Determination of ideal water activity and powder temperature after spray drying to reduce *Lactococcus lactis* cell viability loss

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ABSTRACT

Spray drying presents a promising technology for preserving bacteria despite a low survival rate of heat-sensitive cultures when subjected to the drying process. The aim of this study was to determine the ideal powder parameters [water activity (A_w) and temperature ($T^{\circ}C_{\text{powder}}$)] needed to produce dehydrated *Lactococcus lactis* ssp. *lactis* with a high viability after drying. Cell concentrates injected into a spray dryer using varying cell concentrate flow rates ($F_{\text{concentrate}} = 0.3$ to 1.0 kg/h), inlet air temperatures ($T^{\circ}C_{\text{inlet air}} = 115$ to $160^{\circ}C$), and outlet air temperatures ($T^{\circ}C_{\text{outlet air}} = 70$ to $115^{\circ}C$) resulted in powders with different values of A_w and $T^{\circ}C_{\text{powder}}$, and levels of cell viability loss. Lower cell viability reduction (~ 0.43 log cycles) was obtained in conditions of $A_w = 0.198$ and $T^{\circ}C_{\text{powder}} = 52^{\circ}C$, which can be met by using $T^{\circ}C_{\text{inlet air}} \sim 126^{\circ}C$ and $T^{\circ}C_{\text{outlet air}} = 88.9^{\circ}C$ regardless of $F_{\text{concentrate}}$ values. After 60 d of storage at room temperature, cell population varied from 7.0×10^5 to 1.1×10^8 cfu/g. The initial powder A_w had no influence on cell death rate, but $T^{\circ}C_{\text{powder}}$ influence was observed. The approach adopted in this study can be applied to other bacteria or spray dryer equipment to determine optimal drying conditions.

Key words: starter culture, water activity, powder temperature, drying conditions, storage

INTRODUCTION

In dairy technology, lactic acid bacteria (**LAB**) are added to food to obtain desirable sensory characteristics through the production of lactic acid, flavor compounds, thickening agents, and enzymes with lipolytic or proteolytic activities (van Hylckama Vlieg et al., 2006). Furthermore, LAB as starter culture helps standardize

food production as well as aiding biopreservation/bioprotection via the synthesis of antimicrobial molecules, which protect against deteriorating and pathogenic bacteria (Frantzen et al., 2018).

In most cases, industrial use of LAB as starter culture relies on dehydration preservation methods that ensure high viability and cell metabolic activity during drying, storage, and industrial applications. Freeze-drying or lyophilization is commonly used to preserve LAB because it causes less stress to the cells and results in ferments with higher viability. However, industrial production of freeze-dried bacteria is limited by the high energy costs of batch drying (Ghandi et al., 2012a).

Alternately, the energy costs for spray drying are 10 times lower than freeze drying and the production can be carried out in a continuous process (Schuck et al., 2013). As an example of how this technique can be performed successfully, the production of spray-dried probiotics with low cell viability reduction after drying (approximately 1 log cfu/g) was reported (Ranadheera et al., 2015). Despite the promising results, the cell viability reduction varied from one probiotic to another; the variations were probably due to each species having different tolerance levels for heat (Ranadheera et al., 2015).

Among LAB food production applications, *Lactococcus lactis* strains stand out due to their high acidification potential, caseinolytic and lipolytic activities, and their ability to both produce compounds with organoleptic qualities and inhibit pathogenic microorganisms via bacteriocins such as nisin, lactacin, and lactococcin (Attar et al., 2018). Nevertheless, the thermal sensitivity of these bacteria remains a drawback to spray-dried production of the ferments (Ghandi et al., 2012a).

Inactivation of *L. lactis* strain during spray drying procedures is generally linked to thermal damage suffered by the cells; however, the effect of water activity (A_w) on cell death has not often been covered (Kang et al., 2015; Dijkstra et al., 2018). Certain studies claim that A_w presents an important parameter for bacterial

Received January 14, 2019.

Accepted March 6, 2019.

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heat inactivation in dried food matrices, although this element has often been overlooked in research on LAB spray drying techniques (Lian et al., 2015; Smith et al., 2016; Syamaladevi et al., 2016).

Based on the current lack of information, the objective of this work has been to determine the ideal *Aw* and temperature required during LAB powder production to produce dehydrated *L. lactis* cells with low viability loss during the drying process. The effect of *Aw* and powder temperature on cell viability loss during storage was also investigated.

