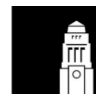

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Effect of the carrier material, drying technology and dissolution media on the viability of Lactobacillus fermentum K73 during simulated gastrointestinal transit

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1 **Effect of the carrier material, drying technology and dissolution media on the viability of**
2 ***Lactobacillus fermentum* K73 during simulated gastrointestinal transit**
3

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16

17 **ABSTRACT**

18 The goal of this study was to determine the effect of the carrier material, drying
19 technology and dissolution media during the passage of *L. fermentum* K73 through a dynamic in
20 vitro digestion system (IViDiS). The carrier materials were (i) culture medium with growing
21 micro-organisms and (ii) culture medium with maltodextrin:sweet whey [0.6:0.4]. The carrier
22 materials were dried by spray-drying and freeze-drying to obtain four types of powders. The
23 dissolution media consisted of water and 1% fat milk. The powders were tested using an in vitro
24 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.

48 Microencapsulation was originally used in the food industry as a way to protect bioactive
49 compounds during food processing and ultimately attain several objectives such as widening the
50 storage temperature range, extending shelf life and providing protection against the
51 gastrointestinal environment.[7] Microencapsulation was later used for probiotic protection. Drying
52 processes are used for probiotic strains, since the resulting products are physic-chemically stable,
53 simpler to store and inexpensive to transport.[8] Two widely used techniques are freeze-drying and
54 spray-drying.[9–12] Freeze-drying has often been used as a conservation technology, but its use as
55 an encapsulation method for probiotics is a relatively innovative concept.[13,14] In contrast, spray-
56 drying has been successfully used as a microencapsulation technique since 1950.[15] The inlet and
57 outlet temperatures and the speed of water migration from the inside of the particle to the drying
58 chamber gas flow may cause damage to the cell membrane.[8]
59 Maltodextrin and sweet whey proteins can be used as carriers in both freeze-drying and
60 spray-drying. Maltodextrin is a D-glucose polymer that has been partially hydrolyzed by means of
61 an acid or an enzymatic process. The final properties and molecular weight of maltodextrin
62 depend on the process used.[16] Sweet whey is a by-product of the cheese industry. Proteins, on
63 account of their globular structure, stability and affinity with hydrophobic groups represent a
64 more versatile carrier for microencapsulation and support the efficient delivery of probiotics.[17]
65 These compounds, used alone or in combination with the appropriate drying technology and
66 conditions, provide an effective carrier for successful delivery of the micro-organisms to the
67 lower gastrointestinal tract.
68 In order to develop a suitable probiotic microencapsulation process (combining the right
69 drying technology with an effective carrier), it is necessary to study the activity of the micro-
70 organisms at the final delivery point—the lower portion of the human digestive tract—using a
71 realistic dynamic digestion system.[18] In vitro digestion systems have been designed to simulate

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

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

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

93

94 **2. Materials and methods**

95

96 *2.1. Materials*

97 The analytical reagents, sodium chloride and potassium phosphate dibasic trihydrate,
98 were purchased from Laboratoire MAT (Beauport, QC, Canada). Potassium chloride was
99 obtained from BDH Chemicals (Toronto, ON, Canada) and 6N HCl was acquired from Fisher
100 Scientific (Trenton, NJ, USA). Pancreatin (from porcine pancreas P1750), porcine bile extract
101 (B8631), phosphatidylcholine (P3556), mucin (from porcine stomach M1778), α-amylase (from
102 porcine pancreas A3176), pepsin (P7000) and sodium bicarbonate were obtained from Sigma-
103 Aldrich (St. Louis, MI, USA). Yeast lipase (YL 100c) was acquired from BIO-CAT (Troy, VA,
104 USA). Commercial natural spring water (Eska, Saint-Mathieu-d'Harricana, QC, Canada) and
105 milk with 1% fat (Québon, Saint-Laurent, QC, Canada) were purchased from a local market.
106 Sweet whey powder (for the spray-drying technique) was obtained from a local company (Sopó,
107 Colombia) and had the following composition: 11.67% w/w protein, 2.0% w/w lipids, 51.64%
108 w/w lactose[25], and 10.9% w/w ash. Maltodextrin was purchased from Shandong WNN Industrial
109 Co., Ltd (Shandong, China), and yeast extract from Oxoid Ltd. (Basingstoke, UK). De Man,
110 Rogosa and Sharpe (MRS) broth, together with agar and peptone water, were obtained from
111 Scharlau Microbiology (Barcelona, Spain).

