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Probiotic survival and in vitro digestion of *L. salivarius* spp. *salivarius* encapsulated by high homogenization pressures and incorporated into a fruit matrix

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Probiotic survival and in vitro digestion of *L. salivarius* spp. *salivarius* encapsulated by high homogenization pressures and incorporated into a fruit matrix.

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1 **Probiotic survival and in vitro digestion of *L. salivarius* spp. *salivarius* encapsulated by high**  
2 **homogenization pressures and incorporated into a fruit matrix.**

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12 **Highlights**

13 Encapsulated *L. salivarius* by HPH was successfully incorporated into an apple matrix.

14 *L. salivarius* content in dried apple was enough to have a probiotic effect.

15 Encapsulation exerted a protective effect after 14 days of storage.

16 Encapsulation exerted a protective effect against simulated gastrointestinal conditions.

17

18 **Abstract**

19 High pressure homogenization allows encapsulating microorganisms ~~in continuous conditions.~~

20 Microencapsulation of probiotic microorganisms may enhance their viability during food  
21 processing, storage and gastrointestinal passage. The aim of this work was to evaluate the probiotic

22 survival and in vitro digestion of non-encapsulated and encapsulated *Lactobacillus salivarius* spp.

23 *salivarius* by ~~homogenization pressures~~ homogenization at 70 MPa, included into an apple matrix

24 by vacuum impregnation, dried by hot air drying and stored during 30 days. *Lactobacillus*

25 *salivarius* spp. *salivarius* was encapsulated with alginate as a coating by homogenization pressures  
26 at 70 MPa and it was added to mandarin juice. Juices with *L. salivarius* spp. *salivarius* encapsulated  
27 and non-encapsulated were used as impregnation liquid to incorporate the probiotic microorganisms  
28 in apple discs. Impregnated apple discs were dried at 40°C during 24 h and water activity, moisture,  
29 counts of viable cells and survival during gastrointestinal simulation for the storage period of 30  
30 days were evaluated. Dried apple discs with encapsulated *L. salivarius* spp. *salivarius* resulted with  
31 higher amount of viable cells than in those non-encapsulated. Gastrointestinal simulation results  
32 evidenced a protection of the microorganism due to the capsule effect.

33

34 **Keywords:**

35 Microencapsulation, hot air drying, high pressure homogenization, probiotic, gastrointestinal  
36 simulation.

37 **1. Introduction**

38 The benefits of probiotic microorganism consumption are increasingly known as scientific  
39 evidences demonstrate more and more that probiotic can protect host against a broad range of  
40 diseases from infection to psychological and even degenerative diseases (Avershina et al., 2017;  
41 Anderson et al., 2017; Pirbaglou et al., 2016).

42 In the development of functional foods with probiotic microorganisms, formulation, processing  
43 and storage should favour microorganism survival. Both, technologies and food matrix must be  
44 aimed at protecting microorganism cells against external stress factors. In addition, once the food is  
45 consumed, the effect of digestion through the gastrointestinal system must be taken into account.

46 The inclusion of probiotic microorganisms into the structure of a food matrix can help to  
47 maintain the integrity of the microbial cells. Moreover, hot air drying technology permits increasing  
48 the product shelf life by reducing the water activity and therefore the development of pathogenic

49 microorganism and conferring specific characteristics (Betoret et al., 2015). Under heat treatment  
50 conditions there is a loss of probiotic viability and a stress response is activated which mechanism  
51 are under study (Cappozzi et al., 2016; Fiocco et al., 2010; De Angelis et al., & Gobetti, 2004).  
52 Afterwards, in a dehydrated regime, the probiotic viability increases while decreasing the water  
53 activity (Ubbink & Krüger, 2006)

54 Microencapsulation of probiotic bacteria can be a very useful strategy to maintain survival rates  
55 and viability higher during processing over the shelf life and after consumption when compared to  
56 non-encapsulated cells (Burgain et al., 2011; Capela et al., 2006). The production of microcapsules  
57 containing probiotics falls into three main categories: extrusion, emulsion and spray drying. Spray  
58 drying technologies are well established, since the size of microcapsules are reduced (few hundred  
59 of microns) and homogeneous (Cook, 2012). However, spray drying impart physical stresses to the  
60 cells such as heat and also increase their exposure to oxygen thus greatly reducing the viability of  
61 the sensitive probiotic bacteria (Lee and Heo, 2000). The emulsion method of encapsulation by  
62 agitation is considered a more gentle process which can be easily monitored thus more viable cells  
63 can survive the encapsulation process (Capela et al., 2005). However, the obtained capsules are  
64 bigger and less homogeneous than in the other technologies. In order to minimize these  
65 disadvantages, high-pressure valve homogenizers can create small droplets by forcing liquids  
66 through a narrow valve under pressure. Homogenizers are already a well established technology in  
67 some food industries such as milk or fruit juices and can operate in continuous thus being not  
68 expensive and facilitating the up-scaling (Ding & Shah, 2009; Calabuig-Jiménez et al. 2019).

69 ~~High pressures homogenization is a reliable, not expensive and quite simple technology that~~  
70 ~~allows encapsulating microorganism in continuous conditions with the advantage of its industrial~~  
71 ~~up-scaling (Ding & Shah, 2009; Calabuig-Jiménez et al. 2019).~~

72 In 2009, Ding and Shah applied 70 or 138 MPa for the encapsulation of *L. salivarius* starting  
73 from an emulsion of sodium alginate and vegetable oil. These process conditions gave  
74 microcapsules having a diameter 85 – 66 µm with an encapsulation yield of 77 % (Ding and Shah,

2009). Patrignani et al., studied in 2017 the application of 50 MPa to encapsulate *L. salivarius*, using sodium alginate in emulsion with vegetable oil. Authors obtained an encapsulation yield of 87 – 83 % and the diameters of the capsule obtained, sphere like and quite rough were < 100 µm (Patrignani et al., 2017). Tolerance to high pressure vary according to the species, strain and suspending mediums used (Abee & Wouters, 1999) but generally, the application of pressure under 100 MPa was not able to induce stresses to the microbial cells (Lanciotti et al., 2007; Burns et al., 2015) and cell death occurred in the range 130 – 800 MPa (De Angelis & Gobetti, 2004).