MATERIALS AND METHODS

Drying Medium Preparation

An aliquot of 200 g of whole milk powder was dispersed in 250 g of sterile distilled water to obtain concentrated reconstituted milk with a final solid content of 45° Brix when measured with a refractometer (Biobrix, São Paulo, Brazil). The concentrate was heated to 85°C for 1 h to eliminate any possible microorganism contaminants as proposed, with modifications, by Ananta et al. (2005). The drying medium was then refrigerated until the temperature reached 40°C.

Preparation of Cell Concentrate

Lactococcus lactis ssp. *lactis* (ATCC 7962), a nisin-producing LAB with potential applications in the dairy industry, was used as a model for thermal-sensitive bacteria in this study.

One hundred microliters of the strains kept at –20°C in Eppendorf tubes containing Man, Rogosa and Sharpe broth (MRS, Difco, Bordeaux, France) and 60% (vol/vol) glycerol was transferred to a tube containing 10 mL of MRS broth and incubated at 30°C for 24 h. For a second activation, all tube contents were inoculated into 500 mL of MRS broth which was incubated at 30°C during 24 h. The cell culture was centrifuged at $5,000 \times g$ for 10 min at 25°C and the supernatant was discarded. The pellet was washed twice in saline solution 0.85% (wt/vol) and resuspended in a drying medium to obtain a cell concentration of approximately 10^9 cfu per gram of DM (calculated from Equation [2]). The cell concentrate was kept in a water bath at 40°C until drying began.

Drying Conditions and Storage of Dried Cells

The cell concentrate was injected into a pilot scale spray dryer model MSDi 1.0 (Labmaq do Brasil, Ribeirão Preto, Brazil) at varied cell concentrate flow rates ($F_{concentrate} = 0.3$ to 1.0 kg/h), inlet air tempera-

tures ($T^\circ C_{inlet\ air} = 115$ to $160^\circ C$), and outlet air temperatures ($T^\circ C_{outlet\ air} = 70$ to $115^\circ C$; Figure 1). At the end of cyclone recovery, the powders were transferred to sterile glass bottles with thermometers coupled to the bottoms through a custom-drilled hole to monitor the powder temperature ($T^\circ C_{powder}$, in °C; Figure 1). After drying, the thermometer in contact with powder was removed under aseptic conditions and the bottle was hermetically sealed and stored at room temperature ($24.4 \pm 1.3^\circ C$) with a relative humidity of $62.5 \pm 5.0\%$ for 60 d.

Physical Analysis of Powders

Water Activity. The *Aw* of the samples obtained was determined at 25°C using a *Aw* meter (Aqualab, Decagon 3TE, Decagon Devices Inc., Pullman, WA). All measurements were performed in triplicate.

Moisture and Dry Extract Analysis. Three grams of cell concentrate, or 1.5 g of powder, was mixed with sand and heated at 105°C until they attained a constant weight (~5 h; Schuck et al., 2005). Moisture (M%) was calculated by determining weight loss after heating expressed in percentage, whereas the dry extract (DE, in %) values were determined by the following equation:

$$DE = 100 - M\%. \quad [1]$$

Determination of Cell Viability in Concentrate and Powders

Cell viability was evaluated in the cell concentrate and the powders immediately after drying (time: 0 d) and during storage. One milliliter of concentrate or 1 g of powder was dispersed in 9 mL of sterile saline solution 0.85% (wt/vol) and serial dilutions were performed in the same diluent. The enumeration of viable cells was performed by microdroplet technique. An aliquot of 20 µL of appropriate dilution was deposited on the surface of MRS agar and incubated at 30°C for 72 h in jars containing an Anaerocult A sachet (Merck, Darmstadt, Germany). The enumeration of colony-forming units was performed in triplicate and conducted in droplets containing between 8 and 80 colonies. The number of viable cells (N) was calculated in relation to dry material content (cfu/g) of concentrate or powder using Equation [2]:

$$N = n \times DF / \text{aliq} \times DE, \quad [2]$$

where *n* represents the number of colonies counted, DF is the dilution factor, aliq denotes the aliquot plated (0.02 mL for concentrate or 0.02 g for powder), and

DE is the dry extract given in grams per milliliter or grams per gram for the cell concentrate and powder, respectively. The cell viability loss was determined by the following equation:

$$\text{viability loss} = \log N_0 - \log N', \quad [3]$$

where N_0 represents the number of cfu/g of surviving *L. lactis* in the cell concentrate and N' indicates the number of viable cells (cfu/g) just after drying. Viability loss during storage was calculated from the same equation, but in this case N_0 represents the number of colony-forming units per gram of surviving *L. lactis* after drying and N' indicates the number of viable cells (cfu/g) in a determined time of storage.

