Multifunctional Role of the Whey Culture Medium in the Spray-Drying Microencapsulation of Lactic Acid Bacteria

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SUMMARY

This study was conducted to evaluate the multifunctional role of whey culture medium (WCM) during the spray-drying microencapsulation of Lactobacillus fermentum K73. WCM containing growing microorganisms was used to hydrate different mixtures (gum arabic, maltodextrin, and whey). The use of these mixtures as carbon sources and their protective effects on simulated gastrointestinal conditions were evaluated. The optimal mixture was spray-dried while varying the outlet temperature and atomizing pressure using a response surface design. Under these conditions, microorganism survival, tolerated gastrointestinal conditions in vitro, physicochemical properties, morphometric features, and stability at 4°C, 25°C, and 37°C were evaluated. Lactobacillus fermentum K73 replicated in the carrier material. Bacterial change cycles were -1.97 ± 0.16 log CFU/g after the drying process and -0.61 ± 0.08 and -0.23 ± 0.00 log CFU/g after exposure of the capsules to simulated gastric pH and bile salt content, respectively. The physicochemical properties and morphometric features were within the normal ranges for a powder product. The powder was stable at a storage temperature of 4°C. The WCM with growing microorganisms was successfully spray-dried using the optimized drying conditions. This study demonstrates the use of WCM as a component of carrier material or as the carrier material itself, as well as its protective effects during drying, under simulated gastrointestinal conditions, and at varied storage temperatures.

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INTRODUCTION

Probiotics are defined by the World Health Organization as live microorganisms, which when administered in adequate amounts, confer health benefits (1). It is recommended that the probiotic strain be isolated from a targeted population, as the microorganism can adapt to the consumers' nutritional conditions and efficiently exert its health effects (2). *Lactobacillus fermentum* K73 is a strain isolated from Suero costeño, a fermented food in the Colombian Atlantic coast, and was characterized as a potential probiotic and hypocholesterolemic in in vitro studies (3). Therefore, including this microorganism in a food matrix has received interest for the development of functional foods for a specific targeted population.

One method of including the probiotic in a food matrix is microencapsulation (4). Microencapsulation is defined as the coating or entrapment of solid, liquid, or gaseous materials within another material (5). This process can be performed in four stages: i) biomass production (6), ii) mixture of the microorganism with carrier materials (7), iii) drying (8), and iv) inclusion in the food and/or shelf-life studies (9).

In the biomass production stage, the microorganism can be pre-adapted to thermal and osmotic stress conditions to tolerate the drying process (10). The microorganism and carrier material can be mixed using a homogenization processes or pre-heating to dissolve the carrier material (11). Whey proteins (12,13), maltodextrin (14), and gum arabic (15) have been used as carrier materials. Whey is a by-product of the dairy industry and is used as a component of culture medium (6) because it mainly contains lactose and soluble proteins (16). Although whey has important nutritional value, it is treated as dairy wastewater and has negative environmental effects (17). Therefore, using whey in an industrial process such as probiotic encapsulation at different levels (biomass production or carrier material) may provide a use for this dairy waste. In this study, whey culture medium with growing microorganisms was used as a carrier material for probiotic microencapsulation in one step. Maltodextrin is a maltooligosaccharide used to improve the survival of the probiotic after the spray-drying process with industrially acceptable results in terms of physicochemical properties such as dissolution, hygroscopicity, moisture content, and thermal properties (18). Gum arabic is an exudate gum from various Acacia species. It is a complex polysaccharide with a branched β-(1,3)-linked galactose backbone, which branches through the 1,6
positions, with arabinose, rhamnose, and uronic (19). It is used as a dietary fiber to enhance the growth of bifidobacteria in the gastric tract (20) and is a good carrier material that links proteins to protect lactic acid bacteria during thermal processes (15).

The drying stage involves technologies such as spray-drying (21). Spray-drying reduces time and production costs. Studies have shown that the use of spray drying for probiotic microencapsulation with isolated whey proteins can maintain longer cell viability during the shelf-life of the product (7), depending on the outlet temperature, residing time of the microorganism in the dryer chamber, and type of carrier material used (22).

The products obtained from the drying process are incorporated into the final product to ensure that the strains maintain their expected characteristics and number of viable cells (1 x 10^6 to 1 x 10^8 CFU/g daily consumed product) during production and storage (weeks to months) and at the specified storage temperature (freezing, refrigeration, room temperature) (23). Finally, probiotics must survive the physiological conditions of the gastrointestinal tract, including the stomach pH, enzymatic degradation, and presence of bile salts in the small intestine (24,25).

The aim of this study was to evaluate the multifunctional role of whey culture medium in a complete microencapsulation process that guarantees the tolerance to gastrointestinal conditions in vitro of L. fermentum K73 through spray-drying, and obtain a powdered product that can be included in a food matrix.

MATERIALS AND METHODS

Materials

Man, Rogosa, and Sharpe (MRS) broth and agar and peptone water were obtained from Scharlau Microbiology (Barcelona, Spain). The yeast extract was purchased from Oxoid Ltd. (Basingstoke, UK). Sweet whey was provided by a local company (Sopó, Colombia). The composition of the sweet whey (by mass) was as follows: protein 11.67 %, lipids 2.0 %, carbohydrates 51.64 %, and ashes 10.9 %. The maltodextrin and gum arabic were obtained from Shandong WNN Industrial Co., Ltd. (Shandong, China). Bile salt (B8756) was obtained from Sigma-Aldrich (St. Louis, MO, USA).

Bacterial strain and culture conditions

Lactobacillus fermentum K73 (Genbank KP784433.1) was stored at -80 °C with 20 % sterilized glycerol as the cryoprotectant in MRS broth (26). The microorganism was grown in MRS broth for 24 h at 37 °C under aerobic conditions before the experiment.
Biomass production was performed in a 1 L bioreactor (Bioflo 110, New Brunswick Scientific Co., Inc., Edison, NJ, USA) with a workload of 800 mL at 37 °C and agitation at 3354 ×g for 10 h. The culture medium contained 8 % sweet whey and 0.22 % yeast extract, and was adjusted to pH 5.5 and sterilized at 121 °C for 15 min. Lactobacillus fermentum K73 was inoculated at 10 % (by volume). Cells were counted following fermentation, as described below.

