quad (5)$$

$$WAI = PW/DW_{part} \quad (6)$$

Where DW_{sup} is the dry weight of the supernatant, DW_{part} is the initial weight of the microparticles (dry basis), and PW is the weight of the pellet after centrifugation.

The Equation (7) was applied according to Paini et al. (2015) using Equation (5) to determine the swelling capacity (SC):

$$SC = DW_{sup}/[DW_{part} \text{ x } (100 - WSI)] \quad (7)$$

2.4. Production of the synbiotic diet mousse (SDM)

The aerated synbiotic diet mousse (SDM) was prepared according to Buriti et al. (2010). Table 2 shows the proportions of the ingredients used. A commercial freeze-dried DVS probiotic culture of *L. acidophilus* La-5 (Christian Hansen, Hoersholm, Denmark) was used. Skimmed milk powder and FOS were dissolved in ultra-high temperature skim milk on the day before the product preparation to improve dissolution. The pre-mixture was stored at 4 °C until the addition of the remaining ingredients. One portion (40 mL) of this pre-mixture was sterilized and employed for the fermentation at 37 °C for 120 min, for the activation of the probiotic culture (Komatsu et al., 2013). The activated culture was then either added directly to the further ingredients of the mixture (free culture) or after being added to a suspension containing the coating agent in a ratio 1:9 and microencapsulated as described above (section 2.1 - microencapsulated culture, obtained through the optimized spray drying process, as described in section 2.2).

Table 2
Proportions of ingredients used in the production of the synbiotic diet mousse.

Ingredient (g/100 g)	Synbiotic Diet Mousse
Skim milk[a]	61.7
Powdered skim milk[b]	4.0
Sucrose[c]	1.1
FOS[d]	6.0
Inulin[e]	4.0
Pasteurized and frozen guava pulp[f]	20.0
Stabilizer/emulsifier[g]	2.8
Lactic acid[h]	0.4
Lactobacillus acidophilus La-5[i]	0.05
Total	100.0

[a] Latte UHT scremato (COOP, Gmunden, Austrian).
[b] Latte scremato in polvere (Ristora, Montichiari, Italy).
[c] Sucralose (COOP, Casalecchio di Reno, Italy).
[d] Beneo P95 (Orafti, Oreye, Belgium).
[e] Beneo HP (Orafti, Oreye, Belgium).
[f] Fruteiro do Brasil (Nectarvis Processamento de Frutas, Ceará-Mirim, RN, Brazil).
[g] Cremodan Mousse 30 (Danisco, Cotia, SP, Brazil).
[h] Lactic acid (Sigma-Aldrich, Steinheim, Germany).
[i] Strain La-5 (Christian Hansen, Hoersholm, Denmark).

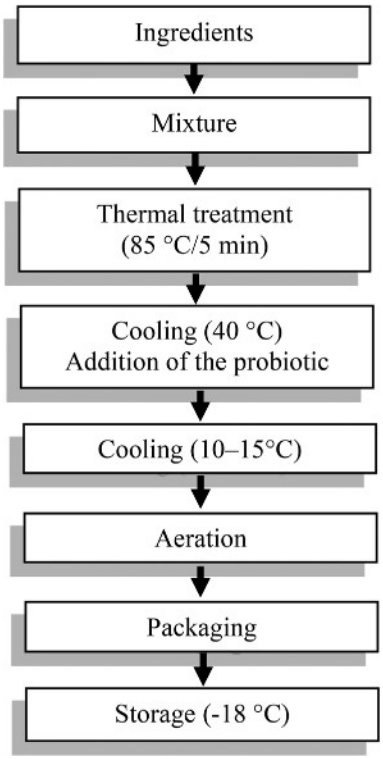

Fig. 1. The main steps employed in the synbiotic diet mousse production.

The other ingredients listed in Table 2 were added and mixed until becoming homogenous. The mixture was pasteurized in the same mixer at 85 °C for 5 min, allowed to cool to 40 °C and supplemented with the fermented milk containing the activated probiotic culture (40 g of milk containing around 10 log CFU/mL) or with the microencapsulated cells (10 g containing around 10 log CFU/g). The mixture was then kept refrigerated (4 °C) for subsequent aeration at a temperature between 10 and 15 °C, during which its volume increased by 80–85%. Fig. 1 schematically illustrates the main steps of the production of the SDM.

