

*Encapsulation in an alginate–goats’ milk–  
inulin matrix improves survival of probiotic  
Bifidobacterium in simulated  
gastrointestinal conditions and goats’ milk  
yoghurt*

Article

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1 **Encapsulation in alginate-goat milk-inulin matrix improves survival of the**  
2 **probiotic *Bifidobacterium* in simulated gastrointestinal conditions and**  
3 **probiotic goat milk yoghurt**

4

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10

11 Abbreviated running headline: **Probiotic capsulation in goat milk-inulin matrix**

12

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24

25 **Abstract**

26 In this work, a new encapsulating matrix alginate-goat milk-inulin was used to encapsulate  
27 *Bifidobacterium animalis* subsp. *lactis* BB-12. The addition of inulin resulted in capsules with  
28 compact structure and higher probiotic cell count under simulated gastrointestinal conditions  
29 and in probiotic goat milk yoghurt during refrigerated storage. Encapsulation of bacteria led to  
30 slower post-acidification yoghurts. The results of this study showed that alginate-goat milk-  
31 inulin matrix has a potential to be utilised as a new encapsulation material to encapsulate  
32 probiotics to be used in goat milk-based probiotic fermented dairy products avoiding the cross-  
33 contamination caused by using capsules based on cow milk.

34

35 **Keywords:** *Bifidobacterium*; Encapsulation; Goat milk; Survival; Refrigeration; Post  
36 acidification

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## 50 **1. Introduction**

51 Functional foods can be defined as food products which are developed using natural food  
52 additives and they are used to provide additional health benefits to the consumer exceeding the  
53 basic nutrition (Prosapio *et al.*, 2016). The demand for functional foods is increasing around  
54 the world which is due to awareness of consumer about the relationship between consumption  
55 of functional foods and health benefits ( Fabersani *et al.*, 2018; Martins *et al.*, 2018; Sperry *et*  
56 *al.*, 2018;). Five main sectors can be identified in relation to the functional food market namely  
57 dairy, beverage, breakfast cereals and bakery, and the dairy sector is considered as the largest  
58 functional food market around the world (Pinto *et al.*, 2014). Most of the functional dairy  
59 products contain probiotic bacteria and these products have become popular and widely  
60 available in functional food markets (Granato *et al.*, 2010). This specific market shows a rapid  
61 growth and there is a huge competition among producers in introducing new probiotic dairy-  
62 based products (Balthazar *et al.*, 2018; Dantas *et al.*, 2016).

63

64 Probiotics are described as “live microorganisms that, when administered in adequate amounts,  
65 confer a health benefit on the host” (Hill *et al.*, 2014) . The most of probiotic strains have been  
66 selected and researched from the genera *Bifidobacterium* and *Lactobacillus*. Bifidobacteria are  
67 commonly used in manufacturing of fermented dairy products (Ranadheera *et al.*, 2010).  
68 Consumption of products containing probiotic bifidobacteria has been reported to exert health  
69 benefits in relation to lowering of serum cholesterol level, enhancing immune system,  
70 alleviation of diarrhoea, reduction of lactose intolerance, modulation of gut microflora, and  
71 prevention of allergy (Prasanna *et al.*, 2014). However, survival of probiotics in the product  
72 and inside the digestive tract depends on many factors such as acidity, culture combination,  
73 sugar concentration, temperature, and oxygen concentration in a particular product. In addition,  
74 higher acidity level in the digestive system can suppress survival of probiotic bacteria

75 (Ranadheera *et al.*, 2014). Therefore, different techniques have been evaluated to enhance  
76 probiotic viability in food systems and the digestive tract, including strain selection, use of  
77 oxygen impermeable packaging systems, two-step fermentation, supplementation with  
78 micronutrients, and encapsulation; the last one is considered as the most effective (Martín *et*  
79 *al.*, 2015).

80

81 Prebiotics are defined as 'a substrate that is selectively utilized by host microorganisms  
82 conferring a health benefit' (Gibson *et al.*, 2017). Inulin is the most popular prebiotic which is  
83 commonly used in dairy products and it has been shown to enhance the viability of  
84 bifidobacteria in the large intestine (Nazzaro *et al.*, 2012). In addition, there are some reports  
85 that prebiotics can improve the stability of alginate-based capsules containing probiotics in  
86 different food products and the digestive system (Krasaekoopt and Watcharapoka, 2014). In  
87 addition, there is a greater interest in using synbiotic which is a combination of prebiotic and  
88 probiotic in food products where prebiotics could improve survival and colonization of  
89 probiotics in the colon (Verruck *et al.*, 2017).

