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Effect of the carrier material, drying technology and dissolution media on the viability of *Lactobacillus fermentum* K73 during simulated gastrointestinal transit

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ABSTRACT

The goal of this study was to determine the effect of the carrier material, drying technology and dissolution media during the passage of *L. fermentum* K73 through a dynamic in vitro digestion system (IViDiS). The carrier materials were (i) culture medium with growing micro-organisms and (ii) culture medium with maltodextrin:sweet whey [0.6:0.4]. The carrier materials were dried by spray-drying and freeze-drying to obtain four types of powders. The dissolution media consisted of water and 1% fat milk. The powders were tested using an in vitro dynamic digestion system (IViDiS). The results showed that powders derived from culture

medium had the highest protective effect on the viability of *L. fermentum* K73 in both dissolution media and that survival increased when the powders were tested in milk. The modified Gompertz model was used to model *L. fermentum* K73 behaviour during the digestion process. The model showed that cells entrapped in culture medium had the longest lag phase and the slowest inactivation rate when evaluated in milk.

Keywords: Food matrix, *Lactobacillus fermentum* K73, probiotic, simulated gastrointestinal system, sweet whey.

1. Introduction

Probiotics are defined as “live micro-organisms which when administered in adequate amounts confer a health benefit on the host.”¹ However, probiotics are sensitive to manufacturing processes such as water activity, pH, oxygen, temperature, hydrostatic pressures and peroxidation^{2,3}, as well as to the physiological conditions of the gastrointestinal tract, including stomach pH and hydrolytic enzyme degradation by α -amylase, pepsin, pancreatin and bile salts.⁴ Therefore, microencapsulation in combination with adequate carrier material can be considered as an alternative approach for improving the survival of probiotics as they travel to their target destination.⁵ *Lactobacillus fermentum* K73 is a probiotic strain isolated from *suero costeño*, a typical fermented dairy food from the Atlantic coast of Colombia. This lactobacillus strain has shown a hypocholesterolemic effect, as it adsorbs cholesterol on its cell membrane, and it has very high bile salt hydrolase activity.⁶ Given its potential health benefits, this strain could be included in functional foods.

Microencapsulation was originally used in the food industry as a way to protect bioactive compounds during food processing and ultimately attain several objectives such as widening the storage temperature range, extending shelf life and providing protection against the gastrointestinal environment.⁷ Microencapsulation was later used for probiotic protection. Drying processes are used for probiotic strains, since the resulting products are physic-chemically stable, simpler to store and inexpensive to transport.⁸ Two widely used techniques are freeze-drying and spray-drying.^{9–12} Freeze-drying has often been used as a conservation technology, but its use as an encapsulation method for probiotics is a relatively innovative concept.^{13,14} In contrast, spray-drying has been successfully used as a microencapsulation technique since 1950.¹⁵ The inlet and outlet temperatures and the speed of water migration from the inside of the particle to the drying chamber gas flow may cause damage to the cell membrane.⁸

Maltodextrin and sweet whey proteins can be used as carriers in both freeze-drying and spray-drying. Maltodextrin is a D-glucose polymer that has been partially hydrolyzed by means of an acid or an enzymatic process. The final properties and molecular weight of maltodextrin depend on the process used.¹⁶ Sweet whey is a by-product of the cheese industry. Proteins, on account of their globular structure, stability and affinity with hydrophobic groups represent a more versatile carrier for microencapsulation and support the efficient delivery of probiotics.¹⁷ These compounds, used alone or in combination with the appropriate drying technology and conditions, provide an effective carrier for successful delivery of the micro-organisms to the lower gastrointestinal tract.

In order to develop a suitable probiotic microencapsulation process (combining the right drying technology with an effective carrier), it is necessary to study the activity of the micro-organisms at the final delivery point—the lower portion of the human digestive tract—using a realistic dynamic digestion system.¹⁸ In vitro digestion systems have been designed to simulate

the complex physicochemical and biological processes occurring in the stomach and duodenum and therefore expose probiotic capsules to realistic enzymatic hydrolysis and pH effects.¹⁹ Some models have a dynamic design that mimics peristaltic movements, shear rate, particle-size reduction, stomach emptying rate and residence time in the stomach and duodenum. Such systems are therefore more appropriate for evaluating the effect of digestion on survival of the micro-organisms after the microencapsulation process.²⁰

One of these dynamic models is the In Vitro Digestion System (IViDiS), which was developed at Agriculture and Agri-Food Canada's Saint-Hyacinthe Research and Development Centre.²¹ IViDiS simulates the human upper gastrointestinal tract, including the constant changes in the pH pattern, enzymes levels, gastric feeding and empty gastric rates that occur during digestion. These changes are called "digestion profiles" and are specific for each type of food, beverage or dissolution medium.²² The digestion profiles are based on clinical data and on the scientific literature, including information from the Guyton and Hall *Textbook of Medical Physiology*.²³ Specific digestion profiles are used to evaluate a micro-organism's stability during its passage through the simulated gastrointestinal tract with or without microcapsules or a food matrix. The results lead to recommendations for consuming a probiotic so that it will survive until it reaches the target site in the gastrointestinal tract.²⁴

The aim of the present study was to evaluate the influence of two microencapsulation parameters (carrier material and drying technology), along with the dissolution medium (water or milk), on the survival of *Lactobacillus fermentum* K73 in the IViDiS, which simulates the upper gastrointestinal environment.