2.5. Survival of microencapsulated Lactobacillus acidophilus La-5 to in vitro simulated gastrointestinal conditions after storage

Free cells were prepared, in triplicates, according to Yonekura, Sun, Soukoulis, and Fisk (2014). The freeze-dried probiotic culture was transferred to MRS broth (Oxoid Ltd., Basingstoke, UK), and aerobically incubated for 48 h at 37 °C. The MRS broth, modified by the addition of a maltose solution (described in section 2.3.1), was centrifuged at 3000 × g for 5 min in aseptic conditions. Supernatants were discarded, and cell pellets were washed with phosphate buffered saline (Sigma-Aldrich, Steinheim, Germany), and re-centrifuged. After discarding the supernatants, the collected pellets were suspended in the drying carrier media.

Microencapsulated and free cells were compared for their ability to survive *in vitro* digestion simulating the human gastric and enteric conditions, according to Liserre, Ré, and Franco (2007), with the modifications suggested by Buriti et al. (2010). Other adjustments were necessary and proceeded as follows.

The microencapsulated and the free probiotic cells or the mousse (10 g) were placed in 90 mL of a saline solution (0.5% w/v). The first 2 h represented the gastric phase (pH 2.0–2.5), with a solution containing 1 N HCl (Merck, Darmstadt, Germany), pepsin (3 g/L) (from porcine stomach mucosa, Sigma-Aldrich, St Louis, USA), and lipase (0.9 mg/L) (Amano lipase G, from *Penicillium camemberti*, Sigma-Aldrich). Bile (10 g/L) (bovine bile, Sigma-Aldrich) and pancreatin (1 g/L) (pancreatin from porcine pancreas, Sigma-Aldrich) were added

to the enteric I (4 h of total assay) and the enteric II (6 h of total assay) phases with the pH adjusted, respectively, to 4.5–5.5 and 6.5–7.5.

After incubation, samples from each phase were removed, and the viability of the entrapped cells was measured in triplicates using the method described in section 2.3.1. The results were presented as log CFU/g of fresh probiotic culture. Each assay was performed in triplicate. The pH of the samples was monitored using a pH meter (Hanna Instruments, Woonsocket, USA).

2.6. Statistical analysis

The response surface modelling was conducted using the STATISTICA v.10.0 software (Statsoft Inc., Tulsa, OK, USA). The statistical analysis of the Box-Behnken model and adjusted coefficient of determination (R^2) was performed through the analysis of variance (ANOVA), followed by the Tukey post-hoc test ($p < 0.05$). The optimization was obtained according to Balasubramani et al. (2015). The optimization techniques of the Design-Expert software (Version 7.0 Trial, Stat-Ease, Minneapolis, MN, USA) were used for the simultaneous optimization of the multiple responses. The desired goals for each variable and response were chosen. All of the independent variables were kept within the range, while the responses were either maximized or minimized.

3. Results and discussion

3.1. Box–Behnken experimental design of the spray drying conditions

The experimental design matrix along with the measured responses for all the 15 experiments is given in Table 3. The probiotic survival rate (in terms of log CFU/g) after spray drying ranged between 62.6% (reduction of 3.8 log CFU/g) and 86.5% (reduction of 1.5 log CFU/g) and its survival rate to acidic conditions ranged between 41.8% (reduction of 4.7 log CFU/g) and 79.3% (reduction of 1.9 log CFU/g). Probiotic counts varied from 6.3 log CFU/g up to 9.3 log CFU/g. The moisture and microencapsulation yield ranged between 4.2% and 10.6% and from 81.5% up to 98.2%, respectively. The water solubility index ranged between 36.9% and 74.0%, the water absorption index from 0.9 g/g_{DP} up to 2.0 g/g_{DP}, and the swelling capacity from 0.059 g/g_{DP} up to 0.031 g/g_{DP}.

Khem, Small, and May (2016) reported an average survival rate for *L. plantarum* A17 after spray drying from 34.7% to 82.8%, using whey protein isolate powder as coating agent and an inlet temperature between 90 °C and 130 °C. Huang et al. (2016) reported a survival of 40.0% for *L. casei* microencapsulated with sweet protein at 140 °C.

Regarding the survival rate in acidic conditions, the results obtained can be attributed to the nature of the coating agent and the porosity of the microparticles (Gomez-Mascaraque et al., 2016). Darjani, Nezhad, Kadkhodaee, and Milani (2016) reported that *L. casei* 431 microencapsulated with a mixture of alginate and inulin in a saline solution with pH 1.5 demonstrated a 69.8% decrease after 120 min.