Calculation of Inlet Energy

The thermal energy used by the equipment to dehydrate the *L. lactis* cells (E_{inlet}) was estimated according the following equation proposed by Silva et al. (2017):

$$E_{inlet} = [T_{inlet\ air}^{\circ}(1.01 + 1.89AH_{inlet}) + 2,500AH_{outlet}] [54.35(1 + AH_{outlet})^{-1}] + 129.933F_{concentrate}, \quad [4]$$

where $T_{inlet\ air}^{\circ}$ corresponds to inlet air temperature; AH_{inlet} and AH_{outlet} are the absolute humidity of air (kg of water/kg of dried air) in the inlet and outlet of equipment, respectively; and $F_{concentrate}$ denotes the cell concentrate flow rate.

Statistical Analysis

The drying processes and simulation powder storage simulations were carried out with 3 repetitions, and each repetition was conducted in duplicate. The results were compared using the Student's *t*-test statistical method with a significant difference at $P < 0.05$. In addition, the results were interpreted using regression analysis and statistically evaluated using Excel (version 2016; Microsoft Corp., Redmond, WA) software.

Figure 1. Schematic representation of the experiment setup. $T^{\circ}C_{inlet\ air}$ = inlet air temperature; $F_{concentrate}$ = cell concentrate flow rate for water activity; and $T^{\circ}C_{powder}$ = powder temperature.

RESULTS AND DISCUSSION

Influence of Drying Parameters on *Aw* and Powder Temperatures

Several food matrixes such as maltodextrin, gum arabic, and maize starch have been considered for use as carrier material for spray drying the bacteria; among them, reconstituted milk was chosen to dry *L. lactis* cells due to its recognized protective properties of dairy proteins, calcium, and lactose when subjected to drying conditions (Rudolph and Crowe, 1985; Zheng et al., 2015; Huang et al., 2016; Reyes et al., 2018).

The *Aw* and the $T^{\circ}C_{\text{powder}}$, measured just after drying, were regulated by varying the operational spray dryer input parameters (Figure 2). When the inlet air temperature was increased ($T^{\circ}C_{\text{inlet air}}$), the powder *Aw* decreased (Figure 2A), which is in line with previous studies (Behboudi-Jobbehdar et al., 2013; A-sun et al., 2016; Dantas et al., 2018). This behavior can be explained by the increased rate of heat transfer into the particles at higher temperatures, which boosts the driving force for moisture evaporation and causes higher water removal from the food matrix (Solval et al., 2012).

In conditions of drying at lower inlet air temperatures (115 to 130°C), water that is not bound to the food matrix will mostly be removed. This results in a relation that is almost inversely proportional between the *Aw* and inlet air temperature (Figure 2A). However, as inlet air temperature increases (145 to 160°C), more water molecules found inside macromolecules or immobilized by hydrogen binding are evaporated, requiring more energy than the previous conditions (Lewicki, 2004). The result is that the *Aw* variation with increasing inlet air temperature is reduced and produces a curve best delineated by a quadratic function (Figure 2A). Thus, the variation of *Aw* in function of $T^{\circ}C_{\text{inlet air}}$ (Figure 2A) is obtained using the following equation:

$$Aw = 10^{-4}(T^{\circ}C_{\text{inlet air}})^2 - 0.029(T^{\circ}C_{\text{inlet air}}) + 2.31, R^2 = 0.98. \quad [5]$$

When the inlet air temperature (from 115 to 160°C) and independent flow rate (from 0.3 to 1.0 kg/h) were increased, the powder temperature increased proportionally as shown in Figure 2B (results obtained using Equation [6]):

$$T^{\circ}C_{\text{powder}} = 0.288(T^{\circ}C_{\text{inlet air}}) + 15.4, R^2 = 0.98. \quad [6]$$

According to Bimbenet (1978), a low pressure system promotes water evaporation without high heating of

the particle during initial contact between a particle and inlet air. In other words, when water is evaporated from particles at around 45°C (Bimbenet, 1978), significant cell inactivation is not expected due to the low heat levels (Dittman and Cook, 1977). However, after free water molecules had been completely evaporated, the thermal energy from the air gradually increased the dried particle temperature, thus promoting progressive thermal damages to the bacteria.