90

91 Encapsulation of different strains of *Bifidobacterium* has been used to improve their viability  
92 in various food systems and in simulated gastrointestinal conditions (Fritzen-Freire *et al.*,  
93 2013). Sodium alginate is a common material which is used to capsule probiotics. However,  
94 this material is very easily disintegrated at low pH leading to the release of microorganisms  
95 entrapped in beads to the environment (Krasaekoopt *et al.*, 2004). Therefore, alginate is mixed  
96 with other materials to improve stability of alginate capsules in food systems (Etchepare *et al.*,  
97 2016). Probiotics encapsulated in alginate-cow milk matrix were shown to improve their  
98 performances in simulated gastrointestinal conditions and in different food systems (Gbassi *et*  
99 *al.*, 2009; Rajam *et al.*, 2012; Shi *et al.*, 2013). Milk proteins have a higher buffering capacity

100 and these have been shown to increase count of probiotics during digestion (Würth *et al.*, 2015).  
101 In addition, encapsulation of probiotics in milk based materials could improve their viability  
102 in dairy foods (Ranadheera *et al.*, 2016). In our previous study, encapsulated *Bifidobacterium*  
103 *longum* subsp. *infantis* CCUG 52486 in alginate-goat milk based matrix was observed to  
104 increase their survival rate in simulated gastrointestinal conditions, goat milk and cow milk  
105 (Prasanna and Charalampopoulos, 2018).

106

107 Goat is considered as an important livestock species in rural areas many developing countries  
108 around the world. They can live in harsh environmental conditions where cattle cannot be  
109 reared. Therefore, goat farming is popular in many remote parts of the world where they are  
110 used for their milk, meat, and skin (Joshi *et al.*, 2004). Dairy goats are basically used as a key  
111 food source in low income countries of the Indian subcontinent and the industry is spreading  
112 in the developed countries. The worldwide goat milk production has been doubled during the  
113 last 50 years and it is predicted to increase by 53% by 2030 (Pulina *et al.*, 2018). Goat milk is  
114 considered as an excellent food source; it is used as raw material in producing different cheeses,  
115 ice cream and yoghurt (Milani and Wendorff, 2011). Consumption of goat milk is  
116 recommended for children and elderly people and it is also recommended as an alternative milk  
117 type for people showing allergy to cow milk (Ribeiro and Ribeiro, 2010). Goat milk is  
118 considered to have similar properties to human milk. It has the higher amount of small fat  
119 globules which are very important in human nutrition. However, goat milk produces a softer  
120 curd during the fermentation process (Clark and García, 2017). Non-bovine dairy products  
121 including goat milk are considered as excellent probiotic carriers and there is an increasing  
122 demand for such products (Ranadheera *et al.*, 2018).

123

124 This new alginate-goat milk-inulin matrix may have an advantage that probiotics encapsulated  
125 in the new material could be directly used as an inoculum for goat milk based products ensuring  
126 minimum contamination with cow milk which is considered to cause cow milk allergy in some  
127 consumers. In this study, we report on some properties of capsules made of new alginate-goat  
128 milk-inulin matrix and survival of encapsulated probiotic *B. animalis* subsp. *lactis* BB-12 in  
129 goat milk yoghurt stored at 4 °C for 28 days. Furthermore, the capsules were evaluated under  
130 simulated gastrointestinal conditions (SGC).

131

## 132 **2. Materials and methods**

### 133 *2.1. Activation of microorganism*

134 *B. animalis* subsp. *lactis* BB-12 was provided by Chr. Hansen Company (Horsholm, Denmark)  
135 and the freeze-dried culture was activated using MRS broth (Oxoid, Hampshire, UK), under  
136 anaerobic condition at 37 °C for 18 h, using an inoculum at the rate of 1% (w/v). The preculture  
137 was produced using two successive cultures of *B. animalis* subsp. *lactis* BB-12 where the  
138 inoculum level of was 1% (v/v). Thereafter, 200 mL of Wilkins-Chalgren (WC) anaerobe broth  
139 (Oxoid, UK) was inoculated with 1% (v/v) of the preculture and the incubation was completed  
140 using the same conditions. The broth was centrifuged at 10,000 rpm for 10 min at 4 °C to  
141 harvest cells. At the end of the centrifugation, sterile phosphate buffered saline (PBS) (Oxoid,  
142 UK) was used to wash the pellet twice. The pellet was mixed with 10 mL of PBS to make the  
143 concentrated cell suspension.

144

### 145 *2.2. Preparation of capsules*

146 Capsules were prepared using sterilized goat milk and sodium alginate (2%, w/v, low viscosity,  
147 Sigma-Aldrich, UK). Five treatments were prepared by mixing sodium alginate and inulin at  
148 the level of 0%, 0.5%, 1%, 1.5% and 2% (w/v) and the mixture were sterilized (121 °C for 15



149 min). The encapsulation mixture was prepared by mixing sodium alginate mixture and  
150 sterilized goat milk at the ratio of 1.5/1 (v/v). Thereafter, each formulation was thoroughly  
151 mixed with the concentrated cell suspension at the ratio of 4/1 (v/v). The capsules were  
152 produced as described by Prasanna and Charalampopoulos (2018).