The moisture content might be influenced by several process variables, including the inlet temperature, the coating agent properties, and the feed flow rate (Khem et al., 2016). In our study, when the feed was injected at the lowest flow rate (7 mL/min), and in the presence of the highest quantity of inulin (20%), the product had the minimum moisture content (4.2%), which is acceptable in food products (Yonekura et al., 2014).

The swelling capacity (SC) is another parameter to investigate the resistance of capsules before their bulk dissolution. A higher SC is associated to the higher physical integrity of capsules before dissolution (Cheow, Kiew, & Hadinoto, 2014). From data shown in Table 3, the highest SC (0.031 g/g_{DP}) corresponded to the highest survival rate after spray drying and also to the highest survival rate under acidic condition, confirming the hypothesis related to the stronger integrity of the particles.

Table 3
Actual and coded levels of independent variables and observed responses for the microencapsulation process of probiotics cells according to the Box-Behnken experimental design.

Standard Run	Feed Flow (mL/min) X_1	Aspiration rate (%) X_2	Inulin concentration (%) X_3	Survival rate after spray drying (%) Y_1	Survival rate to acid conditions (%) Y_2	Probiotic count (log CFU/g) Y_3	Moisture (%) Y_4	Microencapsulation yield (%) Y_5	Water solubility index (%) Y_6	Water absorption index (g/g_{DP}) Y_7	Swelling capacity (g/g_{DP}) Y_8
1	4 (−1)	70 (−1)	15 (0)	64.86	56.59	6.68	8.00	95.73	51.50	1.64	0.0107
2	10 (+1)	70 (−1)	15 (0)	73.51	46.39	7.68	8.00	93.53	38.76	1.78	0.0063
3	4 (−1)	90 (+1)	15 (0)	62.57	54.70	6.33	9.20	94.73	51.80	1.73	0.0105
4	10 (+1)	90 (+1)	15 (0)	75.57	73.15	7.82	4.40	89.27	44.89	1.71	0.0083
5	4 (−1)	80 (0)	10 (−1)	77.26	44.30	8.41	6.93	98.10	57.73	1.04	0.0138
6	10 (+1)	80 (0)	10 (−1)	86.49	79.33	9.30	6.86	93.50	44.41	1.64	0.0080
7	4 (−1)	80 (0)	20 (+1)	64.50	52.90	6.57	4.26	95.25	67.37	0.89	0.0206
8	10 (+1)	80 (0)	20 (+1)	76.22	63.08	7.95	6.45	81.45	73.95	0.98	0.0310
9	7 (0)	70 (−1)	10 (−1)	73.77	63.58	7.89	9.24	92.40	44.54	1.58	0.0080
10	7 (0)	90 (+1)	10 (−1)	77.59	56.57	7.85	10.64	98.20	40.80	1.87	0.0069
11	7 (0)	70 (−1)	20 (+1)	74.03	42.43	7.52	4.34	82.85	41.90	1.69	0.0072
12	7 (0)	90 (+1)	20 (+1)	77.45	44.57	7.87	4.23	94.45	38.93	1.86	0.0064
13	7 (0)	80 (0)	15 (0)	78.01	51.14	7.70	7.64	88.73	61.90	1.03	0.0163
14	7 (0)	80 (0)	15 (0)	78.36	54.41	7.76	9.17	91.33	49.92	1.30	0.0100
15	7 (0)	80 (0)	15 (0)	77.08	41.84	8.00	7.40	91.27	36.87	1.99	0.0059

Table 4
Results of variance analysis.

Factors	Sum of square	df	Mean square	*F*-value	*p*-value	Significance
X_1	678.93	1	678.93	89.80	< 0.0001	*
X_2	18.26	1	18.26	2.41	0.1292	
X_3	196.25	1	196.25	25.96	< 0.0001	*
X_1X_2	14.27	1	14.27	1.89	0.1782	
X_1X_3	4.55	1	4.55	0.60	0.4429	
X_2X_3	0.11	1	0.11	0.01	0.9038	
X_1^2	189.15	1	189.15	25.02	< 0.0001	*
X_2^2	230.05	1	230.05	30.43	< 0.0001	*
X_3^2	65.97	1	65.97	8.73	< 0.0056	*
Error	264.62	35	7.56			
Total Sum of square	1671.37	44				
$R^2 = 0.842$	$R^2_{adj} = 0.801$					

Highly significant (*$p < 0.005$).