2. Materials and methods

2.1. Materials

The analytical reagents, sodium chloride and potassium phosphate dibasic trihydrate, were purchased from Laboratoire MAT (Beauport, QC, Canada). Potassium chloride was obtained from BDH Chemicals (Toronto, ON, Canada) and 6N HCl was acquired from Fisher Scientific (Trenton, NJ, USA). Pancreatin (from porcine pancreas P1750), porcine bile extract (B8631), phosphatidylcholine (P3556), mucin (from porcine stomach M1778), α -amylase (from porcine pancreas A3176), pepsin (P7000) and sodium bicarbonate were obtained from Sigma-Aldrich (St. Louis, MI, USA). Yeast lipase (YL 100c) was acquired from BIO-CAT (Troy, VA, USA). Commercial natural spring water (Eska, Saint-Mathieu-d'Harricana, QC, Canada) and milk with 1% fat (Québon, Saint-Laurent, QC, Canada) were purchased from a local market. Sweet whey powder (for the spray-drying technique) was obtained from a local company (Sopó, Colombia) and had the following composition: 11.67% w/w protein, 2.0% w/w lipids, 51.64% w/w lactose²⁵, and 10.9% w/w ash. Maltodextrin was purchased from Shandong WNN Industrial Co., Ltd (Shandong, China), and yeast extract from Oxoid Ltd. (Basingstoke, UK). De Man, Rogosa and Sharpe (MRS) broth, together with agar and peptone water, were obtained from Scharlau Microbiology (Barcelona, Spain).

2.2. Strain and culture conditions

Lactobacillus fermentum K73 (GenBank accession No. KP784433.1) was previously isolated and characterized in vitro as a probiotic bacteria.^{6,26} The micro-organisms were grown in MRS broth for 24 h at 37 °C before every assay.

The biomass acquisition process was performed in a 1-L bioreactor with a working volume of 800 mL at 37 °C and under agitation at 100 rpm for 10 h. The culture medium consisted of 8% w/v sweet whey and 0.22% w/v yeast extract. The culture medium was adjusted to pH 5.5 and sterilized at 121 °C for 15 min. After the sterilization process, the culture medium contained denatured sweet whey. *Lactobacillus fermentum* K73 was inoculated at 10% v/v from a pre-culture with a concentration of $6.27 \pm 0.34 \log_{10}$ CFU mL⁻¹ in mid-exponential phase.⁶ The cell count was carried out after the fermentation process, as explained below in section 2.7.²⁷

2.3. Maltodextrin:sweet whey solution

The culture medium was harvested in mid-exponential phase and mixed immediately with the powdered mixture of maltodextrin and sweet whey (0.6:0.4). The mixture was homogenized using magnetic agitation (130 rpm) for 20 min. The final solution contained 40% total solids (dry weight).²⁸

2.4. Drying processes

The culture medium with 8.22% total solids (dry weight) was dried at the end of the fermentation (mid-exponential phase) to determine its potential as a carrier material and as a cryoprotectant agent under the same conditions as the encapsulated micro-organism.

2.4.1. Spray-drying

The maltodextrin:sweet whey and the culture medium were spray-dried. The spray dryer (GEA Niro Mobile Minor; GEA Process Engineering, Skanderborg, Denmark) was operated using a pneumatic co-current two-fluid nozzle atomizer with an orifice diameter of 1 mm. The inlet air-drying temperature was set to 175 °C. The outlet air-drying temperature was kept constant at 90 °C. The atomizing air pressure was set to 1.1 bar. The drying air rate was set to

80 kg h⁻¹, and the feed flow rate of the culture medium was set to 450 mL min⁻¹.²⁹ The cell count was performed and moisture was determined.²⁵ The powder from the culture medium was named SDWO, and the name SDWM was used for the maltodextrin:sweet whey dried solution.²⁸

2.4.2. Freeze-drying

The maltodextrin: sweet whey solution and the culture medium were placed on sterilized trays with a 0.3-cm thickness. The trays were frozen at -40 °C for 12.5 h with a cooling rate of 1.5 °C min⁻¹. The trays were then freeze-dried using a Labconco FreeZone 12 Liter Console Freeze Dry System (Labconco; Kansas City, MO, USA) at a collector temperature of -80 °C for 21 h. The sublimation phase was carried out at -10 °C for 18 h with a ramp of 1.5 °C min⁻¹, and the desorption phase was carried out at 25 °C for 6 h with a ramp of 1.5 °C min⁻¹. The chamber pressure was 0.03 mbar during both phases.

After the spray-drying and freeze-drying processes, the powders were collected and placed in metallic (140-μm) bags. The bags were vacuum-packed (9 mbar) (C200 chamber machine; Multivac) and stored at 4 °C. The cell count was performed and moisture was determined.²⁵ The powder derived from culture medium was named FDWO, and the 40% total solids solution was named FDWM.³⁰

2.5. IViDiS digestion

The IViDiS system is an in-house in vitro digestion system.^{20,21} The IViDiS is composed of a stomach, an upper duodenum and a tubular lower duodenum. The system is maintained at 37 °C using jacketed vessels for the stomach and the upper duodenum and a water bath for the lower duodenum. A computer controls the flow rate of each pump according to programmable profiles. For this study, 10 g of powder and 500 mL of spring water or 1% milk fat were added to

the IViDiS. The gastric solutions (Fig. 1) were then pumped at flow rates simulating in vivo conditions for 2.5 h for the water or 3 h for the milk.²³ The contents from the stomach vessel were gradually passed to the upper duodenum vessel, and intestinal fluids were added according to their individual profiles (Fig. 1). Time 0 samples were taken when the contents entered the stomach vessel, and thereafter samples were taken every 15 min in the stomach and every 30 min in the duodenum, in order to calculate the survival curves for the micro-organisms. The cell count of each sample was performed as described below.