153

### 154 *2.3. Determination of encapsulation yield and size of capsules*

155 The encapsulation yield (EY) of different matrices and size of capsules were determined as  
156 described previously (Prasanna and Charalampopoulos, 2018).

157

### 158 *2.4. Assessment of viability of free and encapsulated bacteria*

159 Bifidobacteria selective medium (BSM) agar (Sigma-Aldrich, UK) was used to enumerate free  
160 bifidobacteria at 37 °C for 72 h under the anaerobic condition while the capsulated bacteria  
161 were enumerated as reported by Prasanna and Charalampopoulos (2018).

162

### 163 *2.5. Viability of free and encapsulated bacterial cells during sequential incubation in SGC*

164 The method explained by Sun and Griffiths (2000) was used to prepare simulated gastric juice  
165 (SGJ), by dissolving 0.2% NaCl (w/v) in 0.08 M HCl, at pH 2 whereas simulated intestinal  
166 juice (SIJ) was prepared as described by Chávarri *et al.* (2010). The viability of free and  
167 encapsulated bacteria under SGC were conducted as described by Krasaekoopt *et al.* (2004).  
168 Glass tubes containing 9 mL of sterilized SGJ were mixed with capsules (1 g) or the free cells  
169 (1 mL). The samples were placed and incubated in a water bath at 37 °C. Sampling was carried  
170 out at 0, 30, 60 and 120 min, during the incubation. The capsules were separated by filtration  
171 while free cells were separated using centrifugation (10,000 rpm for 10 min, at 4 °C).  
172 Thereafter, the free cells or the capsules were placed in glass tubes containing 9 mL of SIJ and  
173 the incubation was carried out at 37 °C for 120 min. After the incubation period, the free cells

174 and capsules were separated as described above. The free and the capsulated bacteria were  
175 enumerated as described previously (Prasanna and Charalampopoulos, 2018).

176

#### 177 2.6. Preparation of probiotic goat milk yoghurt

178 Probiotic goat milk yoghurts were produced as described by Costa *et al.* (2014). UHT goat  
179 milk was inoculated with thermophilic yoghurt cultures (YoFlex, YC-X11, Chr. Hansen,  
180 Hoersholm, Denmark) composed of *Streptococcus thermophiles* and *Lactobacillus delbrueckii*  
181 subsp. *bulgaricus* at a rate of 1% (w/v). The inoculated milk was incubated at 43 °C, until the  
182 pH reached ~ 4.5. Thereafter, 10 g of the encapsulated or 10 mL of the free cells of *B. animalis*  
183 subsp. *lactis* BB-12 was separately mixed with 100 g of goat milk yoghurt in plastic cups and  
184 the cups were stored at 4 °C for 28 days. The sampling was carried out on 0, 7, 14, 21 and 28  
185 days to analyse pH and the viability of bifidobacteria. A sample (10 g) of each treatment was  
186 collected from a well-mixed yoghurt cup. The sample was mixed with 90 mL of sterilized 50  
187 mM sodium citrate (Sigma-Aldrich, UK) solution (pH, 7.5) in a stomacher. Bacterial cells were  
188 separated and enumerated as reported by Prasanna and Charalampopoulos (2018).

189

#### 190 2.7. Determination of pH of yoghurt during storage

191 The pH changes of probiotic yoghurts were measured weekly during the storage period using  
192 a benchtop pH meter (Mettler Toledo, UK) as explained by Prasanna *et al.* (2013) and the  
193 measurements were taken at room temperature.

194

#### 195 2.8. Scanning electron microscopic (SEM) analysis of cross sections of capsules

196 Dehydration of capsules was carried out sequentially in a series of ethanol solutions (30, 50,  
197 70, 80, 90, and 100%). For this purpose, capsules were soaked for 15 min. in each solution.  
198 Thereafter, a critical point dryer (Balzers CPD 030, Liechtenstein, Germany) with liquid

199 carbon dioxide was used to dry capsules. Dried capsules were cut into two halves to obtain  
200 cross sections using a sterilized scalpel. Coating of samples and examination of samples using  
201 a scanning electron microscope (FEI, Quanta 600 F, USA) were carried out as described earlier  
202 (Prasanna and Charalampopoulos, 2018).