In the final stage of spray drying, specifically at the end of the cyclone, the powder temperature tends to enter into a balance with the outlet air temperature (Ozmen and Langrish, 2003) and shows low variation during the processing time as represented by a model curve in Figure 2C. Furthermore, an increase in outlet air temperature proportionally increases powder temperature (Figure 2D):

$$T^{\circ}C_{\text{powder}} = 0.418(T^{\circ}C_{\text{outlet air}}) + 14.85, R^2 = 0.99. \quad [7]$$

It can therefore be concluded that increasing the inlet air temperature proportionally increases drying energy (Figure 3A), which results in both higher outlet air and powder temperatures once processing has been completed.

By contrast, cell concentrate flow rates ($F_{\text{concentrate}}$) had no significant influence on the *Aw* and powder temperatures in the tested range (Figures 2E and 2F). Although an increase in cell concentrate flow rate contributes positively to the thermal energy increase applied to drying (see Equation [4]), the flow rate ranges tested showed little effect on the inlet energy (between 39 and 130 kJ/kg).

Effect of *Aw* and $T^{\circ}C_{\text{powder}}$ on Viability Loss After Drying

Cell viability was significantly influenced by *Aw* variations and powder temperatures after drying (Figures 4A and 4B). The relation between loss of cell viability and powder *Aw* followed a quadratic function pattern (Figure 4A) represented by this adjusted Equation [8]:

$$\text{viability loss} = 59.65(Aw)^2 - 23.66Aw + 2.76, R^2 = 0.99. \quad [8]$$

For low *Aw* values, the cell inactivation mechanism can be associated with lipid oxidation, which promotes the release of free radicals that are harmful to cells (França et al., 2007). Furthermore, lipid oxidation can occur on the cell membrane, thus causing irreversible damage to

its structure and resulting in loss of viability (Ezraty et al., 2017).

Alternately, the inactivation of cells in the powders with elevated A_w values could be explained by the higher thermal conductivity of water molecules, which transfer heat more efficiently to cells when the powder is in the drying chamber. Higl et al. (2007) suggest that in higher A_w situations, cells may enter a partially active state in which some enzyme systems are active,

but others are not. Consequently, the intracellular accumulation of intermediate metabolites that may occur cause detrimental effects on the microorganisms. Also in concordance with our findings, Vesterlund et al. (2012) demonstrated that the viability of *Lactobacillus rhamnosus* GG in dry food matrices during storage was reduced in powders containing high A_w values (0.43).

To estimate the ideal A_w for cell preservation in a given powder, a derivative of Equation [8] should equal

Figure 2. Relation between inlet air temperature ($T^{\circ}C_{inlet\ air}$), outlet air temperature ($T^{\circ}C_{outlet\ air}$) and cell concentrate flow rate ($F_{concentrate}$) for water activity (A_w) and powder temperature ($T^{\circ}C_{powder}$). Error bars indicate SEM.

zero. This makes it possible to determine that an $Aw = 0.198$ ensures the highest *L. lactis* survival rate after drying.

A direct relation between cell viability loss and powder temperature was found (Figure 4B) as determined by Equation [9]:

$$\text{Viability loss} = 0.045(T^\circ C_{\text{powder}}) - 1.67, \\ R^2 = 0.93. \quad [9]$$

According to Silva et al. (2018), the temperature is the most important process variable in the microbial inactivation and it is a direct measurement of the energy transferred by a substance in the form of heat. In this case, the drying conditions should be regulated to reduce the maximum as possible the temperature of powder after drying. This result suggests that powder temperature is associated with thermal damage suffered by cells that results in a more pronounced death rate with the increasing in the $T^\circ C_{\text{powder}}$. In accordance with this result, Perdana et al. (2012) and Khem et al. (2015) also verified that the viability loss of *Lactobacillus plantarum* cells increased in powders subjected to higher temperatures after drying. Ananta et al. (2004) also concluded that the viability loss of *Lactobacillus rhamnosus* in skim milk powder was directly proportional to powder temperature.

