



Use of milk protein and maltodextrin in the microencapsulation of *Lactobacillus acidophilus*: a model approach

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ABSTRACT. The microcapsules containing the *Lactobacillus acidophilus* La-5 probiotic were obtained by spray drying technique, using maltodextrin and milk powder concentrate as encapsulating agents, whose conditions were optimized using Central Composite Rotatable Design (CCRD), obtaining maximum performance with yield $87,59 \pm 0,27\%$ employing the inlet air temperature of 100 to 106°C and maltodextrin concentration between 21 to 23.7% (w v⁻¹). The microcapsules revealed by optical microscopy as spherical and homogeneous particles with varying diameters. The results in differential scanning calorimetry corroborate with the optical microscopy for the formation of microcapsules.

Keywords: optimization, spray drying, probiotic.

Uso de proteínas do leite e maltodextrina na microencapsulação de *Lactobacillus acidophilus*: otimização do processo

RESUMO. As microcápsulas que contêm o probiótico *Lactobacillus acidophilus* La-5 foram obtidas pela técnica de secagem por *spray drying*, utilizando maltodextrina e solução de leite em pó concentrado como agentes encapsulantes, cujas condições foram otimizadas por meio do planejamento experimental do tipo Delineamento Composto Central Rotacional (DCCR), em que obtiveram máximo rendimento de $87,59 \pm 0,27\%$, sendo empregada a temperatura de ar de entrada de 100 a 106°C e a concentração de maltodextrina entre de 21 a 23,7% (m v⁻¹). As microcápsulas revelaram-se na microscopia óptica como partículas esféricas e homogêneas, com diâmetros variados. Os resultados obtidos na calorimetria diferencial de varredura corroboram os da microscopia óptica quanto à formação das microcápsulas.

Palavras-chave: otimização, secagem por atomização, probiótico.

Introduction

The foods with added probiotics are gaining popularity, as consumers look for foods that are not only source of nutrients and sensory appeal, but also contribute to the well-being and provide health benefits. One of the prerequisites required for the commercial use of probiotic microorganisms is the survival in sufficiently large numbers during the production and storage of food products and until they reach the human intestine (Saad, Delattre, Urdaci, Schmitter, & Bressollier, 2013).

In this context, microencapsulation has often been studied in order to protect the probiotic microorganisms against physiological and environmental degradation, and prevent their multiplication in food, with consequent change in sensory properties. Among the techniques, the microencapsulation by spray drying involves the dispersal of cells in a polymer solution which is

atomized in a drying chamber resulting in the evaporation of the solvent, and consequently in the formation of the microcapsules (Kent & Doherty, 2014). The use of the atomizer enables more easily the microencapsulation on an industrial scale, producing large amount of microcapsules economically and effectively (Maciel, Chaves, Grosso, & Gigante, 2014).

Various materials are used as encapsulating agents, especially the milk protein, which present gelifying and emulsifying properties becoming interesting in the microencapsulation of probiotics (Doherty et al., 2011). The combined use of proteins with starch derivatives such as maltodextrin, which, besides having a low cost is characterized by low hygroscopicity avoiding agglomeration of particles, may be interesting, since they can improve the surface properties necessary to achieve effective microencapsulation (Anekella & Orsat, 2013).

This study aimed to optimize the microencapsulation of probiotic *Lactobacillus acidophilus* La-5 by atomization technique, investigating the use of milk protein and maltodextrin as encapsulating agents, as well as evaluate the morphology, diameter and the thermal behavior of the microcapsules.

Material and methods

Microencapsulation of *L. acidophilus* La-5

It was employed with two variables, input air temperature ($^{\circ}\text{C}$) and maltodextrin concentration ($\% \text{ w v}^{-1}$), and as the response variable Encapsulation Yield ($\%$) *L. acidophilus* La-5. The schedule was carried out with four axial points ($-1,68$ and $+1,68$) and triplicated in the central point, totaling 11 assays (Table 1).

Table 1. Variables of Central Composite Rotatable Design (CCRD) for the study of optimization of microencapsulation *L. acidophilus* La-5 (coded and real values) by Spray Drying.