203

### 204 *2.9. Statistical analysis*

205 The experiment was conducted in triplicate. One-way analysis of variance (ANOVA) with  
206 Turkey's multiple comparison tests (SAS, version 9.2, SAS Institute Inc., Cary NC, USA) was  
207 used to analyse size and EY of capsules. Split-plot in time design using the General Linear  
208 Model (GLM) procedure of SAS was used to analyse results of viable count and pH of goat  
209 milk yoghurt (version 9.2, SAS Institute Inc., Cary NC, USA).

210

## 211 **3. Results and discussion**

### 212 *3.1. Size, EY and surface morphology of cross sections of capsules*

213 As shown in Table 1, mixing of inulin into alginate-goat milk based matrix significantly  
214 ( $p < 0.05$ ) increased the size of capsules compared to the control (0% inulin); more specifically,  
215 the capsule sizes increased as the inulin concentration increased. For example, capsule sizes  
216 were increased from 2.98 to 3.4 mm for 0.5% inulin and 2% inulin respectively. This may be  
217 due to changes in viscosity of five different matrices where a higher level of inulin  
218 concentration can lead for higher viscosity which can result in larger capsules as describe by  
219 Cheow *et al.* (2014). This observation is consistent with the findings of Chávarri *et al.* (2010)  
220 and Krasaekoopt and Watcharapoka (2014) who observed that prebiotic addition into alginate-  
221 based material resulted in larger capsular size.

222

223 The results further revealed that the incorporation of inulin into alginate-goat milk based matrix  
224 had no significant ( $p>0.05$ ) influence on the EY of capsules (Table 1). Values of EY ranged  
225 from 87 – 91%. Moreover, this high encapsulation yield reveals that alginate-goat milk-inulin  
226 is a compatible matrix which can be used to encapsulate probiotics such as *B. animalis* subsp.  
227 *lactis* BB-12. Our results are consistent with findings of Shi *et al.* (2013) during the  
228 microencapsulation of probiotic *Lactobacillus buguricus* with alginate milk microsphere,  
229 where the EY values were around 100%. It was observed that the addition of prebiotics and  
230 milk proteins in the matrix can lead to higher EY (Soukoulis *et al.*, 2014). In addition, the  
231 higher encapsulation yield may be due to the mild conditions such as room temperature (25  
232 °C) with all natural substances which have a minimum detrimental effect on the  
233 microorganism.

234

235 Cross sections of capsules are shown in Fig.1 and each type of matrix showed a distinctive  
236 morphology. The cross section of alginate-goat milk showed a porous structure [Fig.1, (a)].  
237 The addition of inulin into alginate-goat milk resulted in more compact capsules with less  
238 visible pores [Fig.1, (b), (c), (d) and (e)]; the most densely packed capsules were observed with  
239 2% inulin [Fig.1, (e)]. This may be due to the ability of inulin to make a compact network with  
240 proteins of alginate-goat milk matrix leading to densely packed capsules. In general, inulin is  
241 a water-soluble fibre and it was observed to form complexes with proteins of goat milk leading  
242 to a part of a strong structural network (Costa *et al.*, 2015). Similarly, de Souza Oliveira *et al.*  
243 (2011) observed a reaction of inulin and dairy proteins leading for higher firmness of the  
244 mixture. The compact structure of capsules observed with the mixing of inulin to alginate-goat  
245 milk matrix is very important in food applications and during the digestion process since this  
246 can limit exposure of highly vulnerable probiotic bacteria to the harsh external environmental  
247 conditions. Furthermore, this property of new capsules may support to increase the survival of

248 bacteria in food products in the manufacturing process and storage of food items and to deliver  
249 the probiotic to the colon at a higher survival rate.

250

### 251 3.2. Performance of free and encapsulated bifidobacteria during sequential incubation in SGC

252 Free and the encapsulated cells were exposed to *in vitro* SGC and the results are presented in  
253 Table 2. A continuous reduction of number of free probiotic cells was observed and the cell  
254 number dropped to a value which was undetectable ( $< 10^1$  cfu mL<sup>-1</sup>) after the sequential  
255 exposure of free cells to SGJ (90 min) followed by SIJ (120 min).

256

257 Encapsulation has been recommended to deliver viable cells to the gastro intestinal track  
258 (Champagne *et al.*, 2018). Addition of inulin to alginate-goat milk matrix during encapsulation  
259 increased the resistance of the probiotic to the SGC, resulting in higher viable cell count than  
260 without inulin under all conditions. Similarly, supplement of inulin to alginate during  
261 encapsulation of *Lactobacillus acidophilus* 5 and *Lactobacillus casei* 01 was observed to  
262 improve the survival of bacteria under SGC (Krasaekoopt and Watcharapoka, 2014). In  
263 addition, a higher survival rate of *B. bifidum* and *B. longum* encapsulated in alginate-  
264 fructooligosaccharides under SGC was reported by Chen *et al.* (2005). In another study, spray  
265 drying was used to produce microcapsules containing *B. bifidum* BB-12 and the probiotic was  
266 observed to improve their survival under SGC (Verruck *et al.*, 2017). Milk proteins have been  
267 characterized to have a good buffering capacity which can protect probiotics from the harsh  
268 environment which exists in the gastrointestinal tract (Anthony *et al.*, 2015). Guérin *et al.*  
269 (2003) also described that milk-based proteins could improve survival of bifidobacteria  
270 capsulated in pectin, alginate and whey proteins than free bacteria under the SGC.