112

113 *2.2. Strain and culture conditions*

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

117 The biomass acquisition process was performed in a 1-L bioreactor with a working
118 volume of 800 mL at 37 °C and under agitation at 100 rpm for 10 h. The culture medium
119 consisted of 8% w/v sweet whey and 0.22% w/v yeast extract. The culture medium was adjusted
120 to pH 5.5 and sterilized at 121 °C for 15 min. After the sterilization process, the culture medium
121 contained denatured sweet whey. *Lactobacillus fermentum* K73 was inoculated at 10% v/v from a
122 pre-culture with a concentration of 6.27 ± 0.34 log_{10} CFU mL^{-1} in mid-exponential phase.[6] The
123 cell count was carried out after the fermentation process, as explained below in section 2.7.[27]
124 *2.3. Maltodextrin:sweet whey solution*
125 The culture medium was harvested in mid-exponential phase and mixed immediately with
126 the powdered mixture of maltodextrin and sweet whey (0.6:0.4). The mixture was homogenized
127 using magnetic agitation (130 rpm) for 20 min. The final solution contained 40% total solids (dry
128 weight). [28]
129
130 *2.4. Drying processes*
131 The culture medium with 8.22% total solids (dry weight) was dried at the end of the
132 fermentation (mid-exponential phase) to determine its potential as a carrier material and as a
133 cryoprotectant agent under the same conditions as the encapsulated micro-organism.
134
135 *2.4.1. Spray-drying*
136 The maltodextrin:sweet whey and the culture medium were spray-dried. The spray dryer
137 (GEA Niro Mobile Minor; GEA Process Engineering, Skanderborg, Denmark) was operated
138 using a pneumatic co-current two-fluid nozzle atomizer with an orifice diameter of 1 mm. The
139 inlet air-drying temperature was set to 175 °C. The outlet air-drying temperature was kept
140 constant at 90 °C. The atomizing air pressure was set to 1.1 bar. The drying air rate was set to

141 80 kg h^{-1}, and the feed flow rate of the culture medium was set to 450 mL min^{-1}.[29] The cell count
142 was performed and moisture was determined.[25] The powder from the culture medium was named
143 SDWO, and the name SDWM was used for the maltodextrin:sweet whey dried solution.[28]
144
145 *2.4.2. Freeze-drying*
146 The maltodextrin: sweet whey solution and the culture medium were placed on sterilized
147 trays with a 0.3-cm thickness. The trays were frozen at −40 °C for 12.5 h with a cooling rate of
148 1.5 °C min^{-1}. The trays were then freeze-dried using a Labconco FreeZone 12 Liter Console
149 Freeze Dry System (Labconco; Kansas City, MO, USA) at a collector temperature of −80 °C for
150 21 h. The sublimation phase was carried out at −10 °C for 18 h with a ramp of 1.5 °C min^{-1}, and
151 the desorption phase was carried out at 25 °C for 6 h with a ramp of 1.5 °C min^{-1}. The chamber
152 pressure was 0.03 mbar during both phases.
153 After the spray-drying and freeze-drying processes, the powders were collected and
154 placed in metallic (140-μm) bags. The bags were vacuum-packed (9 mbar) (C200 chamber
155 machine; Multivac) and stored at 4 °C. The cell count was performed and moisture was
156 determined.[25] The powder derived from culture medium was named FDWO, and the 40% total
157 solids solution was named FDWM.[30]

158 *2.5. IViDiS digestion*
159 The IViDiS system is an in-house in vitro digestion system.[20,21] The IViDiS is composed
160 of a stomach, an upper duodenum and a tubular lower duodenum. The system is maintained at
161 37 °C using jacketed vessels for the stomach and the upper duodenum and a water bath for the
162 lower duodenum. A computer controls the flow rate of each pump according to programmable
163 profiles. For this study, 10 g of powder and 500 mL of spring water or 1% milk fat were added to

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

171 **Fig. 1**. Schematic of in vitro dynamic digestion system (IViDiS). Adapted from Tompkins et al.
172 (2011).