The adequacy of experimental results to fit quadratic polynomial models was analyzed using the statistical software (data not shown). The fitted models were not significant ($R^2 < 65\%$), and the results of ANOVA for the only significant model related to the survival rate are listed in Table 4. In this model ($R^2 = 0.842$), all three variables had a significant influence on the response ($p < 0.0001$). The F-value of the quadratic model was also significant ($p < 0.0001$). The quadratic model (Eq. (1)) showed that the two process variables, i.e. feed flow (x_1) and aspiration rate (x_2), had a positive effect, while inulin concentration (x_3) had a negative effect on the survival rate after spray drying. The offset term ($\beta_0 = 73.65$) which corresponds to the predicted value of the survival rate after spray drying at the central point ($x_1 = 0$; $x_2 = 0$; $x_3 = 0$), and its value is not signigicantly different from the experimental result (78.9%), hence confirming the suitability of this model.

$$\text{Survival rate after spray drying (\%)} = 73.65 + 10.64x_1 + 1.74x_2 - 5.72x_3 + 4.13 \times {}_1{}^2 + 4.56x_2{}^2 - 2.44 \times {}_3{}^2 \quad (8)$$

The factors which had the greatest impact were the feed flow ($\beta_1 = 10.64$; ${\beta_1}^2 = 4.13$) and the aspiration rate ($\beta_2 = 1.74$; ${\beta_2}^2 = 4.56$), while inulin concentration ($\beta_3 = -5.72$; ${\beta_3}^2 = -2.44$) was the parameter with the least influence throughout the microencapsulation process. Response surfaces of survival rate after spray drying as a function of three variables are illustrated in Fig. 2.

This result may be due to the high feed flow rate which reduces the microencapsulation process time and the cell exposure to the high air inlet temperature. Serantoni, Piancastelli, Costa, and Esposito (2012) reported that high aspiration rate positively influenced the contact time of the granulated material with the cyclone hot air in the drying chamber.

3.2. Optimization of the spray drying conditions

In order to point out the optimal operative parameters for encapsulation of *L. acidophilus* La-5 using spray drying, a numerical optimization was performed. The criteria of optimization (Table 5) were chosen to maximize the survival rate after spray drying, the probiotic cell count, and the survival rate to acidic conditions. The optimum conditions predicted by the model were: inulin concentration of 10%, aspiration rate of 82%, and feed flow of 10 mL/min. In these conditions a high survival rate after spray drying (86.5%), the probiotic cell count (9.0 log CFU/g), and the survival rate to acidic conditions (78.7%) were predicted.

3.3. In vitro simulated gastrointestinal conditions

Free and microencapsulated probiotic cells were added during production of the mousse formulation, and their viability before and after the addition to the mousse over a 6 h-assay of *in vitro* simulating gastrointestinal conditions was monitored (Table 6).

In general, Table 6 shows that microencapsulated probiotic cells incorporated in the mousse presented the lowest reductions (approximately 1 log cycle) in *L. acidophilus* La-5 population throughout the *in vitro* simulated gastrointestinal conditions, whereas free cells were not stable. The samples were statistically different ($p < 0.05$).

In the gastric phase, after 2 h, a decline in the survival of *L. acidophilus* La-5 in the gastric phase was observed in all the samples ($p < 0.05$). The greatest reduction of cell counts occurred for the free cells (6.2 log cycles), followed by mousse with free cells (3.0 log cycles), microencapsulated cells (1.9 log cycles), and mousse with microencapsulated cells (1.1 log cycles) after 2 h of incubation.

In the enteric phase I (after 4 h of *in-vitro* incubation) the lower reductions in relation to the initial population occurred for the mousse containing microencapsulated cells (1.2 log cycles) and microencapsulated cells (2.1 log cycles), while mousse with free cells (3.3 log cycles) and free cells (7.3 log cycles) presented the greatest reductions.

In the enteric phase II, after 6 h, the lowest decrease ($p < 0.05$) in probiotic population was observed for mousse with microencapsulated cells (1.3 log cycles), followed by microencapsulated cells (2.0 log cycles), mousse with free cells (3.0 log cycles), and free cells (7.4 log cycles), indicating the efficiency of inulin as protective covering agent.

The resistance of the *L. acidophilus* La-5 strain during the *in-vitro* simulated gastrointestinal conditions can be considered suitable when compared to those of other similar studies. Buriti et al. (2010) reported a high susceptibility of *L. acidophilus* La-5 during the gastric phase containing HCl and pepsin when the strain was incorporated in a synbiotic light mousse containing sugar and a lower content of guava pulp (12.5%) and inulin (2.0%). The free probiotic cells were drastically reduced after 30 min of the *in vitro* assay. Gomez-Mascaraque et al. (2016) observed a viability loss for *L. plantarum* CECT using whey protein concentrate (WPC) powder as coating agent during the gastric phase. Similar results have been previously reported using *L. casei* ATCC 393 microencapsulated with reconstituted skim milk that increased the cell viability at low pH (Dimitrellou et al., 2016).