The aim of this work was to determine the probiotic survival and *in vitro* digestion of *Lactobacillus salivarius* spp. *salivarius* encapsulated by homogenization pressures, included into an apple matrix by vacuum impregnation, dried by hot air drying and stored during 30 days.

## 2. Material and methods

### 2.1. Strain and food materials

The strain used in this study was *Lactobacillus salivarius* spp. *salivarius* CECT 4063 provided by the Spanish Type Culture Collection (CECT, Valencia, Spain) in lyophilized form.

Juice was obtained from mandarin fruits cv. Ortanique (*Citrus sinensis* x *Citrus reticulata*) provided by a local cooperative (Rural S. Vicent Ferrer, Benaguacil, Valencia, Spain). Low pulp juice was prepared following the procedure described in WO/2007/042593 with some modifications (Calabuig-Jiménez et al., 2019).

Apples (cv. *Granny Smith*) were purchased from a local market. In this experimental study apple discs with 5 mm thick and 20 – 60 mm of internal and external diameter were used.

### 2.2. Microencapsulation

99 To microencapsulate *L. salivarius* spp. *salivarius* the method described by (Ding & Shah, 2009)  
100 was followed with some modifications (Calabuig-Jiménez et al., 2019). Briefly, an emulsion  
101 containing 25 mL of microorganism with  $10^9$  CFU/ml, 100 mL of sodium alginate (3%) (Sigma-  
102 aldrich, Steinheim, Germany), 1 mL of tween 80 (Sharlau, Sentmenat, Spain) and 200 mL of  
103 sunflower oil was homogenized in two passes at 70 MPa with a homogenizer (Panda Plus Niro  
104 Soavi, Parma, Italy). After homogenization calcium chloride 0.1 M (Sigma-aldrich, Steinheim,  
105 Germany) was added and microcapsules were isolated by centrifugation at 7700 x g for 15 min at  
106 10°C (Beckman Coulter Avanti™ J-25, California, United States).

107

### 108 *2.3. Mandarin juice with probiotic microorganisms*

109 Mandarin juices with *L. salivarius* spp. *salivarius* encapsulated and not were used as  
110 impregnation liquids. Mandarin juice with non-encapsulated *L. salivarius* spp. *salivarius* was  
111 prepared following the methodology described in 13–(Betoret et al., 2017) following inoculation  
112 with  $10^9$  CFU/mL and incubation at 37°C for 24 h. Mandarin juice with microencapsulated *L.*  
113 *salivarius* spp. *salivarius* was prepared by adding microcapsules prepared as described above into  
114 the juice at a ratio of 1.45 juice/microcapsules (w/w) (Calabuig-Jiménez et al., 2019). The mixture  
115 was maintained in agitation at room temperature for 1 h.

116

### 117 *2.4. Process to produce L. salivarius* spp. *salivarius* enriched dried apple

118 Dried apple discs with *L. salivarius* spp. *salivarius* encapsulated and not, were obtained  
119 following the methodology described previously (Betoret et al., 2012). A vacuum pressure of 50  
120 mbar for 10 min was applied to immersed fresh apple discs following an atmospheric pressure  
121 restoration during further 10 min. Impregnated apple discs were dried using an air drier (POL-EKO

122 model CLW400 TOP, Controltecnica Instrumentación Científica, S.L., Madrid, Spain) at 40 °C for  
123 24 h. The values provided are the average of three replicates.

124

## 125 2.5. Physicochemical characterization

126 Impregnated and dried apple discs were characterized by measuring pH, water activity and  
127 moisture content. To determine pH, a pHmeter (Crison GLP21, Barcelona, Spain) was used. Water  
128 activity was measured using a dew point hygrometer (DECAGÓN Aqualab CX-2, Washington,  
129 United States). Water content was quantified by vacuum drying at 60 °C until a constant weight.  
130 The values provided are the average of three replicates.

131

## 132 2.6. Microbial content

133 *L. salivarius* spp. *salivarius* was determined in MRS agar (Scharlab, Barcelona, Spain) on  
134 double layer incubated 24 h at 37 °C. In encapsulated samples the first dilution was done in  
135 phosphate buffer solution stirred during 30 min. Values provided are the average of four replicates.

136

## 137 2.7. Gastrointestinal digestion

138 The effect of the gastrointestinal digestion on the microorganism survival was determined  
139 following the procedure described in (Calabuig-Jiménez et al., 2019).  $T_i$  was the *L. salivarius* spp.  
140 *salivarius* content;  $t_i$  was a moment during the gastrointestinal digestion. Briefly, ten grams of  
141 sample were mixed with 10 mL of pepsine (0.6% w/v) (Sigma-aldrich, Steinheim, Germany)  
142 adjusted to pH 3 with HCl 4 M ( $t_1 - T_1$ ) and mixed at 37°C for 90 min ( $t_2 - T_2$ ). Phosphate buffer  
143 solution (pH 8) with 10% of bile (Sigma-aldrich, Steinheim, Germany) was added ( $t_3 - T_3$ ).  
144 Phosphate buffer with 0.3% of bile and 0.1% pancreatine (Sigma-aldrich, Steinheim, Germany) was



145 added following an incubation at 37°C for 90 min ( $t_4 - T_4$ ). The results provided are the average of  
146 four replicates.

147

## 148 *2.8.Storage*

149 Dried samples were stored in closed opaque plastic bags at room temperature and analyses were  
150 performed weekly during 30 days.

151

## 152 *2.9.Statistical analysis*

153 The significant effect of the process variables, at 95% confidence level, was determined with an  
154 ANOVA analysis using Statgraphics centurion XVI software (StatPoint Technologies, Virginia,  
155 US).