271

272 Furthermore, it was reported that milk fat can play a role in protecting probiotics from the  
273 acidic environment since fat can reduce diffusion of H<sup>+</sup>, organic acid and O<sub>2</sub> (Picot and Lacroix,  
274 2004). As it was observed in this study using SEM micrographs [Fig.1 (b), (c), (d) and (e)],  
275 inulin modified the capsule structure by interacting with proteins leading a compact alginate-  
276 goat milk matrix which may limit exposure of bacterial cells to the external environment and  
277 diffusion of chemical substances. Furthermore, the addition of inulin may improve the strength  
278 of the matrix and reduce the dissolution of capsules, consequently protecting the probiotic cells  
279 within the matrix.

280

### 281 *3.3. Changes of survival rate of encapsulated and free bacterial cells in probiotic goat milk* 282 *yoghurt at 4 °C*

283 The changes in the viable count of encapsulated and free probiotic in probiotic goat milk  
284 yoghurts during the refrigerated storage for 28 d (Fig.2.). The results clearly revealed that there  
285 was a significant ( $p<0.05$ ) loss of the viable count of free bacteria over a period of 28 d where  
286 there was 3.67 log cfu g<sup>-1</sup> loss in viable counts of free bacterial cells during the storage period.  
287 Addition of inulin to the matrix led for better survival of probiotic bacteria in probiotic goat  
288 milk yoghurt; specially there was an increase of cell concentration in yoghurts when inulin  
289 concentration of capsules ranged from 0.5 to 2%. The cell concentration of yoghurt containing  
290 encapsulated probiotics did not decrease below the recommended level ( $10^6$ - $10^7$  cfu/mL or g)  
291 over 28 d of storage. The higher survival rate observed in inulin containing capsules in goat  
292 milk yoghurt may be due to the better protection provided by compact structure of alginate-  
293 goat milk-inulin matrices observed in SEM micrographs [Fig.1 (b), (c), (d) and (e)].  
294 Furthermore, the higher viability of encapsulated bacteria in inulin-based matrices in the goat  
295 milk yoghurt, may be due to the limited potential of passing capsule wall by growth inhibiting  
296 substances which can be resulted during the fermentation process including acids and hydrogen

297 peroxide as reported by Krasaekoopt and Watcharapoka (2014). Furthermore, it was observed  
298 that capsules containing prebiotics could provide the carbon and nitrogen sources for  
299 encapsulated probiotics leading for higher survival rate of *Bifidobacterium* and *Lactobacillus*  
300 in milk (Chen *et al.*, 2005). Similarly, other encapsulating materials such as alginate (Kebary  
301 and Hussein, 1999), alginate-starch (Adhikari *et al.*, 2000) and  $\kappa$ -carrageenan were observed  
302 to improve survival of probiotic *Bifidobacterium* species in fermented dairy foods under  
303 refrigerated storage. Moreover, different prebiotics such as inulin (Akhiar and Aqilah, 2010),  
304 fructooligosaccharides and raftilose (Iyer and Kailasapathy, 2005) with alginate-based  
305 capsules have been shown to be effective in improving probiotic viability in some dairy  
306 products. In addition, goat milk is considered as a suitable vehicle to deliver probiotic to  
307 humans. The properties of goat milk including appropriate pH, higher nutrient content and  
308 good buffering capacity lead for viability of probiotic during shelf life (Ranadheera *et al.*,  
309 2018). Furthermore, the market share of functional yoghurt continues to grow and functional  
310 yoghurt containing probiotics, prebiotics and various plant extracts are being developed and  
311 introduced to satisfy consumer demand (Fazilah *et al.*, 2018). Therefore, this new goat milk  
312 yoghurt containing a novel capsule containing probiotic may have a good market demand.