173

174 *2.6. Mathematical models for L. fermentum K73 survival curves from IViDiS systems*

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

179 $$LogN = LogN_0 - Log\frac{N_0}{N_f}\, e\left(-e\left(\left(\frac{ke(1)}{Log\left(\frac{N_0}{N_f}\right)}\right)(L-t)+1\right)\right) \quad \text{Eq. 1}$$

180

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

184

185 $$Log\frac{N}{N_0} = Log\left(\frac{F(1+e^{-bL})}{1+e^{b(t-L)}} + \frac{(1-F)(1+e^{-cL})}{1+e^{c(t-L)}}\right)$$ Eq. 2

186

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

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

194

195 *2.7. Microbiological analyses*

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

202

203 *2.8. Statistical analysis*

204 The estimation of the survival ratio was done by calculating the difference between the
205 real value in $\log_{10}$ CFU g^{-1} obtained from the directly measured cell count (in the stomach vessel
206 and the lower duodenum vessel) and the theoretical value in $\log_{10}$ CFU g^{-1} obtained from mass
207 balance calculations based on the number of micro-organisms that should be present at that

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

216 **3. RESULTS AND DISCUSSION**

217

218 *3.1. Drying process*

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

221 The bacterial population was 9.672 ± 0.148 $\log_{10}$ CFU mL^{-1} after the fermentation
222 process. The viable cell count was 6.129±0.033 $\log_{10}$ CFU g^{-1} for SDWO and 6.290±0.133 $\log_{10}$
223 CFU $g^{-1}$for SDWM with a moisture content (dry basis) of 12.24±0.428 and 1.818±0.025,
224 respectively. In comparison, the cell counts in the powders obtained from freeze-drying were
225 7.408±0.341 $\log_{10}$ CFU g^{-1} in FDWO and 7.301±0.301 $\log_{10}$ CFU g^{-1} in FDWM; the moisture
226 content (dry basis) was 2.600±0.18 % and 2.263±0.115 %, respectively. The viable cell count
227 was lower when the material was spray-dried than when it was freeze-dried, regardless of the
228 type of material evaluated. A one-way ANOVA using the Tukey HSD test (results not shown)
229 confirmed a significant difference ($p < 0.05$) between the two types of drying processes in terms
230 of cell count value.[28,30] The Tukey's test did not show significant differences between the two
231 types of carrier material used. Hence, using a carrier material solution with 40% total solids in the

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

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

240

241 *3.2. IViDiS Digestion*

242

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

252 The repeated ANOVA measures of the complete 2^3 factorial design are shown in Table 1.
253 This type of ANOVA is used to evaluate the differences in mean scores for one dependent
254 variable across two or more within-group conditions of an independent variable.[34] The
255 *L. fermentum* K73 survival rate (after gastric and duodenal conditions) over time was the

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

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

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

280 environments, the buffering capacity and the composition of the fermented milk may have a
281 significant protective effect on the survival of the lactobacilli.[35] In our study, the results suggest
282 that an alternative strategy for increasing the survival rate of the micro-organisms would be to use
283 1% fat milk as a food matrix to protect lactobacilli from gastrointestinal conditions (Table 1).

284 When a comparison was performed for the gastric conditions at 120 min specifically (not
285 as a result of time), the results suggested that the powders that had a protective effect on
286 *L. fermentum* K73 were SDWO (Fig. 2A) tested in water and FDWO (Fig. 2E) tested in milk,
287 with the survival rate decreasing by only 2.478 and 2.318 $\log_{10}$ CFU g^{-1}, respectively.
288 Conversely, the powders with the least protective capacity against the harsh conditions in the
289 stomach were SDWM (Fig. 2B) and FDWM (Fig. 2F) tested in water, with decreases of 5.620
290 and 5.505 $\log_{10}$ CFU g^{-1}, respectively. Therefore, the culture medium powders showed a
291 beneficial effect in both food matrices by improving cell survival.