Preparation of the carrier material

All suspensions proposed by the model (Table 1) were prepared at a final concentration of 40 % soluble solids (27). The culture medium containing growing microorganisms (10 h of incubation) was used to hydrate each mixture proposed by the experimental design (Table 1). The hydrated mixture was homogenized using a magnetic stirrer for 30 min at 130 rpm. The initial cell count of the mixtures was measured. Next, the mixtures were incubated for 24 h at 37 ± 2 °C. After incubation, cells were counted, and the bacterial change cycles under simulated gastrointestinal conditions (acid pH and bile salts) were evaluated. All experiments were performed in triplicate. The final cell count after homogenization was 10.29 ± 0.18 log CFU/mL.

Experimental design to select the carrier material

The optimal mixture experimental design for obtaining the proportion of carrier material that improves bacterial survival under simulated gastrointestinal conditions was determined using Design Expert version 8.0.7.1 (Stat-Ease Inc., Minneapolis, MN, USA). The design examined 12 mixtures (runs), with mixtures 2, 3, 9, 10, and 15 using one repetition (runs 16, 8, 14, 13, and 17, respectively) (Table 1), and so, 17 total runs were evaluated. Maltodextrin, gum arabic, and sweet whey were selected as carrier materials (4,22,28). The bacterial change cycles after incubation (log CFU/mL) with the mixture proposed by the design and after simulated gastrointestinal conditions (log CFU/mL) were the response variables.

The selected optimal mixture enabled the microorganisms to tolerate the simulated gastrointestinal conditions and showed a desirability equal or close to 1 (29). The criterion of desirability is a general approach in which the value of each response variable is transformed to a measurement ranging from 0–1; values close to 1 represent maximization processes (d = 1), while values close to 0 represent minimization processes (d = 0) (29,30).
Drying process

For the experimental design of the drying process, two factors were considered: i) temperature of the outlet air, 90–110 ± 3°C, which was controlled by regulating the inlet flow, and ii) atomizing air pressure, 0.08–0.15 MPa. The inlet air temperature for each run varied between 190 and 200°C. The optimal mixture was a feed containing 40% total solids in the pilot-scale spray-dryer (GEA Process Engineering, Mobile Minor™, GEA Niro, Denmark) for drying. The equipment was operated with a pneumatic co-current two-fluid nozzle as atomizer system (diameter of 1 mm) with an evaporation capacity of 5 L/h. The response variables to be evaluated were: bacterial change cycles after the drying process (log CFU/g), bacterial change cycles (log CFU/g) after exposure of the capsules to simulated gastrointestinal conditions (acid pH and bile salts), physicochemical properties, and some morphometric features. The design included 12 runs with five replicates (run 1 = 16, run 4 = 9, run 7 = 13, and run 8 = 10 = 14) (Table 2). Analysis of variance (ANOVA), the adjusted determination coefficients, and contour plot generation were performed using Design Expert version 8.0.7.1 (29,30). The conditions that maximized the survival of *L. fermentum* K73 after drying and minimized the effects on cell viability after exposure to simulated gastrointestinal tract conditions, with a desirability equal or close to 1, were selected. The culture medium (CM) with growing microorganisms was dried under the optimized spray-drying conditions to evaluate if it could be used as carrier during the spray-drying process. Cells were counted before and after the drying process and the results are shown in Table 2.

Cell count

The cell count of *L. fermentum* K73 for all proposed experiments was determined by plate counting in MRS agar after culture at 37 ± 2°C for 24 h under aerobic conditions (31). Serial 1:9 dilutions in peptone water (0.1%) were prepared. To count encapsulated cells, the capsule was dissolved (28). First, 0.1 g of powder was added to 9.9 mL sterile phosphate buffer (0.1 M, pH 7.0) and then the capsule was hydrated for 15 min followed by vortexing for 10 min. The bacterial change cycles were determined as: Log (final [CFU / mL or g], after experiment) – Log (initial [CFU / mL or g], before the experiment). So, the bacterial change cycles could show positive or negative values.

Tolerance to simulated gastrointestinal conditions in vitro

The simulated gastrointestinal conditions were prepared using MRS broth with some modifications. The MRS broth was adjusted to pH 2.0 with 6 M HCl (32) to evaluate the tolerance to gastric pH. The resistance to bile salts was evaluated by supplementing the
media with 0.3 % bile salts (33,34). Both experiments were conducted by inoculating 100 µL of the culture medium or mixture or 0.1 g of powder in modified MRS broth for 2 h at 37 °C. The initial and final cell counts were considered to determine the cell viability and bacterial change cycles.

Physicochemical parameters

The powders obtained by spray-drying were characterized according to their water activity (\(a_w\)) (AquaLab®, Model Series 4, Decagon Devices, Inc., Pullman, WA, USA), moisture [\(w\) (moisture)/%] (35), solubility (s) (36,37), hygroscopicity [hygroscopicity/(g/100 g)] (36), and bulk density [\(\rho_b\)/(g/mL)] (38). All analyses were performed in triplicate.

Scanning electron microscopy (SEM)

The powder morphology from the culture medium powder and optimal conditions powder were observed by scanning electron microscopy (SEM, Phenom G2 Pro, Eindhoven, the Netherlands). Powders were dried in phosphorus pentoxide for 2 days and fixed on carbon tape (Ted 57, Ted Pella, Inc., Redding, CA, USA). SEM was conducted at an accelerating voltage of 10 kV at 500 X and 1500 X magnification.

Morphometric features

The morphometric features were determined using the SEM images at a 500 X and 1500 X magnification (39). The 500 X images were converted to grey scale with brightness values between 0 and 106 and saved as bitmap files (40). These files were then converted to binary images (black and white) using ImageJ 1.34 software (National Institutes of Health, Bethesda, MD, USA) (40). The tool “Analyze particles” from ImageJ software was used to calculate the particle area, and the mean diameter \([d/\mu m]\) of the particle was estimated using this value.

The shifting differential box-counting method (SDBC plugging for the ImageJ software) was used to determine the fractal dimension texture (FDt). The 1500 X magnification images were converted to bitmap files and cropped to obtain a square area of 22.86 × 18.29 µm. The FDt was calculated using this file (39).

Shelf-life

Culture medium and optimal conditions powders were packed into flexvac metallic bags (140 µm). The bags were vacuum-closed (0.0009 MPa. C200. Multivac, Barcelona,
Spain) and stored at 4 °C, 25 °C, and 37 °C for 36 days (41). Cell count, measured content, and water activity were determined every 5 days.