156

# 157 **3. Results and discussion**

158

## 159 *3.1.Physicochemical characterization*

160 Physicochemical characteristics of the impregnated and dried apple discs with *L. salivarius* spp.  
161 *salivarius* were evaluated during 30 days of storage (table 1). Generally, the physicochemical  
162 properties of dried apple with *L. salivarius* spp. *salivarius* encapsulated and not, were maintained  
163 similar during all the storage time. The pH values of dried apple with encapsulated *L. salivarius*  
164 spp. *salivarius* were higher, showing less variability than that obtained in samples with non-  
165 encapsulated microorganisms. Samples with encapsulated *L. salivarius* spp. *salivarius* had less  
166 amount of mandarin juice impregnated than those samples with non-encapsulated microorganisms.  
167 Additionally, the encapsulation process could decrease the activity of *L. salivarius* spp. *salivarius*

168 resulting in a lower fermentation activity of the microencapsulated cells which would produce less  
169 acidic compounds (Bilenler et al., 2017; Ribeiro et al., 2014). At the end of the storage there were  
170 not differences between both samples.

171 The rate of food reactions and spoilage microorganisms activity is reduced with lower moisture  
172 content, being retarded and even inhibited with a water activity as or below 0.3 (Smith, 2008). In  
173 our case, despite obtained water activity was higher than 0.3, ~~any~~ moulds or harmful bacteria were  
174 not developed during the storage. Our results were similar to that obtained previously by (Betoret et  
175 al., 2012). Water activity values ranged between 0.48 and 0.54 in both cases, with more variability  
176 observed in samples with encapsulated *L. salivarius* spp. *salivarius* and a tendency to increase with  
177 storage time. In samples with non-encapsulated *L. salivarius* spp. *salivarius*, the values of water  
178 activity were maintained practically constant during 21 days from which had a tendency to increase.  
179 The same behaviour was observed for moisture content values. The presence of oil coming from the  
180 emulsion to encapsulate *L. salivarius* spp. *salivarius* in the apple slices could difficult the water flux  
181 during drying, resulting in a less homogeneous product. An unequal distribution of water content  
182 during drying could cause further water migrations during storage, explaining then the differences  
183 observed between both samples.

184

### 185 3.2. *Effect of technological operations on probiotic survival*

186 Microbial content of the encapsulated and non-encapsulated *L. salivarius* spp. *salivarius* in the  
187 mandarin juice, in the impregnated apple and in the impregnated and dried apple are shown in  
188 figure 1. The content of encapsulated *L. salivarius* spp. *salivarius* in mandarin juice was managed to  
189 be the same as that obtained in samples with non-encapsulated microorganisms in order to compare  
190 its degradation during the processing. The obtained results were similar to that obtained in previous  
191 studies (Calabuig-Jiménez et al., 2019; Betoret et al., 2012; Betoret et al., 2017). The amount of  
192 mandarin juice with *L. salivarius* spp. *salivarius* encapsulated and not, incorporated into the apple,

193 using vacuum impregnation, was estimated by mass balances using the equation 1. Calculated and  
194 experimental obtained values were  $8.71 \pm 0.02 \text{ Log CFU/g}_{IV}$  -  $7.23 \pm 0.02 \text{ Log CFU/g}_{IV}$  and  $7.62 \pm$   
195  $0.04 \text{ Log CFU/g}_{IV}$  -  $7.3414 \pm 0.0014 \text{ Log CFU/g}_{IV}$  in samples with encapsulated *L. salivarius* spp.  
196 *salivarius* and not, respectively. Similar calculated and experimental values, as in samples with non-  
197 encapsulated *L. salivarius* spp. *salivarius*, indicated that the liquid flux into the intracellular pores  
198 of apple was homogeneous and only due to pressure gradients. A homogeneous vacuum  
199 impregnation means that all components of the mandarin juice were incorporated equally. Pressure  
200 levels applied during the vacuum impregnation operation in this study do not affect significantly  
201 microorganisms<sup>2</sup> survival (Betoret et al., 2012). Thus, the differences observed between calculated  
202 and experimental values in samples with encapsulated *L. salivarius* spp. *salivarius* indicated that the  
203 vacuum impregnation operation was not homogeneous and this could be due to three possible  
204 reasons: a not homogeneous distribution of the encapsulated microorganisms, an accumulation of  
205 microorganism in some areas of the apple structure where the pore sizes are smaller than the  
206 capsules, an irregular flows of juice through the structure due to local pressure gradients. ~~the~~  
207 ~~particle size of the capsules was bigger than some porous channels in the cellular structure of apple,~~  
208 ~~the suspended particles were not stable in the cloud and could precipitate or blocked the juice flow~~  
209 ~~inside the porous matrix, the rheological properties of the liquid media did not assure an~~  
210 ~~homogeneous flow inside the apple structure (Castagnini et al., 2015).~~

$$x_{aIV} = (x_{mJ} \cdot X \cdot (\rho_{mJ} / \rho_{fa})) / (1 + X \cdot (\rho_{mJ} / \rho_{fa})) \text{ equation 1}$$

212 Where:

213 **x**; microorganism content (CFU/g or CFU/ml)

214 **X**; incorporated liquid ( $\text{cm}^3/\text{cm}^3_{\text{sample}}$ )

215  **$\rho$** ; density ( $\text{g}/\text{cm}^3$ )

216 **aIV**; impregnated apple

217 **fa**; fresh apple

218 **mJ**; mandarin juice

219 The content of *L. salivarius* spp. *salivarius* encapsulated and not in dried apple samples was  
220 significantly different and high enough to have a potential probiotic effect (International Dairy  
221 Federation, 1992). In order to calculate the degradation of microorganism during drying it is  
222 necessary that quantities of microorganisms are expressed in the same basis. Thus, the total losses  
223 degradation of *L. salivarius* spp. *salivarius* encapsulated and not during air drying operation was  
224 ~~were~~ 6.20 – 6.38 Log CFU/gIV respectively. Considering the initial values of microorganisms, the  
225 degradation of *L. salivarius* spp. *salivarius* encapsulated and not during air drying operation was  
226 0.85 and 0.87 respectively. Heat damage, water losses linked to structural changes and oxidation  
227 reactions due to the air exposure affect both cellular plant tissues and microbial cells. Excessive  
228 heat unfolds the higher order structure of macromolecules such as protein and nucleic acid, breaks  
229 the linkage between monomeric units and eventually causes the destruction of the monomeric units  
230 (Corcoran et al., 2008; Santivarangkna et al., 2008). Water losses linked to structural modifications  
231 and oxidation reactions mainly affects the cytoplasmic membrane of microbial cells by changing its  
232 fluidity or the physical state as well as causing lipid peroxidation (Crowe et al., 1992). Cells  
233 entrapped within the droplets formed by alginate would obtain additional protection by the capsule.  
234 However, as according to (Fu & Chen, 2011), the protection of cell viability during drying given by  
235 this type of microencapsulation is quite limited. In this study, a mild drying was employed, with an  
236 air temperature of 40 °C in order to limit drying stress in bacterial cells but more oxidation reactions  
237 could be promoted due to the long air exposure time.