Fig. 1. Schematic of in vitro dynamic digestion system (IViDiS). Adapted from Tompkins et al. (2011).

2.6. Mathematical models for *L. fermentum* K73 survival curves from IViDiS systems

Cell counts were performed using samples taken in the stomach vessel and at the outlet of the lower duodenum at specific times during digestion of two different profiles (water or milk as the accompanying dissolution medium). The data were fitted using the modified Gompertz model (Eq. 1) and the Whiting and Buchanan model (Eq. 2).^{31,32}

$$\text{Log}N = \text{Log}N_0 - \text{Log}\frac{N_0}{N_f} e \left(-e \left(\left(\frac{ke(1)}{\text{Log}\left(\frac{N_0}{N_f}\right)} \right) (L - t) + 1 \right) \right) \quad \text{Eq. 1}$$

where N represents the cell density at time t (in seconds); N_0 and N_f are the initial and final cell density, respectively; k is the maximum inactivation rate constant; and L is the time parameter (the shoulder or lag phase).

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$$\text{Log}\frac{N}{N_0} = \text{Log}\left(\frac{F(1 + e^{-bL})}{1 + e^{b(t-L)}} + \frac{(1-F)(1 + e^{-cL})}{1 + e^{c(t-L)}}\right)$$

Eq. 2

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where N is the cell density at time t (in seconds), N_0 is the initial cell density (at time = 0), L is the duration of the lag period prior to initiation of inactivation (in seconds), b is the inactivation rate of the major population group, and c is the inactivation rate of the minor population group.

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The goodness of fit of the models was evaluated by comparing the bias factor (Bf), accuracy factor (Af), sum of squares (SS), mean square (MS), correlation coefficient (R^2) and adjusted correlation coefficient (adjusted R^2) between the experimental and predicted values.

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2.7. Microbiological analyses

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For the first dilution and digestion aliquots in the IViDiS, dried powder samples were homogenized in a vortex for 10 min. For colony-forming unit (CFU) analyses, serial 1:9 dilutions in peptone water (0.1 %) were performed. The dilutions were then pour-plated in MRS agar and incubated at 37 °C for 24 h in aerobic conditions. The cell count was expressed as log₁₀ CFU mL⁻¹ for the fermentation process and carrier systems and as log₁₀ CFU g⁻¹ for the drying process and digestion in the IViDiS.³³

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2.8. Statistical analysis

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The estimation of the survival ratio was done by calculating the difference between the real value in log₁₀ CFU g⁻¹ obtained from the directly measured cell count (in the stomach vessel and the lower duodenum vessel) and the theoretical value in log₁₀ CFU g⁻¹ obtained from mass balance calculations based on the number of micro-organisms that should be present at that

location and at that specific moment if there was perfect mixing without adsorption or mortality. The experiment was planned as a complete 2^3 factorial design, where the factors were two different carriers (culture medium and culture medium mixed with maltodextrin:sweet whey), two different drying processes (spray-drying and freeze-drying) and two different dissolution media (water or milk). The data were subjected to repeated measures ANOVA using the STATISTICA software, version 12 (StatSoft, Tulsa, OK, USA). The survival curves (mathematical models) were analyzed with the SAS software, version 2.0.4 (SAS Institute Inc., Cary, NC, USA).

3. RESULTS AND DISCUSSION

3.1. Drying process

The viable cell count and moisture content were measured after the spray-drying and freeze-drying processes, in order to evaluate the viability of the micro-organism after drying.

The bacterial population was $9.672 \pm 0.148 \log_{10}$ CFU mL⁻¹ after the fermentation process. The viable cell count was $6.129 \pm 0.033 \log_{10}$ CFU g⁻¹ for SDWO and $6.290 \pm 0.133 \log_{10}$ CFU g⁻¹ for SDWM with a moisture content (dry basis) of 12.24 ± 0.428 and 1.818 ± 0.025 , respectively. In comparison, the cell counts in the powders obtained from freeze-drying were $7.408 \pm 0.341 \log_{10}$ CFU g⁻¹ in FDWO and $7.301 \pm 0.301 \log_{10}$ CFU g⁻¹ in FDWM; the moisture content (dry basis) was 2.600 ± 0.18 % and 2.263 ± 0.115 %, respectively. The viable cell count was lower when the material was spray-dried than when it was freeze-dried, regardless of the type of material evaluated. A one-way ANOVA using the Tukey HSD test (results not shown) confirmed a significant difference ($p < 0.05$) between the two types of drying processes in terms of cell count value.^{28,30} The Tukey's test did not show significant differences between the two types of carrier material used. Hence, using a carrier material solution with 40% total solids in the

drying processes did not significantly improve cell survival. This result suggests that the culture medium could be adequate as a carrier material.