The plot of relative viability logarithmic (log $N_t/N_0$) versus storage time was fitted to a first order equation (Eq. 1) (42):

$$\log (N_t / N_0) = k_m t$$

where $N_t$ is the total viability of bacteria at time $t$ (days) during storage, $N_0$ is the viability of bacteria at time zero, and $k_m$ is the cellular viability loss specific rate (days$^{-1}$) at the three evaluated temperatures.

The Napierian logarithm from the moisture content ($w$ (moisture)/%) and water activity ($a_w$) values were plotted versus storage time. The values were fitted to a linear model (Eq. 2):

$$\ln (x or w) = A + k x or w t$$

where $\ln(x)$ is the Napierian logarithm of moisture content, $\ln(w)$ is the Napierian logarithm of water activity, $k_x$ is the constant of moisture content “change rate,” $k_w$ is the constant of water activity “change rate,” $A$ is the intercept of the curve and $t$ is time (days) during storage. The effect of storage temperature on $k_m$ was fit to the Arrhenius equation (Eq. 3) (43).

$$\ln (k) = \ln (k_m) – \left(\frac{E_a}{R} \right) \left[\frac{1}{T_{ref}}\right]$$

where $T_{ref}$ is the storage temperature, $E_a$ is the activation energy (J/mol), and $R$ is the universal gas constant (J/mol·K) and $k_m$ is the cellular viability loss specific rate (days$^{-1}$).

The values from Eq. 3 were used in Eq. 4 to predict the storage duration under the three study temperatures (43).

$$\text{Storage time} = \frac{\left[\ln (E_a) – \ln (x_{\text{CFU/g}})\right]}{\exp \left( A_a + k_y/T_{ref}\right)}$$

where $x_{\text{CFU/g}} = 1 \times 10^6$ CFU/g, is the amount of probiotic accepted in a functional food before ingestion by the final consumer (44), and $A_a$ is the intercept of the curve ln($k_m$) versus $\left(1/T_{ref}\right)$ ($R^2$ of culture medium powder curve = 0.965 and $R^2$ of optimal conditions powder curve = 0.981).

RESULTS AND DISCUSSION

The selection of the culture medium plays an important role in maintaining probiotics viability during the microencapsulation process (45). Therefore, we proposed using culture medium because it i) can be added to the food matrix (16), ii) contains the probiotic and is part of the carrier material (46), and iii) can be dried with the growing microorganism directly (47); therefore, the microorganism does not require treatment in downstream processes that increase the production costs and may affect the probiotic’s viability (48). Sweet whey culture medium has multifunctional roles during the microencapsulation process.
Selection of carrier material

One stage of probiotic microencapsulation is mixing of the microorganism with the carrier material (45). In this study, the microorganism grew on an average of $1.25 \pm 0.029 \log \text{CFU/mL}$ in all suspensions, and thus, the model was not significant ($p$-value = 0.1772, Table 1) for the response variable bacterial change cycles after the mixture process. This may be because lactose in the whey from the carrier material was used as a carbon source (6) and small peptides or amino acids in the medium are delivered to the culture medium as metabolizable substrates (49). Lion and Shah (2005) used maltodextrin in culture medium. They found that Lactobacillus casei ASCC 292 metabolized maltodextrin by fermentation, which enhanced the production of propionic acid (50). Thus, maltodextrin can be used as a carbon source for L. fermentum K73 in the carrier material. Using maltodextrin in the powder may have advantageous effects on taste according to in vivo assays. Olano-Martín et al. (2000) used a three-stage continuous culture cascade system that simulates different regions of the large intestine containing human gut microorganism. They reported that after incubation with maltodextrin as a carbohydrate source, the population of bifidobacteria and lactobacilli increased (51). Therefore, maltodextrin may have probiotic effects. Gum arabic is a dietary fiber that improves the growth of bifidobacteria (20,52) and E. faecium NCIMB 30183 (20), but does not have growth-stimulating effects on L. paracasei NFBC338 (19). In contrast to the results of Desmond et al. (2002), L. fermentum K73 grew for 2.28 cycles (run 15 and 17, Table 1). This suggests that the microorganism uses gum arabic and should be further examined.

Additionally, the proportions of maltodextrin, gum arabic, and sweet whey influenced the survival of L. fermentum K73 under simulated gastrointestinal conditions. The response variable bacterial change cycles after gastric pH conditions were fitted to a cubic mixture model, and the response of bacterial change cycles to the presence of bile salts was fitted to a quadratic model (Table 3).

The models were highly significant ($p < 0.0001$) with a non-significant lack of fit ($p = 0.2729$ and $p = 0.1283$, respectively). The regression coefficients and significance test results are shown in Table 3. The combination of components maltodextrin + gum arabic, maltodextrin + sweet whey, and the third-order interaction between the compounds had a significant effect ($p < 0.0001$) on the tolerance of the microorganism to an in vitro simulated gastric pH environment. Additionally, the interaction between maltodextrin + whey ($p = 0.0001$) and gum arabic + whey ($p = 0.0003$) influenced the survival of L. fermentum K73 in medium simulating the concentration of bile salts in the small intestine. The regression
equations of the model with the interactions between maltodextrin, gum arabic, and whey (C) are shown in Table 3.

Fig. 1 shows the simplex plots for the response variables evaluated under in vitro simulation of gastrointestinal conditions. Fig. 1a shows that the lowest difference between the final and initial cell count under gastric pH conditions was observed in the binary mixtures of maltodextrin + whey and maltodextrin + gum arabic. However, when analyzing the estimated coefficients of the three factors (Table 3), the results showed that the maltodextrin and sweet whey favored survival of the microorganism under gastric pH conditions. This effect was largely observed in estimating second-order effects found on the side of the simplex between vertices A–C. Fig. 1b shows, in apex A of the simplex, the strong and positive effect of maltodextrin and whey on the microorganism under bile salts conditions, which agrees with the coefficient analysis and regression coefficient shown in Table 3.

The model showed that the interactions between maltodextrin and sweet whey had a protective effect on the microorganism under gastrointestinal conditions (after incubation in carrier material and during spray-drying process). This can be attributed to the abilities of maltodextrin and whey lactose to bind to polar residues of proteins and maintain the integrity of cell membranes (53). However, the mixture reconstituted with culture medium, which contains denatured whey following the sterilization process, exposes a higher number of functional groups that interact with the non-denatured whey and maltodextrin, forming a strong crosslink between the compounds. Its materials also interact with cell membrane components, generating a viscous layer on the microorganism and reducing the mobility of water across the membrane. This mixture protects the phospholipid of the cell membrane during spray-drying (9) and prevents intracellular acidification by HCl and colic acid, which are produced by the hydrolysis of sodium taurocholate and sodium glycolate, and thus maintains the viability of microorganisms in the carrier material (41,54).