238

### 239 3.3. Probiotic content during storage time

240 The content of *L. salivarius* spp. *salivarius* encapsulated and not, stored during 30 days at room  
241 temperature and maintained in closed opaque plastic bags, was determined (table 2). During the first  
242 14 days of storage a decrease in 60 % of the microorganisms<sup>2</sup> content was observed. This results  
243 agree with (Weinbreck et al., 2010; Moumita et al., 2017) that observed a decrease of 3-5 log in the

244 microorganism content encapsulated and not, after 14 days of storage. From this point, significant  
245 differences were observed between both samples, with an improvement in the microorganism  
246 survival in encapsulated samples of 39 versus 19 % of non-encapsulated at the end of storage.

247 During storage, cell survival is particularly affected when the food matrix has an elevated water  
248 activity ( $a_w > 0.25$ ) (Teixeira et al., 1995). Storage temperature and the presence of atmospheric  
249 oxygen might also contribute to reductions in viable cell amounts (Anal & Singh, 2007). Our  
250 results, showed up that capsules were not able to protect significantly *L. salivarius* spp. *salivarius*  
251 from degradation reactions during the first 14 days of storage. As pointed out by (Dianawati &  
252 Shah, 2011) alginate is a porous material that is not able to isolate encapsulated microorganisms  
253 from water migrations. According to (Crittenden et al., 2006) presence of atmospheric oxygen was  
254 not a significant factor in the microorganisms degradation encapsulated in alginate and maintained  
255 at room temperature during storage. However, after 14 days of storage, capsules were able to  
256 protect *L. salivarius* spp. *salivarius* from degradation reactions.

257

#### 258 3.4. Gastrointestinal simulation

259 In order to exert a positive effect on the host, probiotic microorganisms should maintain their  
260 active form during digestion process, being able to survive the action of lytic enzyme and adverse  
261 pH until reaching the target point. Moreover, in the case of encapsulated microorganisms the  
262 capsule must be a protection from adverse conditions but should release them at the appropriate  
263 time and place in the organism. The microbial content after each simulated gastro-intestinal  
264 digestion step is shown in table 3.  $T_0$  is the *L. salivarius* spp. *salivarius* content in dried apple.  $T_1$   
265 and  $T_2$  means the microorganism content after simulated stomach conditions, acid pH change and  
266 peristaltic movements respectively.  $T_3$  and  $T_4$  are the microorganism content after the duodenal  
267 shock and intestinal juice mixing.

268 *L. salivarius* spp. *salivarius* demonstrated to have a potential effect against *Helicobacter pylori*  
269 infection. Thus, microorganism survival at gastroduodenal stage, in order to have a potential effect

270 against *H. pylori*, and survival at intestinal step, in order to have a potential probiotic effect, are  
271 both key points to consider.

272 The statistical analysis revealed that all variables studied; the encapsulation procedure, the stage  
273 at the simulated gastrointestinal digestion and the storage time had a significant effect ( $p \leq 0.05$ ) on  
274 *L. salivarius* spp. *salivarius* survival. Generally, encapsulated *L. salivarius* spp. *salivarius*  
275 demonstrated higher resistance to gastrointestinal simulation as compared to their free form. Total  
276 microorganisms content and survival percentage of encapsulated *L. salivarius* spp. *salivarius* was  
277 higher than non-encapsulated one. Degradation tendency of the microorganisms encapsulated and  
278 not was different at each stage of the simulated gastrointestinal process as well as during the  
279 storage. Obtained results were similar to that obtained in other studies (Ribeiro et al., 2014;  
280 Yonekura et al., 2014). Survival of encapsulated *L. salivarius* spp. *salivarius* was mainly affected  
281 by the acidic environment created at  $t_1$  and the addition of bile at  $t_3$ . Moreover, survival of  
282 microorganisms decreased with storage time at gastrointestinal stages  $t_2$ ,  $t_3$  and  $t_4$  but not at  $t_1$  at  
283 which survival percentage remained practically constant. The results obtained in literature on the  
284 protective effect of alginate capsules against acidic environmental conditions are contradictory.  
285 While in some cases, the capsule created protects the microorganisms against acidic conditions  
286 (Ding & Shah, 2009; Cook et al., 2011) in others capsule it does not provide any additional  
287 protection (Hansen et al., 2002). As explained by (Cook et al., 2012) it seems that the method used  
288 to make the capsule significantly influences the final result. In our case, the capsule conferred a  
289 limited protection. A porous capsule surface and its degradation during storage could explain the  
290 observed decrease in the *L. salivarius* spp. *salivarius* survival with storage time. Non-encapsulated  
291 *L. salivarius* spp. *salivarius* was affected by the acidic environment created at  $t_1$  and the addition of  
292 lytic enzymes at  $t_4$ . In this case, survival of microorganisms decreased with storage time mainly at  
293  $t_3$ .

294 It is remarkable the increase in microorganisms content observed at day 14 in encapsulated *L.*  
295 *salivarius* spp. *salivarius* and not, and at day 21 in non-encapsulated *L. salivarius* spp. *salivarius*.

296 As pointed out by (Santivarangkna et al., 2008) upon sudden changes in temperature, osmotic  
297 pressure or pH, a microbial cell is able to adapt itself to the new environment by adjusting the  
298 metabolic flow and genetic expression. After the acidic stress conditions created around cells at pH  
299 3.5 (~~Jin et al., 2012~~) Jin et al. (2012) observed a significant increase in the acid tolerance response  
300 mechanism which would promote their growth when optimal conditions are restored.