Variables/ Levels	Inlet Air Temperature ($^{\circ}\text{C}$)	Maltodextrin ($\% \text{ w v}^{-1}$)
$-\alpha$ (-1.41)	64	1.7
-1	70	2
0	85	11
+1	100	20
$+\alpha$ (+1.41)	106	23.7

Initially, 11 suspensions were prepared containing skim milk powder at 20% (m v^{-1}) (Iloay Vita[®], Argentina) dispersed in sterile water and 1% ($\% \text{ w v}^{-1}$) of probiotic *Lactobacillus acidophilus* La-5 (CHR Hansen[®]) concentrate thawed; the treatments differ in terms of the concentration of maltodextrin (DE 20, Maltogill, Cargil) added (Table 1). Following, the assays remained 12 hours in an incubator type Shaker (SL-221, Solab) at $37 \pm 1^{\circ}\text{C}$; then, aliquots were removed for evaluation of concentration of viable cells. The suspension was atomized in the dryer with atomizer system with double nozzle fluid (LM MSDi 1.0, Labmaq do Brasil), under constant operating conditions (drying air pressure $2\text{--}4 \text{ kgf cm}^{-2}$, compressed air flow 35 kgf cm^{-2} , dispersing the feed flow rate of 0.55 L min^{-1} diameter of the air outlet in the spray dryer system $1 \mu\text{m}$), except inlet air temperature (Table 1). The powder particles produced were separated from the gas stream, collected in glass containers hermetically sealed and kept under refrigeration at $5 \pm 1^{\circ}\text{C}$.

Enumeration of the probiotic microorganism free and microencapsulated

To count the number of microencapsulated probiotic microorganisms, is necessary initially

break the microcapsules. The probiotic was released from microcapsules according to the proposed by Kim et al. (2008), and subsequently quantified in accordance with Fritzen-Freire et al. (2012).

Encapsulation Yield of *L. acidophilus* La-5

The encapsulation efficiency was calculated according to the proposed by Kim et al. (2017).

Morphological characterization and particle size

The assays were photographed with a digital color camera (DP25) coupled to an optical microscope (Olympus B x 51), with 1000-fold increase for visual characterization of microcapsules, and the mean diameter was determined from twenty measurements for each blade, in triplicate.

Analysis of the thermal behavior of *L. acidophilus* La-5 microcapsules by differential scanning calorimetry (DSC)

The DSC curves of the microcapsules, skim milk powder and the maltodextrin were obtained following the methodology proposed by Fritzen-Freire et al. (2012), using the equipment STA 6000 (Simultaneous Thermal Analyzer, PerkinElmer Frontier).

Statistical analysis

The results obtained were submitted to variance analysis and, if found significant differences between treatments at 10% level of significance, the studentized Tukey range test was applied. In those cases in which the variables had significant effects at 10% level of significance, it was calculated the ANOVA and constructed the response surface. For this analysis, the statistical program used was Statistica, version 10.0.

Results and discussion

Microencapsulation of *L. acidophilus* La-5 by spray drying using maltodextrin and milk proteins as encapsulating agents

The species of *Lactobacillus acidophilus* La-5 is widely used in commercial food (Saad et al., 2013) and therefore was chosen to be studied in this research. It was chosen to atomize the suspensions with the microorganisms in stationary phase, because they have greater stability at this stage (Ranadheera, Evans, Adams, & Baines, 2015).

The results for the encapsulation yield were similar to those obtained by Maciel et al. (2014), when evaluating the microencapsulation of *L. acidophilus* La-5 by using spray drying skim milk and sweet whey (average 76.58%); however, higher than those found by Rodrigues et al. (2015), to evaluate

the stability of *L. acidophilus* Ki with or without addition of L-cysteine (average 42.85%) (Table 2).

Table 2. Encapsulation Yield and mean diameter (μm) of microcapsules obtained by spray drying of *L. acidophilus* La-5 using maltodextrin and milk proteins as encapsulating agents.

Trial	Input air temperature ($^{\circ}\text{C}$)	Maltodextrin (% w v ⁻¹)	Encapsulation Yield (%)	Diameter (μm)
1	-1 (70)	-1 (2)	83.69 \pm 0.27	524.8 \pm 310.6
2	1 (100)	-1 (2)	73.04 \pm 0.21	697.9 \pm 422.9
3	-1 (70)	1 (20)	77.98 \pm 0.32	858.3 \pm 586.8
4	1 (100)	1 (20)	87.59 \pm 0.27	529.8 \pm 331.4
5	-1.41 (64)	0 (11)	79.56 \pm 0.08	1172.4 \pm 407.1
6	1.41 (106)	0 (11)	69.74 \pm 0.20	514.2 \pm 220.7
7	0 (85)	-1.41 (1,7)	76.14 \pm 0.47	821.4 \pm 371.6
8	0 (85)	1.41 (23,7)	81.53 \pm 1.53	1090.7 \pm 427.5
9	0 (85)	0 (11)	68.55 \pm 0.12	879.5 \pm 476.9
10	0 (85)	0 (11)	68.81 \pm 0.22	636.8 \pm 432.0
11	0 (85)	0 (11)	74.39 \pm 0.39	523.1 \pm 308.4