Desirability analysis determined that -0.19 log CFU/mL indicated the bacterial change cycles decrease at gastric pH; in the presence of bile salts, the increase in the cell count was 0.02 log CFU/mL, with corresponding values of 0.61 (maltodextrin): 0.39 (whey) for a desirability of 0.97. The results were corroborated experimentally using the optimal mixture. The decrease in population at gastric pH was by -0.12 ± 0.04 log CFU/mL, and the increase in population in the presence of bile salts was by 0.15 ± 0.05 log CFU/mL. These results largely agree with those of the model predictions. Thus, this mixture was used in the spray-drying experiments.
Drying process

After selecting the carrier material, the spray-drying process using the optimal mixture was conducted. The RSM design was performed to determine the drying conditions (outlet temperature and atomizing pressure) that improved the protective effect of the selected mixture.

ANOVA of the experimental design for the bacterial change cycles after the drying process showed significant results with $p < 0.0001$ and a determination coefficient of 0.95 ($R^2$). The linear effect of outlet temperature, quadratic effect of the outlet temperature, and quadratic effect of atomizing pressure were significant within the model ($p \leq 0.05$).

Fig. 2 shows the effects of outlet temperature and atomizing pressure on the capacity of the microorganism to tolerate the drying process and their influence on probiotic properties. Fig. 2a shows that viability decreased as the outlet temperature was increased and atomizing pressure was decreased. As outlet temperature increased, the microorganism viability decreased, which was related to cell damage caused by DNA and RNA denaturation, ribosome damage, cytoplasm dehydration, and cell membrane damage. This is related to the speed at which heat and mass transfer occur from the inside to the outside of the cell (36,45,55). This may explain the highest linear effect ($F$ - value = 149.04) of the outlet temperature on the viability of *L. fermentum* K73 after drying. Furthermore, the low pressure decreased cell viability, possibly because the atomizing pressure directly affected capsule flexibility, causing more resistance of the carrier material mixture to the migration of water, both in the cell and the capsule itself (56).

The results showed that under all drying conditions, the cell count decreased when the powder was exposed to simulated gastrointestinal conditions. The bacterial change cycles under gastric pH and bile salt conditions were fitted to cubic models with $p$-values of 0.013 and 0.0008 and determination coefficients ($R^2$) of 0.92 and 0.97, respectively. In both models, the quadratic effect of atomizing pressure ($F$ - value\textsubscript{gastric pH conditions} = 26.4, $F$ - value\textsubscript{bile salt conditions} = 37.6) was the most influential value on the capacity to tolerate gastrointestinal conditions, followed by the quadratic effect of outlet temperature ($F$ - value\textsubscript{gastric pH conditions} = 20.66, $F$ - value\textsubscript{bile salt conditions} = 29.52). The three types of interaction between the outlet temperature and atomizing pressure influenced the tolerance to gastric pH. In contrast, the outlet temperature and its interaction with the quadratic effect of atomizing pressure influenced the capacity of *L. fermentum* K73 to tolerate bile salts.

The contour diagram in Fig. 2b shows that at an atomizing pressure between 0.13 and 0.15 MPa and outlet temperature close to 90 °C, the tolerance of the microorganism to gastric pH was favored. In contrast, the contour diagram in Fig. 2c shows that a pressure
between 0.08 and 0.12 MPa and outlet temperature of 110 °C favored the ability of the probiotic to tolerate bile salts. This agrees with the coefficients and magnitudes expressed in the regression equations in Table 4. Therefore, after the drying process, the capsule protects the microorganism from the gastrointestinal conditions. The protection may be attributed to physicochemical properties, such as crosslinking between whey proteins and maltodextrin. The increased outlet temperature and atomizing pressure result in the formation of a compact powder, and thus, the microcapsule membrane does not facilitate diffusion of the substances such as HCl or colic acid into the microcapsule, with the microcapsule acting as a physical barrier. Therefore, these conditions enhanced the survival of the microorganism under simulated gastrointestinal conditions, although the outlet temperature of 110 °C injured the cell membrane, as described above.

The optimal conditions for spray-drying were an atomizing pressure of 0.117 MPa, outlet temperature of 90.8 °C, and inlet temperature of 175 °C, with a desirability of 0.999. The experimental data validated the model and showed a decrease of \(-1.97 \pm 0.16 \log \text{CFU/g}\) after the drying process or \(8.31 - 0.61 \pm 0.08 \log \text{CFU/g}\) after exposure to \textit{in vitro} conditions of gastric pH, and decrease of \(-0.23 \pm 0.00 \log \text{CFU/g}\) after the addition of bile salts.

The optimal drying conditions were used to dry the culture medium and examine if the culture medium could act as a carrier material. Table 2 shows the bacterial changes after the drying process and after the exposure of the microcapsules to simulated gastrointestinal conditions \textit{in vitro} when the culture medium was used as the carrier material. Compared with the optimal conditions powder, the culture medium powder showed the highest viability after the drying process \((-0.96 \pm 0.04 \log \text{CFU/g})\) and under simulated gastrointestinal conditions (gastric pH: \(-0.587 \pm 0.08 \log \text{CFU/g})\), bile salts: \(-0.674 \pm 0.043 \log \text{CFU/g}\)). The low content solids (8 %) from the culture medium form a feed liquid with low viscosity; thus, when beginning the spray process in the drier, the retention time may be increased and microbiorganism exposed to a higher temperature in the drying chamber (57). However, this common interpretation contrasts the results of our study. The most likely explanation of the successful use of the culture medium as a carrier are as follows: i) the hydrophobic groups and sulfhydryl groups are exposed because whey proteins from the culture medium are denatured by sterilization, and these groups begin irreversible aggregation (58), and thus ii) the culture medium is a heterogeneous feed liquid because agglomerates have dense and irregular structures (Fig. 4) (59). When the culture medium containing microorganisms was sprayed in the drier, iii) the inlet and outlet temperature and atomizing pressure removed the water from the feed liquid (culture medium) and compacted the denatured whey aggregates,
giving the microcapsules a rigid surface (60); however, the temperature in the drying chamber was not sufficient to evaporate the water (92 % (wet basis)) and the core of the microcapsules had a high moisture content (12.69 ± 0.20 % (wet basis), Table 2). This high moisture content of >10 % (wet basis) was also reported by Çabuk and Harsa (2015), with high-survival rates of L. acidophilus NRRL B-4495, and they concluded that it is not possible to establish a correlation only based on the moisture content (61). However, iv) the capsules with high moisture content may create a micro-environment in which the microorganism can repair the damage to the cell membrane after spray-drying. This hypothesis requires further analysis.