The results showed a higher moisture content for SDWO in comparison with the other samples. This was attributed to the fact that during the passage of culture medium through the drying chamber, compaction of the sweet whey aggregates occurred, generating rigid surface capsules. However, the inlet temperature was not high enough to remove all the water content, which generated capsules with a wet core, which could facilitate the repair process of the cell membrane.²⁸

3.2. IViDiS Digestion

Powder products were evaluated in a dynamic gastrointestinal system (IViDiS) to determine the effect of drying technologies (spray-drying or freeze-drying), carrier materials (culture medium or culture medium with maltodextrin:sweet whey) and the type of dissolution medium (water or 1% fat milk) on the survival of *L. fermentum* K73. Powder products prepared with culture medium, regardless of the drying technology used, protected the micro-organism from gastrointestinal conditions, primarily thanks to the resistance of denatured sweet whey proteins to pepsin enzymatic activity during gastric digestion. Additionally, the probiotic product with 1% fat milk as a dissolution medium improved cell survival due to the buffering capacity of the milk proteins.

The repeated ANOVA measures of the complete 2³ factorial design are shown in Table 1. This type of ANOVA is used to evaluate the differences in mean scores for one dependent variable across two or more within-group conditions of an independent variable.³⁴ The *L. fermentum* K73 survival rate (after gastric and duodenal conditions) over time was the

dependent variable, and the type of dissolution medium, the type of drying process and the type of carrier (see section 2.8) were the independent variables. The results for the micro-organism survival rate in the stomach and in the duodenum showed that all factors (independent variables) and the variable time were highly significant ($p < 0.0005$), except except for the interaction between the dissolution medium and the drying process.

Distribution of the variance (using the magnitude of the mean square [MS] factor) showed that the four factors with the greatest influence on the results obtained under gastric conditions were as follows: first, the type of dissolution medium ($MS = 88.6$), followed by the carrier ($MS = 47.9$), the time ($MS = 31.0$), the dissolution medium–carrier interaction ($MS = 17.9$) and, lastly, the drying technology ($MS = 5.5$). With regard to the effect of duodenal conditions on the survival of *L. fermentum* K73, the factors with the greatest influence were the dissolution medium ($MS = 145.8$), followed by the carrier ($MS = 80.2$), the carrier–drying technology interaction ($MS = 18.1$) and, lastly, the dissolution medium–carrier material–drying technology interaction ($MS = 11.9$). A comparison of the effect of the variable time showed that, whether alone or as part of an interaction, time had less of an influence in the duodenum than in the stomach ($MS = 7.8$).

The type of dissolution medium used had a considerable impact on the survival rate of *L. fermentum* K73 during simulated gastrointestinal digestion. The protective effect of milk was evident, given the large number of micro-organisms that survived the passage through the stomach to the duodenum (Fig. 2). This protective effect has been attributed to the buffering capacity of the fat and protein fractions. Tompkins et al. (2011), concluded that probiotics without an enteric coating could be ingested with a dissolution medium containing at least 1% fat, such as milk, in order to achieve higher micro-organism survival in the duodenum. Results have shown that, in addition to the intrinsic tolerance of lactobacilli strains to acidic

environments, the buffering capacity and the composition of the fermented milk may have a significant protective effect on the survival of the lactobacilli.³⁵ In our study, the results suggest that an alternative strategy for increasing the survival rate of the micro-organisms would be to use 1% fat milk as a food matrix to protect lactobacilli from gastrointestinal conditions (Table 1).

When a comparison was performed for the gastric conditions at 120 min specifically (not as a result of time), the results suggested that the powders that had a protective effect on *L. fermentum* K73 were SDWO (Fig. 2A) tested in water and FDWO (Fig. 2E) tested in milk, with the survival rate decreasing by only 2.478 and 2.318 log₁₀ CFU g⁻¹, respectively. Conversely, the powders with the least protective capacity against the harsh conditions in the stomach were SDWM (Fig. 2B) and FDWM (Fig. 2F) tested in water, with decreases of 5.620 and 5.505 log₁₀ CFU g⁻¹, respectively. Therefore, the culture medium powders showed a beneficial effect in both food matrices by improving cell survival.

The effect of the carriers could be explained by their composition. In a previous study, denatured sweet whey proteins, a component of the culture medium powder, were used as a carrier material for the encapsulation of *Lactobacillus rhamnosus* GG and resulted in 99% bacterial survival during simulated gastric digestion for 120 min.³⁶ In another study, microbeads with heat-treated (denatured) sweet whey protein isolate showed stability in gastric conditions and intestinal disintegration, delivering the probiotic to the target site.³⁷ Remondetto and Subirade (2003) suggested that the resistance of denatured sweet whey proteins to pepsin attack in acidic conditions could be attributed to the hydrophobic interaction between aromatic amino acids in the protein complex.³⁸

Therefore, culture medium powder is a promising and economical protective agent for the development of improved probiotics. At the same time, maltodextrin had an important effect on the powders. This polymer is soluble in water and, like other starches, is sensitive to the acidic

conditions in the stomach.³⁹ It has been proposed that heating promotes the formation of protein–carbohydrate conjugates via the Maillard reaction, which can increase protein solubility under acidic conditions owing to a lower isoelectric point.^{40,41} Moreover, the steric structure of the sweet whey proteins (SDWM and FDWM) could be modified by the abundant presence of hydrogen ions in gastric solution (active), which would increase particle porosity, solution uptake^{36,42} and exposure of the micro-organisms to the gastric environment. This could explain the low survival rate of *L. fermentum* K73 when it was included with the carrier material used in this study.