313

#### 314 *3.4. pH Changes of probiotic goat milk yoghurt during storage*

315 Depending on the type of bacterial cells and the level of inulin supplementation during the  
316 encapsulation, the pH of goat milk yoghurt changed (Fig. 3). All goat milk yoghurt types  
317 showed a gradual decrease of pH during the storage period of 28 d. However, goat milk yoghurt  
318 containing free bacterial cells recorded the lowest pH value from 7<sup>th</sup> day to the end of the  
319 storage period. There were no significant differences ( $p>0.05$ ) between final pH values of goat  
320 milk yoghurts produced with encapsulated bacterial cells. The decrease of pH of all types of  
321 goat milk yoghurt during storage is mainly due to growth and metabolic activity of yoghurt

322 starter cultures which are reported to produce lactic acid at refrigerated storage (Shah *et al.*,  
323 1995). In the case of goat milk yoghurt containing free bifidobacteria, in addition to yoghurt  
324 starter bacteria, cells of bifidobacteria are responsible acidifying goat milk yoghurt by  
325 producing both lactic and acetic acids and they have been reported to produce these acids with  
326 yoghurt starter cultures even at refrigerated storage (Samona *et al.*, 1996). Similarly, a decrease  
327 in pH of cow milk yoghurt containing encapsulated *B. lactis* (Kailasapathy, 2006), *B. breve*  
328 R070 (Picot and Lacroix, 2004) and *B. longum* (Adhikari *et al.*, 2003) was observed. The  
329 results of this study revealed that post-acidification in probiotic goat milk yoghurt produced  
330 with capsulated *B. animalis* subsp. *lactis* BB-12 was slower compared with probiotic goat milk  
331 yoghurt containing free bacterial cells.

332

333 However, it is important to conduct a sensory evaluation to have a better understanding of the  
334 effect of this new capsule on the sensory properties of probiotic goat milk yoghurts. This new  
335 capsule may have the effect on sensory attributes of probiotic goat milk yoghurt such as  
336 appearance, aroma, flavour and texture which have been established with some other functional  
337 dairy products (Esmerino *et al.*, 2017; Janiaski *et al.*, 2018; Silva *et al.*, 2018). Therefore, a  
338 sensory evaluation of the goat milk yoghurt containing the new capsule will be carried out to  
339 assess its consumers' acceptability.

340

#### 341 **4. Conclusions**

342 The present study showed that addition of inulin to alginate-goat milk during encapsulation  
343 increased the size of capsules while it had no effect on EY. SEM micrographs revealed that  
344 inulin could lead for compact interior structural characteristics. The addition of inulin to  
345 alginate-goat milk capsules led for a better protection to probiotic cells in simulated  
346 gastrointestinal condition. Inulin could improve the survival rate of capsulated probiotic cells



347 compared to capsules without inulin and free cells in probiotic goat milk yoghurt stored over  
348 28 d. A slower post-acidification of probiotic goat milk yoghurt was observed with  
349 encapsulated probiotic cells compared to that of free probiotic cells. The results revealed that  
350 addition of 1% inulin (w/v) to alginate-goat milk mixture could be used to improve the survival  
351 rate of *B. animalis* subsp. *lactis* BB-12 in probiotic goat milk yoghurt. Nevertheless, a sensory  
352 evaluation should be conducted to have a clear idea about how capsules effect on the sensory  
353 properties of probiotic goat milk yoghurt such as colour, texture, acidity and flavour.

354

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358

359 **The authors declare that they have no conflict of interest.**

360

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543 **Figure captions**

544 Fig.1. Scanning electron micrographs showing the cross section of different capsules. a:  
545 capsules were prepared using alginate and goat milk at a ratio of 1.5:1 (v/v). b: capsules were  
546 prepared using alginate and goat milk at a ratio of 1.5:1 (v/v) and inulin 0.5% (w/v). c: capsules  
547 were prepared using alginate and goat milk at a ratio of 1.5:1 (v/v) and inulin 1% (w/v). d:  
548 capsules were prepared using alginate and goat milk at a ratio of 1.5:1 (v/v) and inulin 1.5%  
549 (w/v). e: capsules were prepared using alginate and goat milk at a ratio of 1.5:1 (v/v) and inulin  
550 2% (w/v). (magnification 10,000×). White → shows the bacterial cells.

551

552 Fig.2. Survival of free and encapsulated *B. animalis subsp. lactis* BB-12 in goat milk yoghurt  
553 at 4 °C for 28 days. Vertical lines represent standard deviations. <sup>ABC</sup>Means with different  
554 uppercase are significantly different ( $p<0.05$ ) between each time, for each type of alginate-  
555 goat milk capsule during the storage. <sup>abcd</sup>Means with different lowercase are significantly  
556 different ( $p<0.05$ ) between each type of alginate-goat milk capsule, for a particular day of the  
557 storage period. Free: Free bacterial cells. 0%: capsules were prepared using alginate and goat  
558 milk at a ratio of 1.5:1 (v/v). 0.5: capsules were prepared using alginate and goat milk at a ratio  
559 of 1.5:1 (v/v) and inulin 0.5% (w/v). 1: capsules were prepared using alginate and goat milk at  
560 a ratio of 1.5:1 (v/v) and inulin 1% (w/v). 1.5: capsules were prepared using alginate and goat  
561 milk at a ratio of 1.5:1 (v/v) and inulin 1.5% (w/v). 2: capsules were prepared using alginate  
562 and goat milk at a ratio of 1.5:1 (v/v) and inulin 2% (w/v). Free: Free cells.