Additionally, the whey proteins have a buffering effect at gastric pH to improve microorganism survival (62). This may cause low bacterial change cycles at acidic pH.

**Final product**

The powder obtained from each drying treatment, the optimal conditions and culture medium powder (Table 2), were characterized based on the a₃, humidity, solubility, hygroscopicity, and bulk density. The a₃ and moisture content were 0.09–0.19 % and 0.66–1.01 %, respectively (Table 2). The values of a₃ were adjusted to a linear model, with a p-value < 0.0001 and determination coefficient of 0.81 (R²) (Table 5). The outlet temperature showed a statistically significant effect (p < 0.0001) on a₃. The moisture content was adjusted to a cubic model with a p-value of 0.0043 and R² of 0.94 (Table 5). The outlet temperature and cubic effect of the outlet temperature had the greatest influence on the moisture content in the microcapsules. Therefore, the higher outlet temperature (110 °C) and inlet temperature (200 °C) produced a powder with lower moisture content, compared with that produced under the other temperatures evaluated, thereby suggesting temperature to be the most important factor (14), which agrees with the results of the contour plot in Fig. 3. Figs. 3a and 3b show that the lowest values of a₃ and moisture content were observed at 110 °C, which is consistent with the coefficients shown in the linear equation and regression equation (Table 5).

The solubility measurement of the powders was adjusted to a quadratic model (Table 5), as shown by ANOVA, with a p = 0.003 and R² = 0.80. The outlet temperature and its quadratic effect influenced the ability to reconstitute the powder. In accordance with the regression coefficients, the longest time to solubilize the sample occurred at temperatures close to 90°C (Fig. 3c).

The hygroscopicity values were adjusted to a cubic model (p = 0.0008) with an R² of 0.97 (Table 5). The first-order interaction between the outlet temperature and atomizing
pressure, quadratic effect of the outlet temperature and atomizing pressure, second-order interaction between the outlet temperature and atomizing pressure squared, and cubic effect of both factors were significant in the model. **Fig. 3d** shows that at temperatures of 105–110 °C, the hygroscopicity of the capsules was increased. The optimal spray-drying was 15.6 ± 0.50 g/100 g.

The data obtained for bulk density were adjusted to a cubic model with \( p = 0.0050 \) and \( p < 0.0001 \) and \( R^2 \) values of 0.94 and 0.99, respectively. For both response variables, the quadratic and cubic effects of the outlet temperature showed significant effects \( (p \leq 0.05) \) (**Table 5**). The bulk density results showed that higher outlet and inlet temperatures decreased the density of powders (**Fig. 3d**) because of more rapid drying of the particle. This fixed the particle dimensions before most of the water content had been evaporated, which agrees with the results of a previous study (63).

The final product from the spray-drying optimal conditions, referred to as SDOC, was characterized according to the physicochemical properties. In this study, we obtained an optimal product for the food industry. The moisture content and \( a_w \) were < 4 % and < 0.2 %, respectively. The isosmotic conditions generated between the wall and cell membrane and the water monolayer that maintains the shape and structure of enzymes and proteins are maintained (64). In contrast, a low moisture content and \( a_w \) prevent the growth of the accompanying microorganisms in the powder, thereby extending its lifetime (65).

The solubility time in water, including the time of 126.99 ± 5.92 s obtained from the dried powder under optimal conditions, was comparable to those reported by Fritzen-Freire et al. (2013) and Pinto et al. (2015), who suggested that during spray-drying, the hydrophilic groups in lactose and whey proteins are more exposed. Moreover, the absence of fat on the surfaces of the particles makes the powder more hydrophilic, causing its water solubility to increase (36,37).

The hygroscopicity of the samples, including the microcapsules obtained by the optimal spray-drying, 15.6 ± 0.50 g/100g, was comparable to those of microcapsules containing 20–30 % of maltodextrin as reported by Tonon et al. (2008). The previous study attributed this property to the high concentration of maltodextrin, which has low hygroscopicity, confirming its effectiveness for use as a carrier material (66).

The physicochemical properties of the culture medium powder were compared with those of the SDOC powder. The solubility time of the culture medium powder (166.66 ± 29.2 s) was higher than that of the SDOC powder (126.99 ± 5.92 s). This can be explained by the hydrophobic properties of the denatured whey proteins, which decrease microparticle solubility. The difference between the hygroscopicity of culture medium powder and SDOC
powder (36.57 ± 0.05 g/100g and 37.65 ± 0.13 g/100g, respectively) was low. The hygroscopicity value of the culture medium powder is attributed to the greater number of hydrophobic amino acid residues from the denatured whey proteins, which affect water adsorption (37). The bulk density of the SDOC was 0.55 ± 0.00 g/mL, whereas that of the culture medium powder was 0.29 ± 0.02 g/mL. Felix et al. (2016) reported that adding maltodextrin to the carrier material increased the final bulk density (14).

Finally, the microencapsulation process proposed in this study was performed using pilot-scale equipment. Performing this type of study using robust equipment would enable the development of microencapsulation processes easily adaptable to the food industry, without the need for large changes in the drying technology, preparation of the carrier material, and preparation of the biomass acquisition process.

**Morphometric features**

ANOVA revealed no significant differences among the diameter and FDt values ($p = 0.76$ and $p = 0.39$, respectively) (Table 2). The powder from all drying conditions and SDOC powder showed a spherical shape with few fractures and concavities on the surface (Fig. 4a), with a smoother surface than the culture medium powder. The culture medium powder presented as rougher particles (Fig. 4b). Adding maltodextrin decreases the roughness because the maltodextrin molecules join with denatured whey proteins, creating stronger steric hindrance against protein aggregation (67) and producing particles with a smoother surface, as observed in this study.

The diameter values from the designed experiment powder, SDOC powder, and culture medium powder were < 13.67 ± 0.3 μm (Table 2). In industrial production, capsule sizes below 100 μm prevent gritty or sandy undesirable textural properties in the food product (61). Therefore, the powder product obtained in this study had the correct size for use in functional foods.