It has been shown that the combined effects of heat and mechanical stress result in cellular damage that lead to a loss of viability of microorganisms (Chávez

and Ledebøer, 2007). These cellular injuries include DNA and RNA denaturation, ribosomal damage, dehydration, and destabilization of plasma membrane due to water removal (Silva et al., 2018). The results showed in Figures 4A and 4B indicate that of *L. lactis* survival depends both on Aw and powder temperatures at the end of processing. Thus, a compromise between these 2 parameters must be made to determine optimal drying conditions. By correlating Aw values and powder temperatures obtained from the experiment results, a second-order polynomial curve can be produced with the following equation (Figure 4C):

$$Aw = 0.0008(T^\circ C_{\text{powder}})^2 - 0.092(T^\circ C_{\text{powder}}) \\ + 2.82, R^2 = 0.99. \quad [10]$$

With an ideal value of Aw (0.198) in Equation [10], 52°C appears to be the ideal temperature for the powder after drying. When $Aw = 0.198$ in Equation [5], it can be estimated that inlet air temperature should be adjusted to 125°C for minimal cell viability loss. Conversely, replacing $T^\circ C_{\text{powder}} = 52^\circ\text{C}$ in Equation [6] would set the inlet air temperature estimate at 127°C. By determining the average of these 2 calculations, higher cell survival can be expected in conditions of inlet air temperature at around 126°C regardless of cell concentrate flow rate.

Although the inlet air temperatures determined by Equations [5] and [6] are close, the difference in the calculated values can be explained by mathematical

Figure 3. Relation between inlet air temperature ($T^\circ C_{\text{inlet air}}$) and cell concentrate flow rate ($F_{\text{concentrate}}$) for inlet thermal energy (E_{inlet}). Error bars indicate SEM.

rounding and measurement error. In our experiments, drying *L. lactis* at $T^{\circ}C_{inlet\ air} = 130^{\circ}C$ resulted in lower mean viability loss (0.43 log cycles) compared with other inlet air temperatures used in this study (viability loss between 0.60 and 1.22 log cycles). Similarly, replacing $T^{\circ}C_{powder} = 52^{\circ}C$ in Equation [7] results in an outlet air temperature of $88.9^{\circ}C$, which should be carried out to ensure higher cell survival.

In concordance with our findings, Ghandi et al. (2012b) reported a higher survival percentage (21.6%) of *Lactococcus lactis* ssp. *cremoris* when spray dried at $130^{\circ}C$ when using lactose and sodium caseinate as the drying medium. However, the ideal outlet air temperature was found by the researchers in that study to be

$65^{\circ}C$, whereas a higher value ($88.9^{\circ}C$) was verified in the present study.

As observed by our results and described by Ozmen and Langrish (2003), increasing the $T^{\circ}C_{inlet\ air}$ is likely to increase the $T^{\circ}C_{outlet\ air}$ and consequently the $T^{\circ}C_{powder}$; thus, it is evident that the control of inlet/outlet air temperatures are factors that should be taken into consideration when drying bacteria. Despite numerous studies that have used the inlet/outlet air temperatures as indicators of cell viability loss in the spray drying process, the combination of these parameters is specific to the equipment or operating conditions tested. In other words, using the same inlet air temperature in 2 different drying towers with different evaporative

Figure 4. Effect of water activity (A_w ; A) and powder temperature ($T^{\circ}C_{powder}$; B) on cell viability loss. Relation between A_w and $T^{\circ}C_{powder}$ of powders (C). Error bars indicate SEM.

Figure 5. Effect of water activity (A_w ; A) and powder temperature ($T^{\circ}C_{\text{powder}}$; B) on the viability loss during storage time. Error bars indicate SEM.

capacities will not necessarily result in the same outlet air temperature (Silva et al., 2017). In this sense, our study proposes verification of cell viability loss based on the powder properties (A_w and $T^{\circ}C_{\text{powder}}$), which may be analyzed independent from the evaporative capacity and design of the spray dryer used.

Effect of A_w and $T^{\circ}C_{\text{powder}}$ on Viability Loss During Storage

In the first part of our experiments, it was demonstrated that the A_w and $T^{\circ}C_{\text{powder}}$ have an important effect on the viability loss of *L. lactis* just after drying. In the second part of our study, the influence of initial A_w and $T^{\circ}C_{\text{powder}}$ values on cell survival during storage was investigated. The dried *L. lactis* cells were placed in hermetically sealed bottles under ambient conditions for 60 d and a progressive reduction in the cell population was observed over time (Figure 5).