Through the results obtained, were calculated the regression coefficients and it was observed that the quadratic term of the variable concentration of maltodextrin ($p < 0.023882$) and interaction of the variables temperature of the inlet air atomizer and concentration of maltodextrin ($p < 0.043781$) were statistically significant ($p \leq 0.10$). Thus, there was obtained Equation 1, which represents the quadratic model of Encapsulation Yield of *L. acidophilus* La-5 as a function of the studied variables. The non-significant parameters were added to the residue to calculate the analysis of variance (ANOVA).

$$Y1(\text{UFC} \cdot \text{g}^{-1}) = 2.18 + 3.19x_2^2 + 3.78x_1 \quad (1)$$

As the $F_{\text{calculated}}$ for reducing was higher than the $F_{\text{tabulated}}$ and the percent variation explained by the model was adequate ($R^2 \approx 81.89\%$), considering the inherent variability of the bioprocess (Haaland, 1989), it can be concluded that the model (Equation 1) set well to the experimental data, allowing to build the response surface of Figure 1A.

According to Rodrigues and Iemma (2005), the indication of a great range of variables is more interesting than just a point value, in that it may allow a variation in concentrations of variables around the optimal values, keeping still, the process in optimal condition. Thus, the results (Figures 1A and B) indicate that the optimum range to maximize the Encapsulation Yield ($87.59 \pm 0.27\%$) of *L. acidophilus* La-5 covers the air inlet temperature range of 100 to 106 $^{\circ}\text{C}$ and maltodextrin concentration between 21 and 23.7% (% w v⁻¹) and the the air inlet temperature range of 64 to 70 $^{\circ}\text{C}$ and maltodextrin concentration between 1.7 to 2% (% w v⁻¹); however, in this last track of study there was greater loss of material adhered to the walls of the atomizer, which can be correlated with the

lowest concentration of maltodextrin. The maltodextrin has a higher glass transition temperature compared to milk powder, which contributes to the reduction of hygroscopicity and losses on the wall of the atomizer (Langrish, Chan, & Kota, 2007).

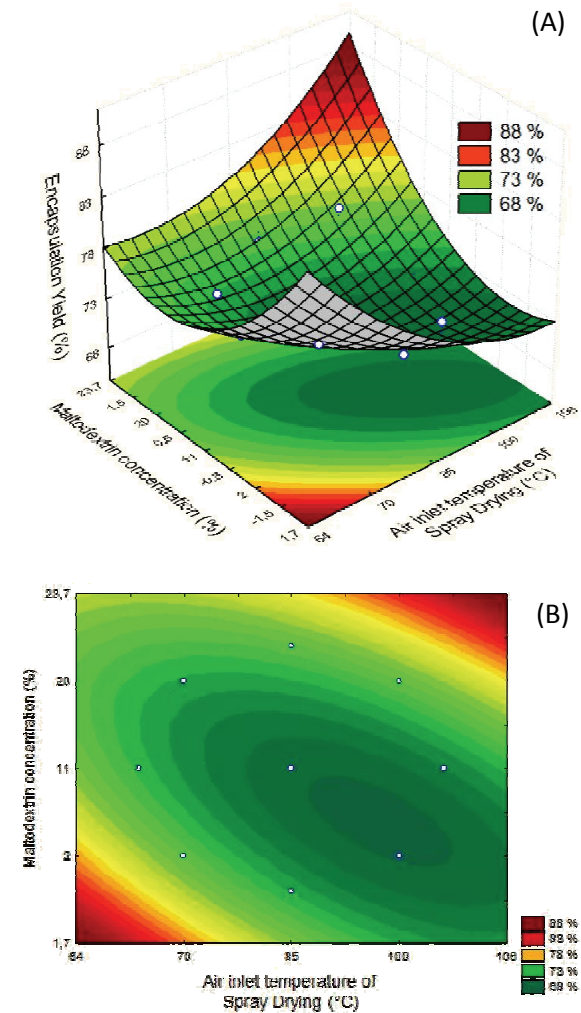


Figure 1. Response surface (A) and contour plot (B) of the effects of two factors (air inlet temperature and maltodextrin) on Encapsulation Yield of *L. acidophilus* La-5.