**Shelf-life**

SDOC powder and culture medium powder were evaluated at 4 °C, 25 °C, and 37 °C for 36 days to determine the cell count, moisture content, and water activity. Fig. 5a shows L. fermentum K73 behaviors at 4 °C, 25 °C, and 37 °C spray-dried with and without carrier material. The results showed that $k_m$, $k_x$, and $k_w$ increased with increasing storage temperature and with the use of maltodextrin:sweet whey (0.61:0.39) (Table 6). The storage of powders showed the highest viability losses at 37°C (Fig. 5a), while lower $k_m$ occurred at 4°C (Table 6).
The behaviors of the microorganisms dried using culture medium as the carrier material were similar at 4 °C and 25 °C (Fig. 5a) and showed similar $k_m$ values (Table 6), although, the $k_{m25^\circ C}$ -0.04 days$^{-1}$ was lower than $k_{m4^\circ C}$ -0.04 days$^{-1}$. Additionally, the prediction time (Table 6) showed that the viability of microorganisms was better at 25 °C (57.51 days) than at 4 °C (54.89 days). The observed increase in viability at 25 °C may have been because the moisture content was high (12.69 ± 0.20 %) in the culture medium capsules but stable during storage (Fig. 5b). This stability may help maintain *L. fermentum* K73 viability at 25 °C, as described above. The high moisture content may help microorganisms repair the injured areas on the cell membrane.

In contrast, the $k_m$ values of the viability loss kinetics (Fig. 5a) of *L. fermentum* K73 with carrier material increased with storage temperature (Table 6). The moisture content of SDOC powder was low (<8.2 ± 0.49 (wet basis)) and constant at all temperatures evaluated, while the $a_w$ values were high (<0.37 ± 0.002). Therefore, the viability loss and short days (23 days at 4°C, Table 6) may be attributed at the $a_w$ values. It has been suggested that $a_w$ values greater than 0.25 considerably increase the $k_m$ of lactic acid bacteria, such as *L. fermentum* K73, likely by stimulating its metabolism that is associated with high molecular mobility in the carrier material (4).

Shelf-life results showed that using culture medium as carrier material protected *L. fermentum* K73 not only from the spray-drying conditions, but also from the storage temperatures. The best conditions were at 25 °C, followed by those at 4 °C. The moisture content may determine the viability of *L. fermentum* K73, but these data must be interpreted with caution. This may occur because of the repair of the damage to the cell membrane at high moisture content after drying, but further studies are needed to confirm this.

**CONCLUSION**

The multifunctional roles of whey culture medium in the spray-drying microencapsulation process for *L. fermentum* K73 were evaluated. The experimental design of the mixtures showed that the carrier materials are a source of substrate for the bacilli, as well as protect against in vitro simulated gastric conditions. The use of culture medium as part of the mixtures improved the physicochemical properties of the powders and increased the survival of the microorganisms under the evaluated conditions by decreasing the downstream processes. Complementarily, the optimal mixture of maltodextrin and sweet whey, spray-dried at an outlet temperature of 90.79 °C and atomizing pressure of 0.117 MPa, maintained microorganism viability after the drying process and allowed microorganisms to survive under in vitro gastrointestinal conditions. Because the protein-carbohydrate complex maintained its
glass state on the microorganism membrane, exerting resistance to water migration, there were no large lesions on the cell membrane of *L. fermentum* K73. The microcapsules produced under different drying conditions were evaluated according to their physicochemical properties (*a*<sub>w</sub>, moisture content, solubility, hygroscopicity, and bulk density) and suitability for inclusion in a food matrix. Therefore, future studies can be conducted to develop products that not only meet the regulatory cellular concentrations but also exert effects on consumer health.

Shelf-life analysis revealed that the drying and storage temperatures are critical factors in bacterial survival. The use of culture medium as a carrier material improved the viability of *L. fermentum* K73 at 4 °C, and thus, the probiotic product can be stored at refrigeration temperature for 87.44 days.

Our results showed that the culture medium is an effective carrier material because the microorganism survived after the drying process and under simulated gastrointestinal conditions, and the powder was stable during the storage duration at 4 °C and 25 °C. The size and some physicochemical features agree with the industry requirements. Thus, our results can be used to develop additional one-step microencapsulation processes that optimize time and cost.

ACKNOWLEDGEMENTS
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https://doi.org/10.1016/j.jfoodeng.2013.01.028.


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Table 1. Optimization mix design to select the carrier material

<table>
<thead>
<tr>
<th>Run</th>
<th>Maltodextrin</th>
<th>Gum</th>
<th>Whey</th>
<th>After mixture process</th>
<th>Under gastric pH conditions</th>
<th>Under bile salt conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5</td>
<td>0.5</td>
<td>0</td>
<td>2.25 ± 0.01</td>
<td>-0.25 ± 0.02</td>
<td>0.16 ± 0.05</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1.21 ± 0.02</td>
<td>-1.31 ± 0.02</td>
<td>0.92 ± 0.01</td>
</tr>
<tr>
<td>3</td>
<td>0.42</td>
<td>0.5</td>
<td>0.5</td>
<td>2.29 ± 0.02</td>
<td>-0.98 ± 0.08</td>
<td>1.09 ± 0.12</td>
</tr>
<tr>
<td>4</td>
<td>0.17</td>
<td>0.67</td>
<td>0.17</td>
<td>0.39 ± 0.09</td>
<td>-0.59 ± 0.10</td>
<td>0.35 ± 0.03</td>
</tr>
<tr>
<td>5</td>
<td>0.67</td>
<td>0.17</td>
<td>0.17</td>
<td>1.58 ± 0.11</td>
<td>-0.56 ± 0.04</td>
<td>0.14 ± 0.04</td>
</tr>
<tr>
<td>6</td>
<td>0.5</td>
<td>0.5</td>
<td>0</td>
<td>2.15 ± 0.01</td>
<td>-0.93 ± 0.07</td>
<td>1.08 ± 0.08</td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0.5</td>
<td>0.43 ± 0.06</td>
<td>-0.16 ± 0.02</td>
<td>0.02 ± 0.00</td>
</tr>
<tr>
<td>10</td>
<td>0.42</td>
<td>0.17</td>
<td>0.42</td>
<td>0.47 ± 0.06</td>
<td>-0.64 ± 0.12</td>
<td>0.31 ± 0.01</td>
</tr>
<tr>
<td>12</td>
<td>0.33</td>
<td>0.33</td>
<td>0.33</td>
<td>0.59 ± 0.03</td>
<td>-0.68 ± 0.06</td>
<td>0.22 ± 0.06</td>
</tr>
<tr>
<td>13</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2.71 ± 0.14</td>
<td>-1.05 ± 0.04</td>
<td>-0.02 ± 0.00</td>
</tr>
<tr>
<td>14</td>
<td>0.5</td>
<td>0</td>
<td>0.5</td>
<td>0.48 ± 0.01</td>
<td>-0.16 ± 0.02</td>
<td>0.08 ± 0.00</td>
</tr>
<tr>
<td>15</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2.28 ± 0.17</td>
<td>-0.67 ± 0.04</td>
<td>0.58 ± 0.02</td>
</tr>
<tr>
<td>16</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1.18 ± 0.07</td>
<td>-1.25 ± 0.03</td>
<td>0.91 ± 0.01</td>
</tr>
<tr>
<td>17</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2.29 ± 0.05</td>
<td>-0.72 ± 0.01</td>
<td>0.42 ± 0.04</td>
</tr>
</tbody>
</table>