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

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

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

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

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

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

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

328 (60 min) should be similar to that in the stomach vessel between 15 and 30 min after the
329 digestion process began (Fig. 2C, D, G and H), if the bile salt and pancreatic solution does not
330 have any detrimental effects on *L. fermentum* K73. Finally, 90 min after the simulation of
331 digestion began, the bacteria was sampled from the duodenal vessel, because there was sufficient
332 volume from the stomach vessel.

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

345 The powders that showed the best protection for *L. fermentum* K73 were SDWO
346 (−3.044 $\log_{10}$ CFU g^{-1}) and FDWO (−3.8 $\log_{10}$ CFU g^{-1}) tested in water and SDWO (−0.230 $\log_{10}$
347 CFU g^{-1}) and FDWO (−0.379 $\log_{10}$ CFU g^{-1}) tested in milk. The comparison was done at the end
348 of each digestion process (150 min). The micro-organisms survived better during milk digestion
349 (mortality was less than 2 log over time). A larger proportion of cells died either sooner
350 (maltodextrin:sweet sweet whey powders) or later (culture medium powders) during digestion
351 with water.

352 The results shown in Fig. 2 suggest that the culture medium has a strong impact on the
353 survival of the micro-organisms with both drying methods. However, the use of spray-drying as a
354 drying technology provides major benefits to the food industry, given that large quantities of
355 probiotic powder can be produced at a lower cost with spray-drying than with freeze-drying.[44]
356
357 **Fig. 2.** Survival kinetics of *Lactobacillus fermentum* K73 in the stomach (A, B, E, F) and the
358 duodenum (C, D, G, H) with different powders. Values represent means ± 95% confidence
359 intervals from three independent digestions. The red and blue lines represent the survival
360 behaviour when milk and water, respectively, are used as the dissolution medium.
361
362 *3.3. Mathematical models for L. fermentum K73 survival curves from IViDiS*
363
364 The Gompertz model[45] and the Whiting and Buchanan model[31] are the tools that were
365 used to describe the behaviour of *L. fermentum* K73 in the stomach and the duodenum using the
366 kinetics parameters faster inactivation rate (k) and lag phase (L).
367 The goodness of fit of the experimental data was calculated using the Bf, Af, SS, RMSE
368 and R^2. In all survival curves evaluated with the Gompertz model, the Bf and Af had a value of
369 nearly 1 (Bf = 0.993 to 1.016; Af = 1.0001 to 1.022). However, in approximately 10 curves
370 analyzed with the Whiting and Buchanan model, the statistical goodness-of-fit values were
371 farther from 1 (Bf = 0.131; Af = 1.025 to 7.612). These parameters explain the uniformity of the
372 distribution.[46] No curve was calculated for SDWM in intestinal conditions, since the micro-
373 organisms did not survive beyond 15 to 30 min of gastric conditions.
374 In the Gompertz model (Table 2), the R^2 values indicate that the experimental values were
375 generally close to the predicted values in both gastric and intestinal conditions for digestions

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

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

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

400 The Gompertz model kinetic parameters, estimated from the survival curves, provided an
401 idea of the microbial susceptibility to gastrointestinal conditions. During water digestion, the
402 maltodextrin:sweet whey powders presented a faster inactivation rate (*k*) in gastric and intestinal
403 conditions, as well as a longer lag phase (*L*) in gastric conditions, than the culture medium
404 powders did (Fig. 2C). When gastric emptying was complete and the powder had passed into the
405 duodenum, the SDWO showed a longer lag phase and a slower inactivation rate in comparison
406 with the other powders (Table 2, Fig. 2C). The lag phase of FDWO was shorter than that of the
407 other powders, but its inactivation rate was slower than for FDWM (Table 2, Fig. 2G and H).
408 For milk digestion, the culture medium powders had longer lag phases (*L*) and faster
409 inactivation rates (*k*) in gastric conditions than the maltodextrin:sweet whey powders did
410 (Table 2, Figs. 2E and 3A). *Lactobacillus fermentum* K73 showed a longer lag time in duodenal
411 conditions for all types of powders. The inactivation rate in FDWM and SDWO had a negative
412 sign (Table 2), as previously discussed; in these cases, the lag phase should be considered the
413 period prior to growth initiation of the bacterial population under intestinal conditions (Fig. 2C
414 and H).
415 The lag phase and the inactivation rate showed a correlation with the pH change in water
416 and milk digestions. At the beginning of the experiments, the pre-prandial conditions in the
417 stomach were simulated by the addition of a basal gastric solution at pH 2.01 and saliva at
418 pH 7.23. After consumption of the dissolution medium, the pH increased owing to the buffering
419 capacity of the dissolution medium. For water digestion, after the dissolution medium entered the
420 stomach, the pH dropped rapidly from 7.01 to 3.1 in the first 30 min and then gradually decreased
421 to 1.9 by 120 min. For milk digestion, the initial pH was 6.3 (after milk entry) and dropped
422 slowly to 3.9 over the following 60 min; the pH then decreased to 1.9 during the next hour