The IViDiS simulates gastric emptying during the fed motility phase. This system has a dynamic flow rate from the stomach vessel to the upper and then the lower duodenum vessels that depends on the dissolution medium. Enzyme addition, peristaltic movements, pH control, temperature control and the volume in the reactors are all factors that are not constant and are intended to re-recreate the events that occur during in vivo digestion. Therefore, the micro-organisms must be in constant movement to reflect the behaviour in the stomach, upper duodenum and lower duodenum.²¹

Owing to the movement of the cells through the IViDiS, the time that the micro-organisms spend in each segment of the system has been calculated theoretically. Because of the dynamic nature of the IViDiS, the concentration of micro-organisms in each segment of the system varies with time. Hence, determining the survival rate involves calculating the ratio of measured CFU to theoretical CFU for each sample taken.

The first samples were taken 15 min after the micro-organism reached the stomach vessel. This 15-min period is the time it takes the probiotic powder to dissolve completely.

The first micro-organisms exited the duodenum after 45 or 60 min, depending on the nature of the dissolution medium (water or milk). The initial survival rate in the duodenum

(60 min) should be similar to that in the stomach vessel between 15 and 30 min after the digestion process began (Fig. 2C, D, G and H), if the bile salt and pancreatic solution does not have any detrimental effects on *L. fermentum* K73. Finally, 90 min after the simulation of digestion began, the bacteria was sampled from the duodenal vessel, because there was sufficient volume from the stomach vessel.

The stomach contents gradually move to the duodenum vessels during the first 120 min. In the upper duodenum vessel, a homogenous mixture of gastric contents and pancreatic solution with bile salts is created. The micro-organisms have a brief residence time in the vessel before passing to the lower duodenum. The bile tolerance of *L. fermentum* is an important characteristic that enables its survival, enhancing its internal damage repair and subsequently allowing its growth to exert an effect on the small intestine.²⁸ Pereira et al. found that *L. fermentum* strains can grow in MRS supplemented with 5 mM conjugated bile salts (oxgall, cholic acid, deoxycholic acid, taurocholic acid, glycocholic acid and taurodeoxycholic acid).⁴³ If the powder has a higher protective effect under gastric conditions, as was the case for SDWO tested in milk, when the micro-organisms reach the duodenal vessel, cellular damage could be repaired during the transit period (30-45 min), increasing the micro-organism survival rate at 90 and 120 min (constant increase until the end of the experiment) (Fig. 2C).

The powders that showed the best protection for *L. fermentum* K73 were SDWO ($-3.044 \log_{10}$ CFU g⁻¹) and FDWO ($-3.8 \log_{10}$ CFU g⁻¹) tested in water and SDWO ($-0.230 \log_{10}$ CFU g⁻¹) and FDWO ($-0.379 \log_{10}$ CFU g⁻¹) tested in milk. The comparison was done at the end of each digestion process (150 min). The micro-organisms survived better during milk digestion (mortality was less than 2 log over time). A larger proportion of cells died either sooner (maltodextrin:sweet whey powders) or later (culture medium powders) during digestion with water.

The results shown in Fig. 2 suggest that the culture medium has a strong impact on the survival of the micro-organisms with both drying methods. However, the use of spray-drying as a drying technology provides major benefits to the food industry, given that large quantities of probiotic powder can be produced at a lower cost with spray-drying than with freeze-drying.⁴⁴

Fig. 2. Survival kinetics of *Lactobacillus fermentum* K73 in the stomach (A, B, E, F) and the duodenum (C, D, G, H) with different powders. Values represent means $\pm$ 95% confidence intervals from three independent digestions. The red and blue lines represent the survival behaviour when milk and water, respectively, are used as the dissolution medium.

3.3. Mathematical models for *L. fermentum* K73 survival curves from IViDiS

The Gompertz model⁴⁵ and the Whiting and Buchanan model³¹ are the tools that were used to describe the behaviour of *L. fermentum* K73 in the stomach and the duodenum using the kinetics parameters faster inactivation rate (k) and lag phase (L).

The goodness of fit of the experimental data was calculated using the Bf, Af, SS, RMSE and R^2 . In all survival curves evaluated with the Gompertz model, the Bf and Af had a value of nearly 1 (Bf = 0.993 to 1.016; Af = 1.0001 to 1.022). However, in approximately 10 curves analyzed with the Whiting and Buchanan model, the statistical goodness-of-fit values were farther from 1 (Bf = 0.131; Af = 1.025 to 7.612). These parameters explain the uniformity of the distribution.⁴⁶ No curve was calculated for SDWM in intestinal conditions, since the micro-organisms did not survive beyond 15 to 30 min of gastric conditions.

In the Gompertz model (Table 2), the R^2 values indicate that the experimental values were generally close to the predicted values in both gastric and intestinal conditions for digestions

performed with water and milk ($R^2 > 0.9225$ for 14 out of 15 conditions). However, SDWM tested in milk showed a low regression coefficient (0.7304). In the Whiting and Buchanan model (Electronic supplementary information), the R^2 values indicate good correlation between predicted and experimental values in gastric conditions ($R^2 > 0.941$ for seven out of eight conditions). However, the model could not effectively predict the behaviour of the micro-organisms under intestinal conditions, as shown by a R^2 value greater than 0.871 in only four of the seven curves analyzed.