*p-value model* 0.1772 <0.0001 <0.0001

Results expressed as mean ± standard deviation
Table 2. Response surface methodology design to evaluate the effects of process conditions on bacterial change cycles, physicochemical properties, and morphometric features.

<table>
<thead>
<tr>
<th>Run</th>
<th>Factors</th>
<th>Response variables</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Outlet temperature/ ºC</td>
<td>After spray drying</td>
</tr>
<tr>
<td></td>
<td>ρ/MPa</td>
<td>N/(log CFU/g)</td>
</tr>
<tr>
<td>1</td>
<td>96</td>
<td>0.15</td>
</tr>
<tr>
<td>2</td>
<td>110</td>
<td>0.08</td>
</tr>
<tr>
<td>3</td>
<td>110</td>
<td>0.15</td>
</tr>
<tr>
<td>4</td>
<td>110</td>
<td>0.116</td>
</tr>
<tr>
<td>5</td>
<td>93</td>
<td>0.106</td>
</tr>
<tr>
<td>6</td>
<td>96.7</td>
<td>0.084</td>
</tr>
<tr>
<td>7</td>
<td>90</td>
<td>0.128</td>
</tr>
<tr>
<td>8</td>
<td>101.29</td>
<td>0.11</td>
</tr>
<tr>
<td>9</td>
<td>110</td>
<td>0.116</td>
</tr>
<tr>
<td>10</td>
<td>101.29</td>
<td>0.11</td>
</tr>
<tr>
<td>11</td>
<td>90</td>
<td>0.08</td>
</tr>
<tr>
<td>12</td>
<td>103</td>
<td>0.137</td>
</tr>
<tr>
<td>13</td>
<td>90</td>
<td>0.128</td>
</tr>
<tr>
<td>14</td>
<td>101.29</td>
<td>0.11</td>
</tr>
<tr>
<td>15</td>
<td>103.4</td>
<td>0.087</td>
</tr>
<tr>
<td>-----</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>16</td>
<td>96</td>
<td>0.15</td>
</tr>
</tbody>
</table>

| p value model | <0.0001 | 0.013 | 0.0008 | <0.0001 | 0.0043 | 0.003 | 0.0008 | 0.005 | 0.761 | 0.399 |

Optimal conditions

| Culture medium | 90.79 | 0.117 | -1.97 ± 0.16 | -0.61 ± 0.08 | -0.23 ± 0.00 | 0.065 ± 0.001 | 4.43 ± 0.19 | 126.99 ± 5.92 | 36.57 ± 0.05 | 0.55 ± 0.00 | 4.619 ± 0.69 | 2.54 ± 0.01 |

Results expressed as mean ± standard deviation. FDt = Fractal dimension texture.
Table 3. Analysis of variance (ANOVA) of mix design and regression equations for bacterial change cycles under gastric pH and bile salt conditions

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of squares</th>
<th>Df</th>
<th>Mean Square</th>
<th>F-Value</th>
<th>p-value</th>
<th>Sum of squares</th>
<th>Df</th>
<th>Mean Square</th>
<th>F-Value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>1.86</td>
<td>6</td>
<td>0.31</td>
<td>246.54</td>
<td>&lt;0.0001*</td>
<td>2.36</td>
<td>5</td>
<td>0.47</td>
<td>60.03</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Linear Mixture</td>
<td>0.27</td>
<td>2</td>
<td>0.14</td>
<td>109.09</td>
<td>&lt;0.0001*</td>
<td>1.86</td>
<td>2</td>
<td>0.93</td>
<td>118.13</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>AB</td>
<td>0.34</td>
<td>1</td>
<td>0.34</td>
<td>272.17</td>
<td>&lt;0.0001*</td>
<td>0.01</td>
<td>1</td>
<td>0.01</td>
<td>0.90</td>
<td>0.36</td>
</tr>
<tr>
<td>AC</td>
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<td>1</td>
<td>1.35</td>
<td>1075.74</td>
<td>&lt;0.0001*</td>
<td>0.26</td>
<td>1</td>
<td>0.26</td>
<td>33.19</td>
<td>0.0001*</td>
</tr>
<tr>
<td>BC</td>
<td>0.00</td>
<td>1</td>
<td>0.00</td>
<td>1.07</td>
<td>0.3245</td>
<td>0.21</td>
<td>1</td>
<td>0.21</td>
<td>26.60</td>
<td>0.0003*</td>
</tr>
<tr>
<td>ABC</td>
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<td>1</td>
<td>0.24</td>
<td>186.77</td>
<td>&lt;0.0001*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residual</td>
<td>0.01</td>
<td>10</td>
<td>0.00</td>
<td></td>
<td></td>
<td>0.09</td>
<td>11</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lack of Fit</td>
<td>0.01</td>
<td>5.00</td>
<td>0.00</td>
<td>1.77</td>
<td>0.27</td>
<td>0.07</td>
<td>6.00</td>
<td>0.01</td>
<td>2.94</td>
<td>0.13</td>
</tr>
<tr>
<td>Pure Error</td>
<td>0.00</td>
<td>5.00</td>
<td>0.00</td>
<td></td>
<td></td>
<td>0.02</td>
<td>5.00</td>
<td>0.00</td>
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<td></td>
</tr>
<tr>
<td>Cor Total</td>
<td>1.88</td>
<td>16.00</td>
<td></td>
<td>2.44</td>
<td>16.00</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>R²</td>
<td>0.99</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.96</td>
</tr>
</tbody>
</table>