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564 Fig.3. Changes in pH of goat milk yoghurt containing free and encapsulated bacterial cells at  
565 4 °C for 28 days. Vertical lines represent standard deviations. <sup>ABCDE</sup>Means with different  
566 uppercase are significantly different ( $p<0.05$ ) between each time, for each type of alginate-  
567 goat milk based capsule during the storage. <sup>abcdef</sup>Means with different lowercase are

568 significantly different ( $p < 0.05$ ) between each type of alginate-goat milk based capsule, for a  
569 particular day of the storage period. For legend explanations see Fig. 2.

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593 **Tables**

594 Table 1. Influence of different concentrations of inulin on the size and encapsulation yield of  
595 different capsules

Concentration of inulin (% w/v)	Size of capsules (mm)	Encapsulation yield (%)
0	2.79 ± 0.33 <sup>b</sup>	90.84 ± 3.10 <sup>a</sup>
0.5	2.98 ± 0.23 <sup>ab</sup>	91.67 ± 1.76 <sup>a</sup>
1	3.11 ± 0.58 <sup>ab</sup>	91.94 ± 3.88 <sup>a</sup>
1.5	3.32 ± 0.35 <sup>a</sup>	90.57 ± 2.04 <sup>a</sup>
2	3.41 ± 0.44 <sup>a</sup>	87.45 ± 2.06 <sup>a</sup>

596 <sup>ab</sup>Mean values (±standard deviation) within the same column not sharing a common superscript  
597 differ significantly (P < 0.05).

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612 Table 2. Survival of free and encapsulated *B. animalis* subsp. *lactis* BB-12 after incubation in  
 613 simulated gastric juice (pH 2) at 37 °C for 30, 60, 90 and 120 min and in simulated intestinal  
 614 juice pH (7.5) at 37 °C for 2h (Value represents both after gastric and intestinal digestion *in*  
 615 *vitro*)

Type of cells	Concentration of inulin (%)	Number of viable cells (log cfu mL <sup>-1</sup> / log cfu g <sup>-1</sup> )				
		0	30	60	90	120
Free		9.43 ± 0.08 <sup>A a</sup>	7.37 ± 0.32 <sup>B b</sup>	3.49 ± 0.09 <sup>C c</sup>	ND	ND
Encapsulated	0	9.49 ± 0.12 <sup>A a</sup>	8.22 ± 0.10 <sup>B b</sup>	8.11 ± 0.11 <sup>B b</sup>	8.09 ± 0.09 <sup>B b</sup>	8.07 ± 0.03 <sup>B b</sup>
	0.5	9.47 ± 0.11 <sup>A a</sup>	8.28 ± 0.26 <sup>B ab</sup>	8.21 ± 0.21 <sup>B ab</sup>	8.14 ± 0.12 <sup>B b</sup>	8.11 ± 0.18 <sup>B b</sup>
	1	9.45 ± 0.23 <sup>A a</sup>	8.38 ± 0.07 <sup>B ab</sup>	8.33 ± 0.05 <sup>B ab</sup>	8.28 ± 0.18 <sup>B a</sup>	8.14 ± 0.11 <sup>B a</sup>
	1.5	9.45 ± 0.14 <sup>A a</sup>	8.62 ± 0.16 <sup>B ab</sup>	8.52 ± 0.18 <sup>BC a</sup>	8.43 ± 0.07 <sup>C a</sup>	8.41 ± 0.19 <sup>C a</sup>
	2	9.44 ± 0.11 <sup>A a</sup>	8.70 ± 0.22 <sup>B a</sup>	8.54 ± 0.06 <sup>BC a</sup>	8.46 ± 0.15 <sup>C a</sup>	8.44 ± 0.10 <sup>C a</sup>

616 <sup>ABCD</sup>Means in the same row without common letter differ significantly ( $p < 0.05$ ) for each type  
 617 of capsules. <sup>abcde</sup>Means in the same column for each type of capsule without common letter  
 618 differ significantly ( $p < 0.05$ ) for a particular time. Data are expressed as mean ± standard  
 619 deviation. ND: Not detected.