423 (Fig. 3, dotted line). The drastic change in the pH observed during water digestion decreased the
424 lag phase and increased the inactivation rate of *L. fermentum* K73 in all carrier systems.
425

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

431

432 **4. Conclusion**

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

444 **Conflicts of interest**

445 There are no conflicts of interest to declare.

446

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451

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589

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		

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

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

[c] Significantly 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.

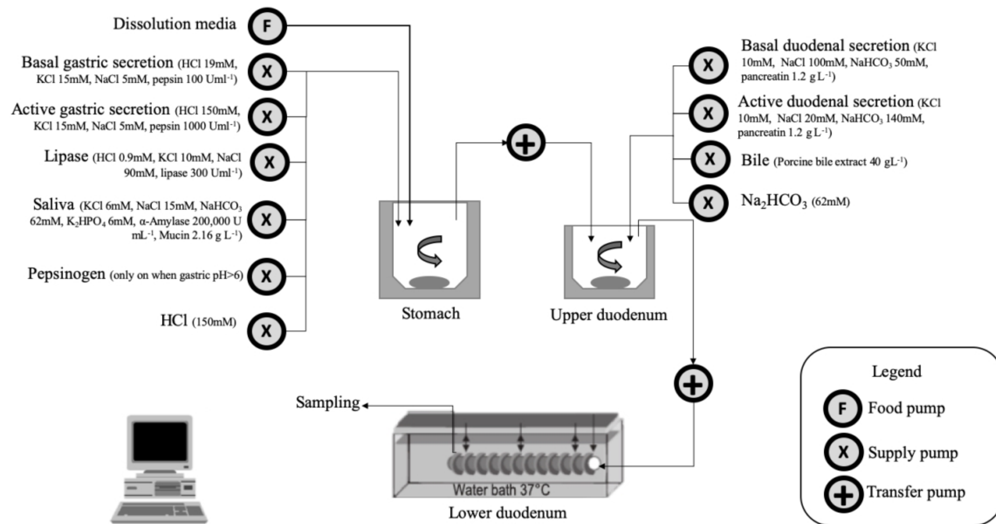

Fig. 1. Scheme of In vitro dynamic digestion system (IViDiS). Adapted from Tompkins et al. (2011).

503x276mm (72 x 72 DPI)