Final equation in terms of real components

- **Bacterial change cycles in gastric pH conditions**
  \[
  \text{N/(log CFU/mL)} = 1.052A + 0.692B + 1.282C - 2.493AB - 3.965AC + 12.521ABC
  \]

- **Bacterial change cycles in bile salt conditions**
  \[
  \text{N/(log CFU/mL)} = 0.006A + 0.504B + 0.935C - 1.589AC + 1.484BC
  \]

A = Maltodextrin; B = Gum arabic; C = Whey. * Significant at an alpha level of 0.05
Table 4. Analysis of variance (ANOVA) of the response surface model and regression equations for evaluating effects of spray-drying process on the viability and probiotic properties of *L. fermentum* K73.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Bacterial change cycles after spray drying</th>
<th>Bacterial change cycles after gastric pH conditions</th>
<th>Bacterial change cycles after bile salt conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>N/(log CFU/g)</em></td>
<td><em>N/(log CFU/g)</em></td>
<td><em>N/(log CFU/g)</em></td>
</tr>
<tr>
<td></td>
<td>Sum of squares</td>
<td>Df</td>
<td>Mean Square</td>
</tr>
<tr>
<td>Model</td>
<td>Quadratic</td>
<td>Cubic</td>
<td>Cubic</td>
</tr>
<tr>
<td>A</td>
<td>4785.13</td>
<td>5</td>
<td>957.03</td>
</tr>
<tr>
<td>B</td>
<td>3825.34</td>
<td>1</td>
<td>3825.34</td>
</tr>
<tr>
<td>AB</td>
<td>1.00</td>
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<td>A²</td>
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<tr>
<td>B²</td>
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<tr>
<td>A²B</td>
<td>247.07</td>
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<td>247.07</td>
</tr>
<tr>
<td>A³</td>
<td>0.37</td>
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</tr>
<tr>
<td>AB²</td>
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</tr>
<tr>
<td>A³B</td>
<td>0.822</td>
<td>1</td>
<td>0.822</td>
</tr>
<tr>
<td>B³</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Residual</td>
<td>256.66</td>
<td>10</td>
<td>25.67</td>
</tr>
<tr>
<td>Lack of Fit</td>
<td>177.47</td>
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<td>35.50</td>
</tr>
<tr>
<td>Pure Error</td>
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<td>15.84</td>
</tr>
<tr>
<td>Cor Total</td>
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<td>15</td>
<td></td>
</tr>
<tr>
<td>R²</td>
<td>0.949</td>
<td></td>
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</tr>
<tr>
<td>Final equation in terms of coded factors</td>
<td><em>Bacterial change cycles after spray drying N/(log CFU/g) = -2.835 - 1.962 * A - 1.057 * A² - 0.698 * B²</em></td>
<td><em>Bacterial change cycles in gastric pH conditions after spray drying N/(log CFU/g) = -1.01 - 0.34 * AB + 0.69 * A² + 0.70 * B² + 0.72 * A²B - 0.61 * AB²</em></td>
<td><em>Bacterial change cycles bile salt conditions after spray drying N/(log CFU/g) = -0.54 + 0.29 * A² + 0.29 * B² - 0.28 * AB²</em></td>
</tr>
</tbody>
</table>

*A = Outlet temperature; B = Pressure. * Significant at an alpha level of 0.05.*
Table 5. ANOVA of the surface response model and regression equations for physicochemical properties

<table>
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<tr>
<th>Factor</th>
<th>Model type</th>
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<td>Mean Square</td>
<td>F-value</td>
<td>p-value</td>
<td>Sum of squares</td>
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<td>Model</td>
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<td>55.97</td>
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<tr>
<td>temperature</td>
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<td></td>
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</tr>
<tr>
<td>Pressure</td>
<td>(B)</td>
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<td>0.00</td>
<td>0.34</td>
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<td>0.94</td>
<td>0.80</td>
<td>0.97</td>
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Final Equation in Coded Factors:

\[ a_x = 0.137 - 0.044A + 0.646 - 0.843A + 0.664A^2 \]


Hygroscopicity/(g/100g) = 33.372 + 10.144A + 14.666B + 5.189A + 5.466A^2 + 4.952B^2

ρb/(g/mL) = 0.327 - 0.165A + 0.090B - 0.067A^2 - 0.089A^2B + 0.158 A^2 - 0.148B^2
Table 6. Predicting shelf life time and specific rate of: bacterial survival, change in % moisture content, and water activity

<table>
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<th>Storage temperature/ Culture medium-powder</th>
<th>Optimal conditions-powder</th>
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<tr>
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<td>Survival bacteria</td>
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<td>°C</td>
<td>k_m (R^2)</td>
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<tr>
<td>25°C</td>
<td>-0.039 0.84</td>
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<tr>
<td>37°C</td>
<td>-1.19 0.98</td>
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</table>

k_m = Specific loss viability rate (days⁻¹); k_x = Specific change rate in % moisture content (days⁻¹); k_w = Specific change rate in water activity (days⁻¹); Pd = Predicted days; R² = R-squared.

Fig. 1. Simplex for bacterial change cycles under gastric pH conditions (a) and bile salt conditions (b) for L. fermentum K73.

Fig. 2. Contour plots of bacterial change cycles after: spray drying (a), gastric pH conditions after spray drying (b), and bile salt conditions after spray drying (c).

Fig. 3. Contour plots of a_w (a), w(moisture content)% (b), solubility/s (c), hygroscopicity/(g/100 g) (d), and ρ_b/(g/mL) (e).

Fig. 4. Micrographs from optimal conditions (a) and culture medium (b) spray-dried powder. The magnification used was 1500X.

Fig. 5. Effect of the cryoprotective matrices on log (N_t/N_0) (a), moisture content (b) and a_w (c) at 4°C (diamond figure), 25°C (square figure), and 37°C (triangle figure). The cryoprotective matrices were culture medium (CM) (white figures) and maltodextrin-whey (MD-W) (black figures). The error barres corresponded to the mean values of the triplicate measurements ± SD.
Fig. 1

a) After gastric pH conditions

b) After bile salt conditions

X1= A. Maltodextrin
X2= B. Arabic gum
X3= C. Whey
Fig. 2
Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

Fig. 5