The A_w of the powders had no significant influence on the death rate of cells during storage, probably due to the fact that powders conserve low available water after drying (Figure 5A). According to Ying et al. (2016), water would be not available for degradative reactions or solubilization/component mobility in the formulation because the powders maintained low A_w values. This helps explain why the cell death rate remains constant even with A_w variations in the powders.

Our findings are corroborated by Farakos et al. (2013), who observed that *Salmonella* cells maintained

a constant death rate when stored in dairy powders with A_w between 0.16 and 0.26. By contrast, other studies involving pathogens and probiotics have demonstrated an increase in cell death rate with an A_w increase in the powders (Lian et al., 2015; Romano et al., 2018). In a study carried out by Vesterlund et al. (2012), it was also demonstrated that an increase in the A_w of a crushed flaxseed matrix resulted in the decrease of *Lactobacillus rhamnosus* GG viability during storage. The bacterium viability was rapidly lost in $A_w = 0.43$, whereas at $A_w = 0.22$ and $A_w = 0.11$ the cell viability reduced in 2.4 and 0.29 log cycles, respectively.

It should be noted that the cited works used a broader range of A_w values (from 0.11 to 0.75) than were tested in this study.

Considering that water availability in the interval is low, cell death can be explained in part by lipid oxidation when storage conditions were not kept free of oxygen and ambient light. By contrast, the higher the $T^{\circ}C_{\text{powder}}$ after drying, the higher the cell death rate during storage (Figure 5B). For example, when $T^{\circ}C_{\text{powder}} = 45^{\circ}C$, the cell population went down by 0.025 log cycles/d, whereas when $T^{\circ}C_{\text{powder}} = 60^{\circ}C$ the reduction was around 0.035 log cycles/d. According to Farakos et al. (2013), heating cells to temperatures above their maximum growth temperature causes damage to the cytoplasmic membrane and ribosome degradation, which can provoke injuries to the microorganisms. In these cases, rigorous thermal treatments probably made the strains more susceptible to cellular

component oxidation over time (van Niel et al., 2002; Santivarangkna et al., 2008).

After 60 d of storage, the cell population decreased from 1.32 to 2.15 log cycles depending on the initial temperatures of the powders (Figure 5B). In conditions where $T^{\circ}C_{\text{powder}} = 45^{\circ}\text{C}$, the viable cell concentration after 60 d was approximately 1.1×10^8 cfu/g, whereas a lower population ($\sim 7.0 \times 10^5$ cfu/g) was found when $T^{\circ}C_{\text{powder}} = 60^{\circ}\text{C}$ (Figure 5B). The viability of *L. lactis* in the powders after storage was still relatively low compared with a sample of commercially available freeze-dried cultures (10^{10} to 10^{11} cfu/g; Huang et al., 2016). Nevertheless, the results are promising when it is considered that a thermal-sensitive bacterium was tested and that the storage conditions were not optimized to guarantee high cell survival.

CONCLUSIONS

Drying heat-sensitive LAB remains a challenge in the dairy industry because of low cell survival rates after drying. Low cell survival occurs especially when elevated inlet air temperatures are used in the process. In this study, it was demonstrated that the survival rate of spray-dried cells is determined by powder parameters such as *Aw* and end-of-process temperatures. This approach has been used in studies involving pathogens in dried food matrices, but evaluating cell viability in drying procedures had not previously been investigated. Under the experimental conditions, the powder should reach an ideal *Aw* value of 0.198 and temperature of 52°C to guarantee maximal cell survival after drying. By means of mathematical tools, it was predicted that the inlet and outlet air temperatures should be set around 126 and 88.9°C , respectively, regardless of cell concentrate flow rate, to obtain the ideal *Aw* and $T^{\circ}C_{\text{powder}}$ parameters. In future research, the effect of drying conditions on the techno-functional properties of *L. lactis* will be evaluated in detail. In addition, other carrier materials and optimized storage conditions will be tested to improve the LAB viability during prolonged storage.

ACKNOWLEDGMENTS

The authors are grateful for the financial support provided by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES; financial code 001), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) to carry out this research.

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