Schell and Beermann (2014) observed an increased survival of microencapsulated *L. reuteri* DSM 20016 (in sweet whey and shellac) during the *in-vitro* gastrointestinal environment, probably due to the cell structure recovery of the injured bacteria to the less stressful conditions of the enteric phase.

In contrast to our results, Gandomi, Abbaszadeh, Misaghi, Bokaie, and Noori (2016) reported the low viability during the gastric and intestinal models of encapsulated *L. rhamnosus* GG with sodium alginate and inulin using the extrusion technique.

In this study, *L. acidophilus* La-5 had a high survival in the *in vitro* gastric environment, due to the slow degradation of inulin microcapsules in acidic conditions, which resulted in a delayed decrease in probiotic survival. The use of prebiotic ingredients may be an alternative to improve probiotic survival through the gastrointestinal tract (Hernandez-Hernandez et al., 2012). Besides, the survival of microencapsulated probiotic cells in this step may be attributed to the resistance of inulin to hydrolysis by the gastrointestinal enzymes, low solubility of the long-chain inulin, and slower rehydration of the powder and release of the bacteria cells into the gastrointestinal tract (Pinto et al., 2015). Some studies reported on the application of inulin as potential coating agent for the microencapsulation of probiotic strains with satisfactory results (Karimi, Azizi, Ghasemlou, & Vaziri, 2015; Silva, Zabot, Bargas, & Meireles, 2016; Zamora-Vega et al., 2013).

4. Conclusions

Spray drying was employed to encapsulate *L. acidophilus* La-5. Optimization of the process parameters demonstrated that inulin