For the two models, the R^2 values were higher in gastric conditions than in intestinal conditions, owing to the fact that Eqs. 1 and 2 were designed to model curves with negative slopes.^{32,45} The bacteria populations increased during milk digestion in the duodenum; therefore, in FDWM and SDWO, the curve slopes were positive. Hence, it was necessary to change the sign of the constant (k) to adjust the model. The Gompertz model was found to be more robust for almost all the conditions in the different digestion profiles. This may be due to the fact that the model is commonly used with asymmetrical sigmoidal shape curves^{32,45,47}, like those obtained in our experiments. Moreover, this model was suitable for describing the survival of *L. fermentum* K73 exposed to dynamic digestion using the IViDiS system with three different powder types.

The fits of the Gompertz model and the Whiting and Buchanan model are presented in Fig. 3 for FDWO and FDWM in the stomach and the duodenum. Those plots were representative of the distribution for all conditions tested. For comparison purposes, the simulated cell count values (solid line) and the real pH values (dotted line) obtained in the stomach and the duodenum, respectively, during the digestion of water or milk are included in the same figure. The fit between the predicted data and the experimental data is better with the Gompertz model than with the Whiting and Buchanan model in the majority of the curves according to the R^2 and adjusted R^2 , as previously discussed.

The Gompertz model kinetic parameters, estimated from the survival curves, provided an idea of the microbial susceptibility to gastrointestinal conditions. During water digestion, the maltodextrin:sweet whey powders presented a faster inactivation rate (k) in gastric and intestinal conditions, as well as a longer lag phase (L) in gastric conditions, than the culture medium powders did (Fig. 2C). When gastric emptying was complete and the powder had passed into the duodenum, the SDWO showed a longer lag phase and a slower inactivation rate in comparison with the other powders (Table 2, Fig. 2C). The lag phase of FDWO was shorter than that of the other powders, but its inactivation rate was slower than for FDWM (Table 2, Fig. 2G and H).

For milk digestion, the culture medium powders had longer lag phases (L) and faster inactivation rates (k) in gastric conditions than the maltodextrin:sweet whey powders did (Table 2, Figs. 2E and 3A). *Lactobacillus fermentum* K73 showed a longer lag time in duodenal conditions for all types of powders. The inactivation rate in FDWM and SDWO had a negative sign (Table 2), as previously discussed; in these cases, the lag phase should be considered the period prior to growth initiation of the bacterial population under intestinal conditions (Fig. 2C and H).

The lag phase and the inactivation rate showed a correlation with the pH change in water and milk digestions. At the beginning of the experiments, the pre-prandial conditions in the stomach were simulated by the addition of a basal gastric solution at pH 2.01 and saliva at pH 7.23. After consumption of the dissolution medium, the pH increased owing to the buffering capacity of the dissolution medium. For water digestion, after the dissolution medium entered the stomach, the pH dropped rapidly from 7.01 to 3.1 in the first 30 min and then gradually decreased to 1.9 by 120 min. For milk digestion, the initial pH was 6.3 (after milk entry) and dropped slowly to 3.9 over the following 60 min; the pH then decreased to 1.9 during the next hour

(Fig. 3, dotted line). The drastic change in the pH observed during water digestion decreased the lag phase and increased the inactivation rate of *L. fermentum* K73 in all carrier systems.

Fig. 3. Experimental values (symbols) and predicted values (continuous line) obtained for the Gompertz model and the Whiting and Buchanan model: freeze-dried culture medium (FDWO) and freeze-dried maltodextrin:sweet whey solution (FDWM) in the stomach (A, B) and the duodenum (C, D) using water (▲ or Δ) and milk (◆ or ◇) as the accompanying dissolution medium. (← →) pH recorded using water; (← →) pH recorded using milk.

4. Conclusion

This study showed that the culture medium was an adequate carrier material for *L. fermentum* K73 ingested with milk or water, independent of the drying technology tested. The advantages associated with the use of denatured sweet whey as the culture medium and carrier material hold promise for designing and developing encapsulation methods from the fermentation process to the final product in only one step. In addition, the Gompertz model was a useful tool for correlating micro-organism behaviour with the kinetic parameter estimates obtained from the survival curves. Lag phase and inactivation rates were affected by pH changes in the water and milk profiles. This research opens up the possibility of developing a new culture medium as a principal component of the carrier material in microencapsulation processes and evaluating microcapsule digestion by the colonic microbiota while using specific profiles to study micro-organism release in the colon and the survival rate under these conditions.

Conflicts of interest

There are no conflicts of interest to declare.

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- 1 **Table 1.** Repeated measures ANOVA on survival ratio of *Lactobacillus fermentum* K73 in the different powders in the stomach and
 2 lower duodenum.

Source	Stomach				Duodenum			
	DF ^a	MS	F	p	DF	MS	F	p
Test for between-subject effects								
Intercept	1	575.983	10,724.290	0.000 ^c	1	447.053	7,258.130	0.000 ^c
Beverage	1	88.583	1,649.330	0.000 ^c	1	145.841	2,367.807	0.000 ^c
Drying	1	5.447	101.410	0.000 ^c	1	5.417	87.952	0.000 ^c
Carrier	1	47.857	891.050	0.000 ^c	1	80.150	1,301.280	0.000 ^c
Beverage*Drying	1	0.036	0.680	0.423 ^b	1	1.720	27.926	0.000 ^c
Beverage*Carrier	1	17.913	333.530	0.000 ^c	1	9.553	155.101	0.000 ^c
Drying*Carrier	1	4.523	84.210	0.000 ^c	1	18.088	293.660	0.000 ^c
Beverage*Drying*Carrier	1	8.154	151.830	0.000 ^c	1	11.900	193.198	0.000 ^c
Error	16	0.054			16	0.062		
Test for within-subject effects								
Time	5	30.981	1,978.190	0.000 ^c	3	7.775	195.553	0.000 ^c
Time*Beverage	5	9.794	625.340	0.000 ^c	3	10.513	264.410	0.000 ^c
Time*Drying	5	1.447	92.410	0.000 ^c	3	7.058	177.519	0.000 ^c
Time*Carrier	5	2.216	141.510	0.000 ^c	3	0.583	14.666	0.000 ^c