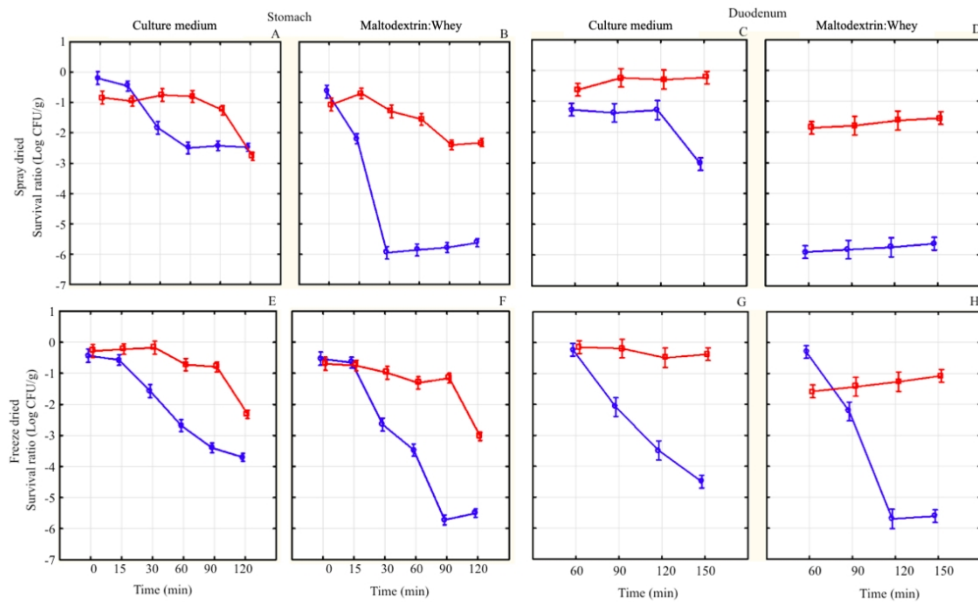

Fig. 2. Survival kinetics of Lactobacillus fermentum K73 in the stomach (A, B, E, F) and the duodenum (C, D, G, H) with different powders. Values represent means ± 95 % confidence intervals from three independent digestions. The red and blue lines represent the survival behaviour using milk and water,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

410x251mm (72 x 72 DPI)

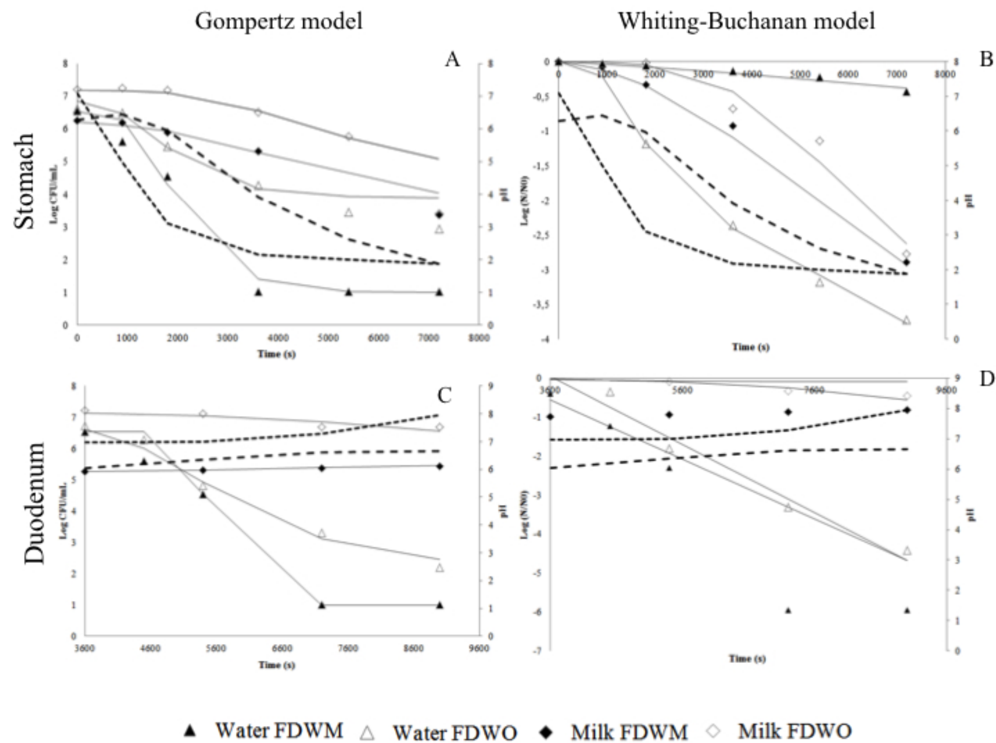

Fig. 3. Experimental values (symbols) and predicted values (continuous line) obtained for the Gompertz model and the Whiting and Buchanan model: culture medium freeze-dried (FDWO) and maltodextrin:sweet whey solution freeze-dried (FDWM) in the stomach (A, B) and the duodenum (C, D) using water (▲ or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

254x190mm (72 x 72 DPI)