Morphological characterization and average diameter of the microcapsules

Optical microscopy is an important tool for the preliminary assessment of the microcapsules morphology, presenting the advantages of speed, simplicity and low cost. As observed in Figure 2, there was formation of microcapsules, resulting in spherical particles, which is interesting since this form favors the flow of material, mostly in smooth surface, with some microcapsules with a slight surface roughness and little structures not encapsulated.

It was also observed that the microspheres had a size range (Table 1), demonstrating they had quite different measures ($p < 0.05$), with diameters ranging from 514.2 ± 220.7 (Trial 6) to 1172.4 ± 407.1 (Trial 5), with an average diameter of $769.11 \pm 451.15 \mu\text{m}$. Large capsules and thus with a greater volume increases the surface and the probability of protection against the conditions of the digestive tract (Doherty et al., 2011).

The mean particle size was not significantly affected by the variables tested ($p > 0.10$). However, the input air temperature in the spray dryer showed the greatest and negative effect, in other words, within the range studied the rise of temperature contributed to the reduction of the particle diameter, as can be seen in Figure 2.

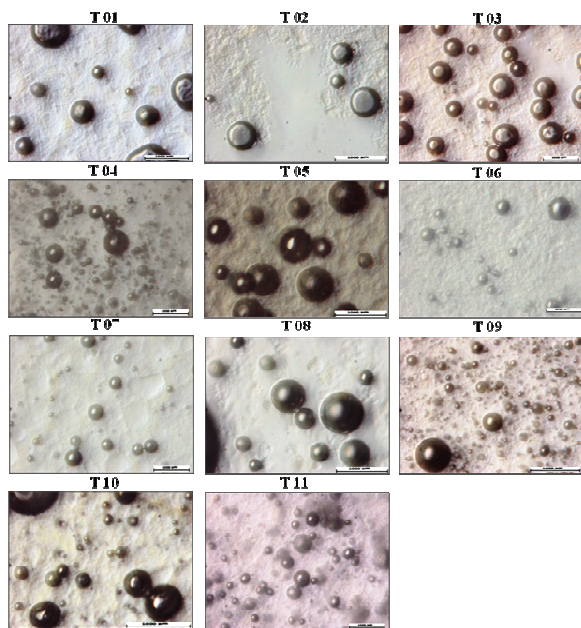


Figure 2. Optical photomicrographs (with an increase of 1000 times) of microcapsules from 11 trials by DCCR, containing *L. acidophilus* La-5, obtained by spray drying. Bar 1000 μM .

Analysis of the thermal behavior of *Lactobacillus acidophilus* microcapsules

Similar to the results obtained by Fritzen-Freire et al. (2012), for all microcapsules, the DSC curves showed a well defined endothermic event, which likely corresponds to the melting point of reconstituted skimmed milk powder (Figure 3) and does not characterize the thermal transitions observed with the pure material (milk powder and maltodextrin). It can be inferred from these experimental data, that the method by atomizing appears to be effective in *L. acidophilus* La-5 encapsulating in particles containing milk powder concentrate solution and maltodextrin.

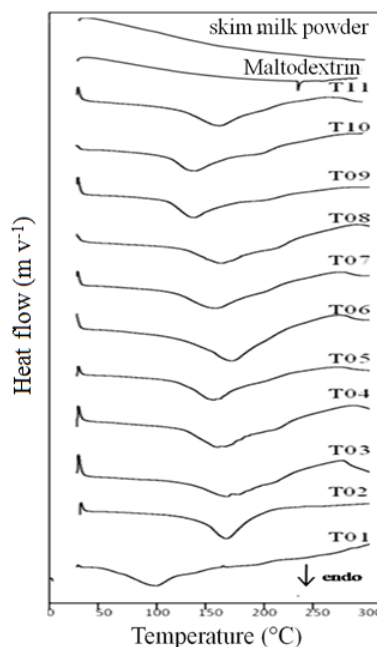


Figure 3. DSC curve of microcapsules from 11 trials by DCCR, obtained by spray drying, using concentrate milk solution and maltodextrin as encapsulating agents.

Conclusion

The Central Composite Rotatable Design allowed the definition of the optimum conditions to microencapsulation of the probiotic microorganism *Lactobacillus acidophilus* La-5 using the drying technique by spray drying and skimmed milk powder solution concentrated along with maltodextrin as encapsulating agents. The microcapsules formed were revealed by optical microscopy as spherical particles, slightly roughened and uniform surface and with varying diameters and may be an alternative mean for obtaining a probiotic powder product to be incorporated in foods.

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