Time*Beverage*Drying	5	1.488	94.990	0.000 ^c	3	5.566	139.988	0.000 ^c
Time*Beverage*Carrier	5	1.234	78.820	0.000 ^c	3	0.705	17.724	0.000 ^c
Time*Drying*Carrier	5	0.533	34.050	0.000 ^c	3	0.557	14.009	0.000 ^c
Time*Beverage*Drying*Carrier	5	0.563	35.970	0.000 ^c	3	1.277	32.123	0.000 ^c
Error	80	0.016			48	0.040		

3

^a DF, degrees of freedom; MS, mean square; F, *F*-statistic; p, *P*-value.

4

^b Not significantly different (p > 0.0005). 1- that 0,000 means p<0,0005 and that 0,423 is not significant at p=0,05

5

^cSignificantly different (p <0.0005)

Table 2. Parameter estimates with the Gompertz model for the behaviour of *Lactobacillus fermentum* K73 in the IViDiS *in vitro* digestion system and regression analysis results.

	Beverage	System ^a	Parameter estimates ^b		Regression analysis ^c					
			k (s ⁻¹)	L (s)	Bf	Af	SS	RMSE	R^2	Adjusted R^2
Stomach	Water	SDWO	0.00150	723.989	0.999	1.001	0.133	0.136	0.991	0.988
		SDWM	0.00973	738.811	0.993	1.007	0.025	0.057	0.9998	0.9997
		FDWO	0.00100	630.481	1.004	1.001	0.101	0.240	0.947	0.933
		FDWM	0.00277	999.999	1.016	1.020	0.023	0.826	0.984	0.980
	Milk	SDWO	0.00130	4779.900	1.002	1.002	0.002	0.101	0.992	0.991
		SDWM	0.00110	2953.460	0.999	1.005	0.008	0.174	0.947	0.933
		FDWO	0.00560	5262.600	1.008	1.008	0.023	0.975	0.960	0.950
		FDWM	0.00041	1293.840	1.000	1.001	0.035	0.262	0.989	0.986
Duodenum	Water	SDWO	0.0003153	7867.854	1.0151	1.0229	0.2647	0.7991	0.9917	0.9876
		SDWM	-	-	-	-	-	-	-	-
		FDWO	0.0013	3995.5	1.000	1.009	0.029	0.095	0.999	0.987
		FDWM	0.02	5300	1.005	1.006	0.0038	0.9201	0.9815	0.9754
	Milk	SDWO	-0.00169	6839	0.9963	1.0061	0.00029	0.03761	0.9225	0.8838
		SDWM	0.0001	7887.6	0.999	1.0001	0.001	0.053	0.7304	0.6406
		FDWO	0.000321	7887.608	1.00042	1.0018	0.00121	0.089	0.9587	0.9484
		FDWM	-0.00004	3855.9	1.00024	1.00029	6.00E-10	0.0104	0.9898	0.9873

^a SDWO, spray-dried without carrier material; SDWM, spray-dried with carrier material; FDWO, freeze-dried without carrier material; FDWM, freeze-dried with carrier material.

^b k , inactivation rate; L , lag phase; -, not examined.

^c Bf, bias factor; Af, accuracy factor; SS, sum of squares; RMSE, root mean square error; R^2 , correlation coefficient; -, not examined.

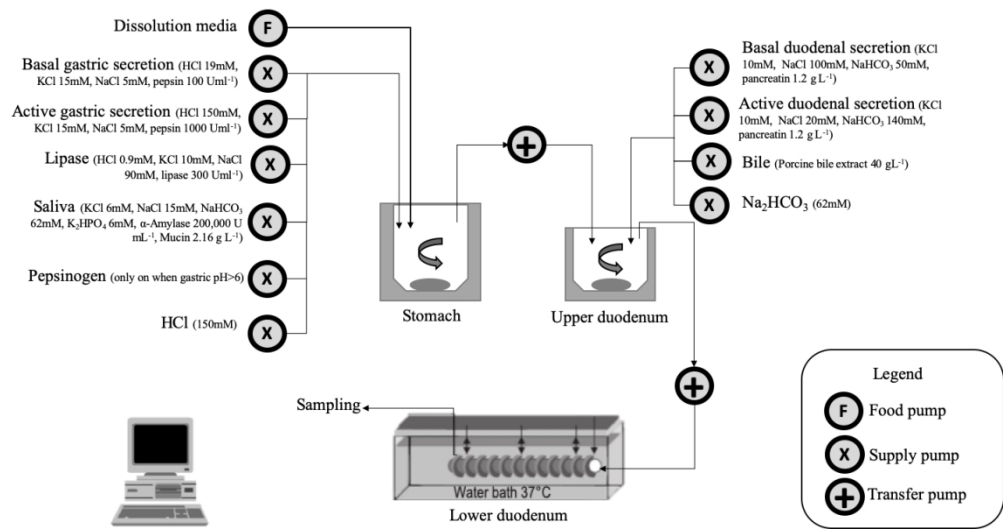


Fig. 1. Scheme of In vitro dynamic digestion system (IVIiDiS). Adapted from Tompkins et al. (2011).