301

#### 302 **4. Conclusion**

303 Incorporation of encapsulated *L. salivarius* spp. *salivarius* using homogenization pressures into  
304 an apple structure by vacuum impregnation operation was successfully done. In spite of the  
305 microorganisms losses during hot air drying operation, the number of *L. salivarius* spp. *salivarius* in  
306 the impregnated and dried apple was enough high to have a potential beneficial effect.

307 Capsules were able to significantly protect *L. salivarius* spp. *salivarius* during the simulated  
308 gastrointestinal digestion and storage. However, further fundamental studies on morphology and  
309 degradation of capsules during processing and storage would be necessary in order to enhance the  
310 microorganisms' protection and thus the industrial utility.

311

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316

#### 317 **6. References**

318 Abee, T., & Wouters, J. A. (1999) International Journal of Food Microbiology, 50, 65-91.

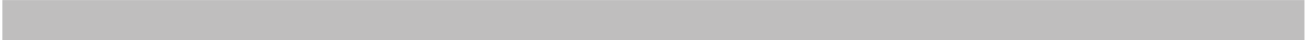
- 319 Anal, A. K., & Singh, H. (2007). Recent advances in microencapsulation of probiotics for industrial  
320 applications and targeted delivery. *Trends in Food Science and Technology*, 18, 240–251.
- 321 Anderson, J. L., Milles, C., & Tierney, A. C. (2017). Effect of probiotics on respiratory,  
322 gastrointestinal and nutritional outcomes in patients with cystic fibrosis: a systematic review.  
323 *Journal of Cystic Fibrosis*, 16(2), 186-197.
- 324 Avershina, E., Rubio, R. C., Lundgård, K., Martinez, G. P., Collado, M. C., Storrø, O., Øien, T.,  
325 Dotterud, C.K., Johnsen, R., & Rudi, K. (2017). Effect of probiotics in prevention of atopic  
326 dermatitis is dependent on the intrinsic microbiota at early infancy. *Journal of Allergy and*  
327 *Clinical Immunology*, 139(4), 1399-1402.
- 328 Betoret E., Betoret N., Rocculi P., & Dalla Rosa M. 2015. Strategies to improve food functionality:  
329 structure-property relationships on high pressure homogenization, vacuum impregnation and  
330 drying operations. *Trends in Food Science and Technology*, 46, 1-12.
- 331 Betoret, E., Calabuig-Jimenez, L., Patrignani, F., Lanciotti, R., & Dalla Rosa, M. (2017). Effect of  
332 high pressure processing and trehalose addition on functional properties of mandarin juice  
333 enriched with probiotic microorganisms. *LWT-Food Science and Technology*, 85, 418-422.
- 334 Betoret, E., Sentandreu, E., Betoret, N., Codoñer-Franch, P., Valls-Bellés, V., & Fito, P. (2012).  
335 Technological development and functional properties of an apple snack rich in flavonoid from  
336 mandarin juice. *Innovative Food Science & Emerging Technologies*, 16, 298-304.
- 337 Bilenler, T., Karabulut, I., & Candogan, K. (2017). Effects of encapsulated starter cultures on  
338 microbial and physicochemical properties of traditionally produced and heat treated sausages  
339 (sucuks). *LWT-Food Science and Technology*, 75, 425-433.
- 340 Burns, P. G., Patrignani, F., Tabanelli, G., Vinderola, G. C., Siroli, L., Reinheimer, J. A., Lanciotti,  
341 R. (2015). Potential of high pressure homogenisation on probiotic Caciotta cheese quality and  
342 functionality. *Journal of Functional Foods*, 13, 126–136.
- 343 Burgain, J., Gaiani, C., Linder, M., & Scher, J. (2011). Encapsulation of probiotic living cells: from  
344 laboratory scale to industrial applications. *Journal of Food Engineering*, 104, 467-483.
- 345 Calabuig-Jiménez L., Betoret E., Betoret N., Patrignani F., Barrera C., Seguí L., Lanciotti R., &  
346 Dalla Rosa M. 2019. High pressures homogenization to microencapsulate *L. salivarius* spp.  
347 *salivarius* in mandarin juice. Probiotic survival and in vitro digestion. *Journal of Food*  
348 *Engineering*, 240, 43-48.
- 349 Capela, P., Hay, T. K. C., & Shah, N. P. (2005). Effect of cryoprotectants, prebiotics and  
350 microencapsulation on survival of probiotic organisms in yoghurt and freeze-dried yoghurt.  
351 *Food Research International*, 39, 203–211.
- 352 Capozzi, V., Arena, M.P., Russo, P., Spano, G. & Fiocco, D. (2016). Stressors and Food  
353 Environment: Toward Strategies to Improve Robustness and Stress Tolerance in Probiotics,