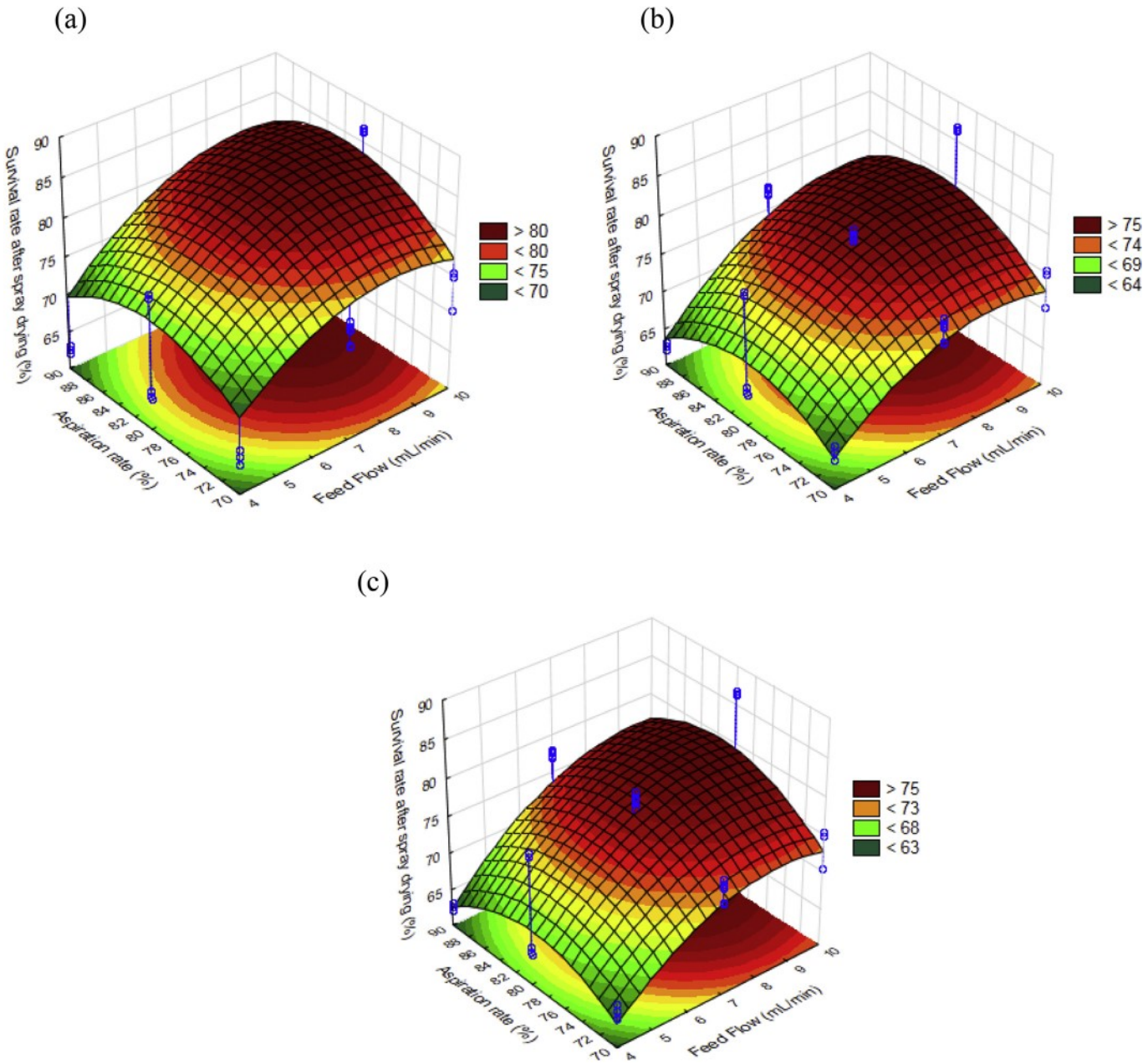

Fig. 2. Response surface plots for survival rate after spray drying as a function of aspiration rate and feed flow at inulin concentration of 10% (a), 15% (b), and 20% (c).

concentration of 10%, an aspiration rate of 82%, and a feed flow of 10 mL/min, ensured a high survival rate after spray drying of 86.5% (reduction of 1.4 log CFU/g), a high probiotic cell count (9.0 log CFU/g), and a high survival rate to acidic conditions of 78.7% (reduction of 1.9 log CFU/g). Furthermore, mousse enriched with microencapsulated cells presented the highest probiotic survival among the samples during the *in vitro* simulated gastrointestinal conditions. The results of this study confirm the appropriateness of the spray drying technique to encapsulate the probiotic strain evaluated using a prebiotic compound such as inulin, especially when the microorganism was incorporated in a synbiotic mousse, leading to an excellent survival rate under *in vitro* simulated gastrointestinal conditions.

Table 5
Criteria for optimization of the process conditions for probiotic microencapsulation along with responses obtained.

Name	Goal	Lower limit	Upper limit	Importance	Solution	Actual response value
X_1: Feed Flow (mL/min)	Is in range	4	10	3	10	–
X_2: Aspiration (%)	Is in range	70	90	3	82	–
X_3: Inulin concentration (%)	Is in range	10	20	3	10	–
Survival rate after spray drying (%)	Maximize	63.6	89.1	3	86.5	86.5
Probiotic cell count (log CFU/g)	Maximize	6.3	9.3	3	9.0	9.0
Survival rate to acid conditions (%)	Maximize	41.8	79.3	5	79.3	78.7

Table 6
Population of *Lactobacillus acidophilus* La-5 (log CFU/g) during exposition to simulated gastrointestinal conditions.

	Mousse with microencapsulated cells	Mousse with free cells	Microencapsulated cells	Free cells
Initial count	6.82 ± 0.05^{a}	6.65 ± 0.22^{a}	8.37 ± 0.06^{a}	8.15 ± 0.01^{a}
Gastric phase	5.68 ± 0.22^{b}	3.64 ± 0.04^{b}	6.46 ± 0.04^{b}	1.92 ± 0.02^{b}
Enteric phase I	5.61 ± 0.03^{b}	3.35 ± 0.10^{c}	6.25 ± 0.04^{b}	0.87 ± 0.05^{c}
Enteric phase II	5.56 ± 0.06^{b}	3.67 ± 0.14^{b}	6.37 ± 0.07^{b}	0.78 ± 0.10^{c}
Population reduction*	1.26 ± 0.11^{A}	2.98 ± 0.25^{B}	2.00 ± 0.05^{C}	7.37 ± 0.09^{b}

*Population reduction = Population $_{\text{initial count}}$ – Population $_{\text{enteric phase II}}$. Within a row, different uppercase letters indicate statistically significant differences ($p < 0.05$) among the four preparations containing *Lactobacillus acidophilus* La-5 to the *in vitro* assay. Within a column, different lowercase letters indicate statistically significant differences ($p < 0.05$) among different sampling periods of the *in vitro* assay for the same preparation.

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