503x276mm (72 x 72 DPI)

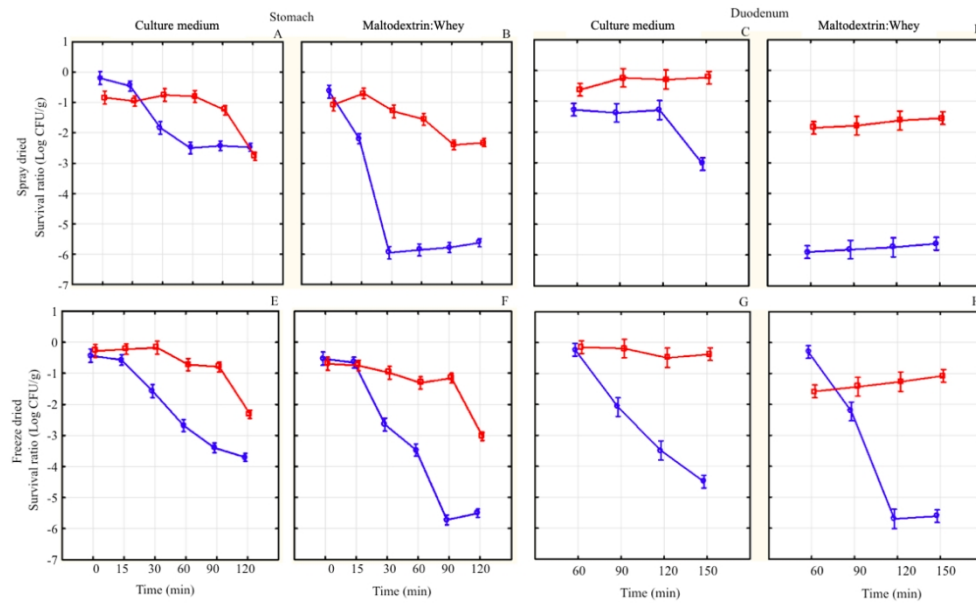


Fig. 2. Survival kinetics of *Lactobacillus fermentum* K73 in the stomach (A, B, E, F) and the duodenum (C, D, G, H) with different powders. Values represent means $\pm$ 95 % confidence intervals from three independent digestions. The red and blue lines represent the survival behaviour using milk and water, respectively, as the dissolution media.

410x251mm (72 x 72 DPI)

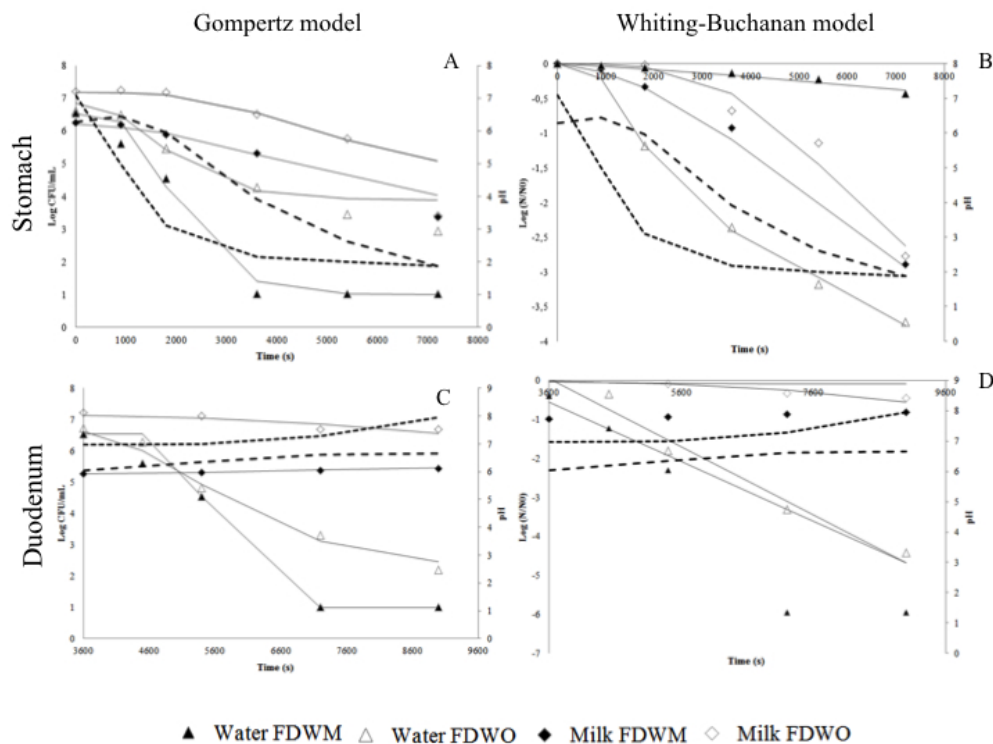


Fig. 3. Experimental values (symbols) and predicted values (continuous line) obtained for the Gompertz model and the Whiting and Buchanan model: culture medium freeze-dried (FDWO) and maltodextrin:sweet whey solution freeze-dried (FDWM) in the stomach (A, B) and the duodenum (C, D) using water (▲ or Δ) and milk (◆ or ◇) as the accompanying dissolution media. () pH recorded using water; () pH recorded using milk.

254x190mm (72 x 72 DPI)