- 354 Probiotics, Prebiotics, and Synbiotics, Chapter 16, Pages 245-256. Academic Press, Cambridge,  
355 United States.
- 356 Castagnini J.M., Betoret E., Betoret N., & Fito P. 2015. Vacuum impregnation and air drying  
357 temperature effect on individual anthocyanins and antiradical capacity of blueberry juice  
358 included into an apple matrix. *LWT-Food Science and Technology*, 64(2), 1289-1296.
- 359 Cook, M., Tzortzis, G., Charalampopoulos, D. & Khutoryanskiy, V.V. (2011). Production and  
360 evaluation of dry alginate-chitosan microcapsules as an enteric delivery vehicle for probiotic  
361 bacteria. *Biomacromolecules*, 12, 2834-2840.
- 362 Cook, M., Tzortzis, G., Charalampopoulos, D. & Khutoryanskiy, V.V. (2012) Microencapsulation  
363 of probiotics for gastrointestinal delivery. *Journal of Controlled Release*, 162, 56-67.
- 364 Corcoran, B. M., Stanton, C., Fitzgerald, G., & Ross, R. P. (2008). Life under stress: the probiotic  
365 stress response and how it may be manipulated. *Current pharmaceutical design*, 14(14), 1382-  
366 1399.
- 367 Crittenden, R., Weerakkody, R., Sanguansri, L. & Augustin, M.A. (2006). Synbiotic microcapsules  
368 that enhance microbial viability during nonrefrigerated storage and gastrointestinal transit.  
369 *Applied and Environmental Microbiology*, 72(3), 2280-2282. doi:10.1128/AEM.72.3.2280-  
370 2282.2006.
- 371 Crowe, J. H., Hoekstra, F. A., & Crowe, L. M. (1992). Anhydrobiosis. *Annual Review of*  
372 *Physiology*, 54(1), 579-599.
- 373 De Angelis, M., & Gobbetti, M. (2004). Environmental stress response in *Lactobacillus*: A review.  
374 *Proteomics*, 4, 106–122.
- 375 Dianawati, D. & Shah, N.P. (2011) Enzyme stability of microencapsulated *Bifidobacterium*  
376 *animalis* ssp. *lactis* Bb12 after freeze drying and during storage in low water activity at room  
377 temperature. *Journal of Food Science*, 76(6), M463-M471.
- 378 Ding, W. K., & Shah, N. P. (2009). Effect of homogenization techniques on reducing the size of  
379 microcapsules and the survival of probiotic bacteria therein. *Journal of Food Science*, 74(6),  
380 M231-M236.
- 381 Fiocco, D., Capozzi, V., Collins, M., Gallone, A., Hols, P., Guzzo, J., Weidmann, S., Rieu, A.,  
382 Msadek, T., Spano, G., 2010. Characterization of the CtsR stress response regulon in  
383 *Lactobacillus plantarum*. *Journal of Bacteriology*, 192, 896–900.
- 384 Fu, N., & Chen, X. D. (2011). Towards a maximal cell survival in convective thermal drying  
385 processes. *Food Research International*, 44(5), 1127-1149.
- 386 Hansen, L.T., Allan-Wojtas, P.M., Jin, Y.L. & Paulson, A.T. (2002). Survival of Ca-alginate  
387 microencapsulated *Bifidobacterium* spp. in milk and simulated gastrointestinal conditions. *Food*  
388 *Microbiology*, 19, 35-45.

- 389 International Dairy Federation (IDF/FIL), 1992. Physiological and functional properties of  
390 probiotics. *Bulletin of the International Dairy Federation*; 272: 17-22.
- 391 Jin, J., Zhang, B., Guo, H., Cui, J., Jiang, L., Song, S., Sun, M., & Ren, F. (2012). Mechanism  
392 analysis of acid tolerance response of *Bifidobacterium longum* subsp. *longum* BBMN 68 by  
393 gene expression profile using RNA-sequencing. *PLoS One*, 7(12), e50777
- 394 Lanciotti, R., Patrignani, F., Iucci, L., Saracino, P., & Guerzoni, M. E. (2007). Potential of high  
395 pressure homogenization in the control and enhancement of proteolytic and fermentative  
396 activities of some *Lactobacillus* species. *Food Chemistry*, 102, 542–550.
- 397 Lee KY, Heo TR. 2000. Survival of *Bifidobacterium longum* immobilized in calcium alginate beads  
398 in simulated gastric juices and bile salt solution. *Applied Environmental Microbiology*, 66:869–  
399 73.
- 400 Moumita, S., Goderska, K., Johnson, E. M., Das, B., Indira, D., Yadav, R., Kumari, S., &  
401 Jayabalan, R. (2017). Evaluation of the viability of free and encapsulated lactic acid bacteria  
402 using in-vitro gastro intestinal model and survivability studies of synbiotic microcapsules in dry  
403 food matrix during storage. *LWT-Food Science and Technology*, 77, 460-467.
- 404 Patrignani, F., Siroli, L., Serrazanetti, D.I., Braschi, G., Betoret, E., Reinheimer, J.A., Lanciotti, R.,  
405 2017. Microencapsulation of functional strains by high pressure homogenization for a potential  
406 use in fermented milk. *Food Research International*, 97, 250–257.
- 407 Pirbaglou, M., Katz, J., de Souza, R. J., Stearns, J. C., Motamed, M., & Ritvo, P. (2016). Probiotic  
408 supplementation can positively affect anxiety and depressive symptoms: a systematic review of  
409 randomized controlled trials. *Nutrition research*, 36(9), 889-898.
- 410 Ribeiro, M. C. E., Chaves, K. S., Gebara, C., Infante, F. N., Grosso, C. R., & Gigante, M. L. (2014).  
411 Effect of microencapsulation of *Lactobacillus acidophilus* LA-5 on physicochemical, sensory  
412 and microbiological characteristics of stirred probiotic yoghurt. *Food research international*, 66,  
413 424-431.
- 414 Santivarangkna, C., Kulozik, U., & Foerst, P. (2008). Inactivation mechanisms of lactic acid starter  
415 cultures preserved by drying processes. *Journal of Applied Microbiology*, 105(1), 1-13.
- 416 Smith, P. G. (2008). Applications of fluidization to food processing introduction (pp. 116-117).  
417 Wiley-Blackwell.
- 418 Teixeira, P. C., Castro, M. H., Malcata, F. X., & Kirby, R. M. (1995). Survival of *Lactobacillus*  
419 *delbrueckii* ssp. *bulgaricus* following spray-drying. *Journal of Dairy Science*, 78(5), 1025-1031.
- 420 Ubbink, J., Krueger, J., 2006. Physical approaches for the delivery of active ingredients in foods.  
421 *Trends in Food Science & Technology*. 17, 244–254.
- 422 Weinbreck, F., Bodnár, I., & Marco, M. L. (2010). Can encapsulation lengthen the shelf-life of  
423 probiotic bacteria in dry products?. *International journal of food microbiology*, 136(3), 364-367.

424 Yonekura, L., Sun, H., Soukoulis, C. & Fisk, I. (2014). Microencapsulation of *Lactobacillus*  
425 *acidophilus* NCIMB 701748 in matrices containing soluble fibre by spray drying: Technological  
426 characterization, storage stability and survival after *in vitro* digestion. *Journal of Functional*  
427 *Foods*, 6, 205-214.



**Figure 1.** *Microorganism content expressed in Log CFU/g with encapsulated and non-encapsulated L. salivarius spp. salivarius. Plotted results are the average  $\pm$  standard deviation of four replicates.*

**Table 1.** Physicochemical properties of the dried apple with encapsulated and non-encapsulated *Lactobacillus salivarius* spp. *salivarius* during the storage time. Mean  $\pm$  standard deviation of three replicates.

Day	pH		$a_w$		Moisture (kg <sub>water</sub> /kg <sub>dried</sub> )	
	Encapsulated	Non-encapsulated	Encapsulated	Non-encapsulated	Encapsulated	Non-encapsulated
1	3.44 $\pm$ 0.05 <sup>ab</sup>	3.21 $\pm$ 0.05 <sup>a</sup>	0.516 $\pm$ 0.002 <sup>c</sup>	0.516 $\pm$ 0.002 <sup>c</sup>	0.107 $\pm$ 0.002 <sup>a</sup>	0.128 $\pm$ 0.006 <sup>ab</sup>
7	3.48 $\pm$ 0.03 <sup>abc</sup>	3.16 $\pm$ 0.08 <sup>a</sup>	0.487 $\pm$ 0.006 <sup>a</sup>	0.516 $\pm$ 0.002 <sup>c</sup>	0.128 $\pm$ 0.012 <sup>b</sup>	0.124 $\pm$ 0.006 <sup>ab</sup>
14	3.39 $\pm$ 0.09 <sup>a</sup>	3.36 $\pm$ 0.08 <sup>b</sup>	0.534 $\pm$ 0.002 <sup>d</sup>	0.500 <del>3</del> $\pm$ 0.002 <sup>a</sup>	0.125 $\pm$ 0.003 <sup>b</sup>	0.117 $\pm$ 0.003 <sup>a</sup>
21	3.55 $\pm$ 0.12 <sup>bc</sup>	3.43 $\pm$ 0.04 <sup>b</sup>	0.544 $\pm$ 0.002 <sup>e</sup>	0.51 <del>2</del> <del>6</del> $\pm$ 0.002 <sup>b</sup>	0.129 $\pm$ 0.006 <sup>b</sup>	0.12 $\pm$ 0.06 <sup>a</sup>
30	3.6 $\pm$ 0.02 <sup>c</sup>	3.6 $\pm$ 0.02 <sup>c</sup>	0.505 $\pm$ 0.002 <sup>b</sup>	0.53 <del>3</del> <del>2</del> <del>5</del> $\pm$ 0.003 <sup>d</sup>	0.132 $\pm$ 0.006 <sup>c</sup>	0.136 $\pm$ 0.003 <sup>b</sup>

<sup>abc...</sup> Values with different superscript letters within the same column are significantly different ( $p \leq 0.05$ ).

**Table 2.** Microbial count (Log CFU/g<sub>dried</sub>) of encapsulated and non-encapsulated dried apple during the storage time. Number in brackets indicates the survival in percentage respect the first day. Mean  $\pm$  standard deviation of four replicates.

	<b>Day 1</b>	<b>Day 7</b>	<b>Day 14</b>	<b>Day 21</b>	<b>Day 30</b>
Encapsulated	7.19 $\pm$ 0.07 <sup>a</sup> (100)	5.85 $\pm$ 0.12 <sup>a</sup> (81.3 $\pm$ 1.7)	3.03 $\pm$ 0.06 <sup>a</sup> (42.2 $\pm$ 0.9)	2.94 $\pm$ 0.03 <sup>a</sup> (40.9 $\pm$ 0.5)	2.78 $\pm$ 0.14 <sup>a</sup> (39 $\pm$ 2)
Non-encapsulated	6.71 $\pm$ 0.08 <sup>b</sup> (100)	5.26 $\pm$ 0.09 <sup>b</sup> (78.2 $\pm$ 1.4)	2.89 $\pm$ 0.09 <sup>b</sup> (43.1 $\pm$ 1.4)	2.37 $\pm$ 0.05 <sup>b</sup> (35.4 $\pm$ 0.7)	1.3 $\pm$ 0.2 <sup>b</sup> (19 $\pm$ 3)

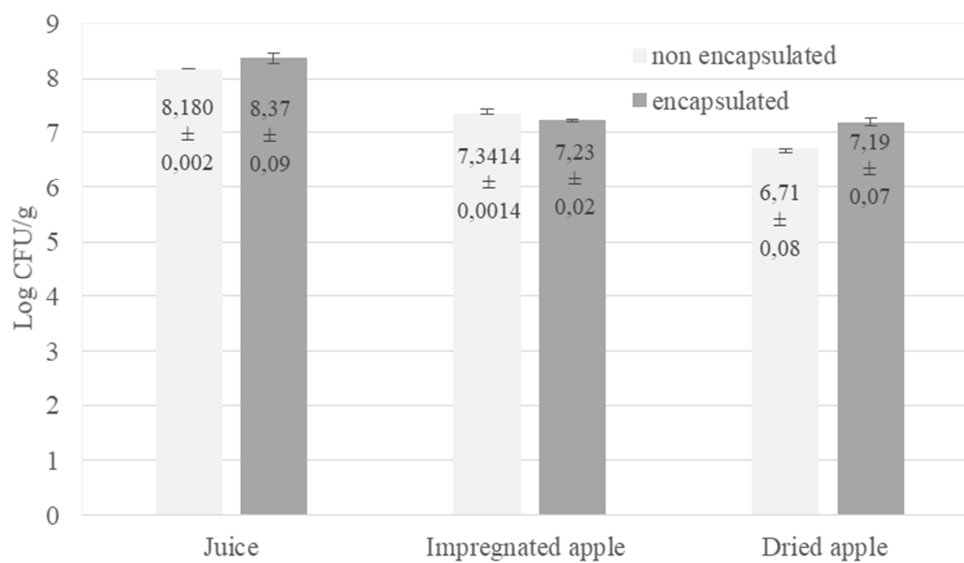
<sup>abc...</sup> Values with different superscript letters within the same column are significantly different ( $p \leq 0.05$ ).

**Table 3.** Microbial content (Log CFU/g<sub>dried</sub>) of encapsulated and non-encapsulated dried apple with *L. salivarius* at the beginning ( $T_0$ ) and at each phase of the gastrointestinal simulation process ( $T_1$  to  $T_4$ ) and over the storage time. Number in brackets indicates the survival in percentage respect the initial content. Mean  $\pm$  standard deviation of four replicates.

		Day 0	Day 7	Day 14	Day 21	Day 30
Encapsulated	$T_0$	7.19 $\pm$ 0.07 <sup>h</sup> <sub>B</sub> (100)	5.85 $\pm$ 0.12 <sup>g</sup> <sub>B</sub> (100)	3.03 $\pm$ 0.06 <sup>cd</sup> <sub>B</sub> (100)	2.94 $\pm$ 0.03 <sup>f</sup> <sub>B</sub> (100)	2.83 $\pm$ 0.14 <sup>f</sup> <sub>B</sub> (100)
	$T_1$	6.03 $\pm$ 0.09 <sup>f</sup> <sub>B</sub> (83.7 $\pm$ 0.8)	5.58 $\pm$ 0.02 <sup>f</sup> <sub>B</sub> (96 $\pm$ 2)	3.71 $\pm$ 0.07 <sup>g</sup> <sub>A</sub> (122 $\pm$ 2)	2.67 $\pm$ 0.09 <sup>e</sup> <sub>B</sub> (90.7 $\pm$ 3)	2.38 $\pm$ 0.09 <sup>ef</sup> <sub>B</sub> (85.6 $\pm$ 1.3)
	$T_2$	5.81 $\pm$ 0.07 <sup>e</sup> <sub>B</sub> (80.8 $\pm$ 0.4)	5.44 $\pm$ 0.06 <sup>ef</sup> <sub>B</sub> (94 $\pm$ 3)	3.84 $\pm$ 0.04 <sup>h</sup> <sub>B</sub> (127 $\pm$ 2)	2.32 $\pm$ 0.13 <sup>c</sup> <sub>A</sub> (79 $\pm$ 4)	2.0 $\pm$ 0.2 <sup>c</sup> <sub>B</sub> (70 $\pm$ 2)
	$T_3$	5.26 $\pm$ 0.02 <sup>d</sup> <sub>B</sub> (73.2 $\pm$ 0.4)	3.99 $\pm$ 0.07 <sup>c</sup> <sub>B</sub> (68 $\pm$ 2)	2.96 $\pm$ 0.06 <sup>bc</sup> <sub>A</sub> (97.7 $\pm$ 0.9)	2.04 $\pm$ 0.12 <sup>b</sup> <sub>B</sub> (69 $\pm$ 3)	0.8 $\pm$ 0.3 <sup>ab</sup> <sub>A</sub> (29 $\pm$ 9)
	$T_4$	5.2 $\pm$ 0.2 <sup>d</sup> <sub>B</sub> (72 $\pm$ 2)	4.20 $\pm$ 0.04 <sup>c</sup> <sub>B</sub> (72 $\pm$ 2)	3.09 $\pm$ 0.12 <sup>d,e</sup> <sub>B</sub> (102 $\pm$ 3)	1.41 $\pm$ 0.13 <sup>a</sup> <sub>A</sub> (48 $\pm$ 4)	0.87 $\pm$ 0.19 <sup>abc</sup> <sub>B</sub> (31 $\pm$ 6)
Non-encapsulated	$T_0$	6.71 $\pm$ 0.08 <sup>g</sup> <sub>A</sub> (100)	5.26 $\pm$ 0.09 <sup>e</sup> <sub>A</sub> (100)	2.89 $\pm$ 0.09 <sup>b</sup> <sub>A</sub> (100)	2.37 $\pm$ 0.05 <sup>cd</sup> <sub>A</sub> (100)	1.3 $\pm$ 0.2 <sup>cd</sup> <sub>A</sub> (100)
	$T_1$	3.89 $\pm$ 0.08 <sup>d</sup> <sub>A</sub> (58.1 $\pm$ 0.4)	4.5 $\pm$ 0.3 <sup>d</sup> <sub>A</sub> (86 $\pm$ 6)	3.75 $\pm$ 0.06 <sup>gh</sup> <sub>B</sub> (130 $\pm$ 4)	2.39 $\pm$ 0.13 <sup>de</sup> <sub>A</sub> (105 $\pm$ 5)	1.0 $\pm$ 0.7 <sup>bc</sup> <sub>A</sub> (77 $\pm$ 5)
	$T_2$	3.55 $\pm$ 0.06 <sup>d</sup> <sub>A</sub> (52.9 $\pm$ 0.3)	4.5 $\pm$ 0.5 <sup>d</sup> <sub>A</sub> (85 $\pm$ 9)	3.18 $\pm$ 0.03 <sup>ef</sup> <sub>A</sub> (109 $\pm$ 3)	2.40 $\pm$ 0.06 <sup>cd</sup> <sub>B</sub> (100 $\pm$ 0.8)	1.8 $\pm$ 0.3 <sup>de</sup> <sub>A</sub> (138 $\pm$ 15)
	$T_3$	3.96 $\pm$ 0.04 <sup>c</sup> <sub>A</sub> (59.1 $\pm$ 0.7)	2.75 $\pm$ 0.12 <sup>a</sup> <sub>A</sub> (52 $\pm$ 2)	3.25 $\pm$ 0.05 <sup>f</sup> <sub>B</sub> (112 $\pm$ 2)	2.0 $\pm$ 0.2 <sup>b</sup> <sub>A</sub> (86 $\pm$ 7)	0.7 $\pm$ 0.8 <sup>abc</sup> <sub>B</sub> (53 $\pm$ 62)
	$T_4$	1.9 $\pm$ 0.06 <sup>a</sup> <sub>A</sub> (28 $\pm$ 0.6)	3.48 $\pm$ 0.05 <sup>b</sup> <sub>A</sub> (66.2 $\pm$ 0.3)	2.67 $\pm$ 0.02 <sup>a</sup> <sub>A</sub> (92 $\pm$ 2)	1.46 $\pm$ 0.06 <sup>a</sup> <sub>B</sub> (61 $\pm$ 2)	0.3 $\pm$ 0.3 <sup>a</sup> <sub>A</sub> (22 $\pm$ 25)

abc... Values with different superscript letters within the same column are significantly different ( $p \leq 0.05$ ).

ABC... Values with different subscript letters within the same column shows significance of encapsulation factor ( $p \leq 0.05$ ).



**Figure 1.** Microorganism content expressed in Log CFU/g with encapsulated and non-encapsulated *L. salivarius* spp. *salivarius*. Plotted results are the average  $\pm$  standard deviation of four replicates.