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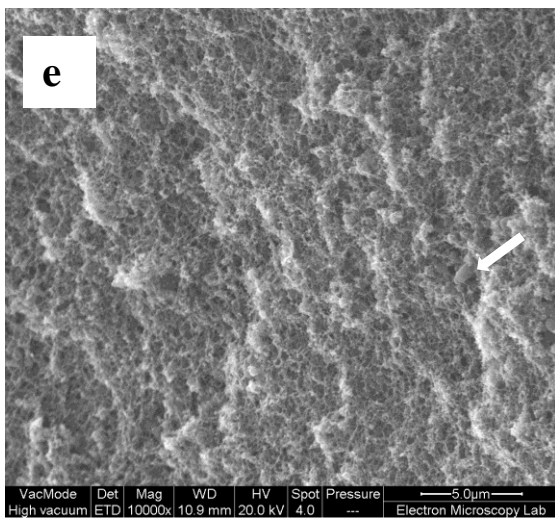
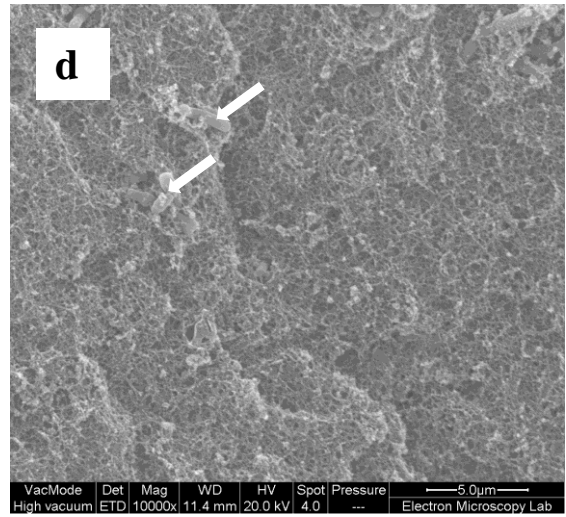
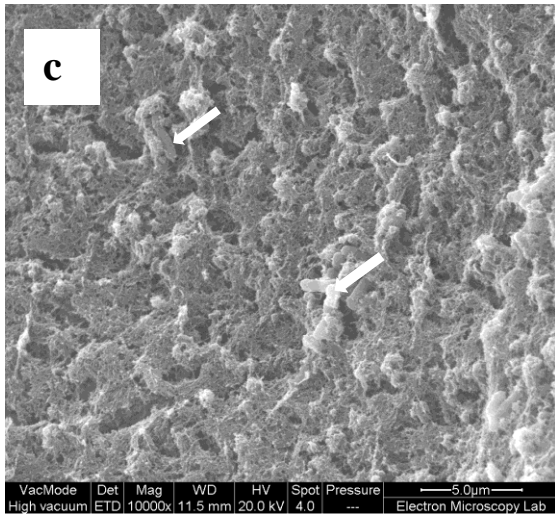
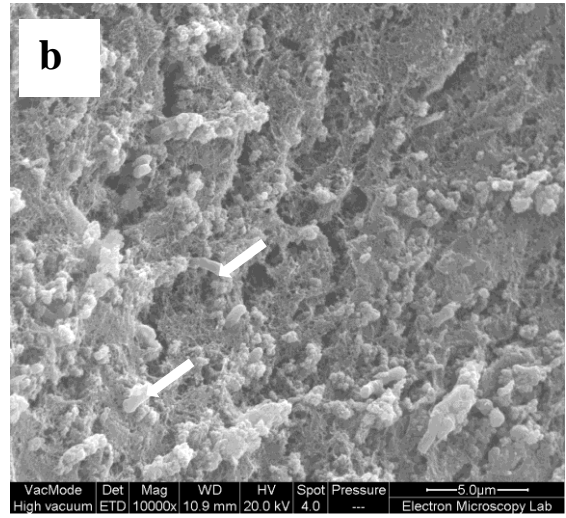
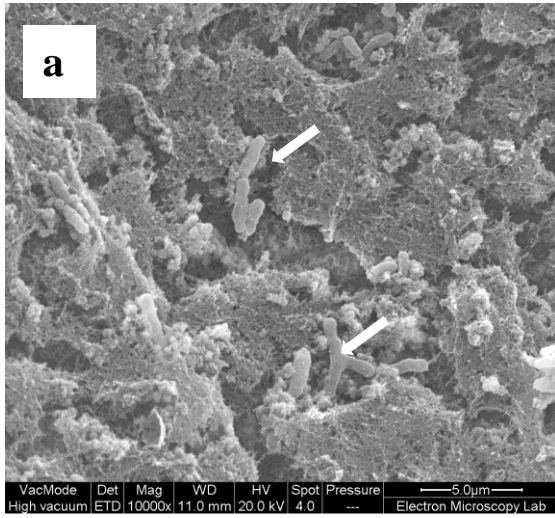


Fig.1.

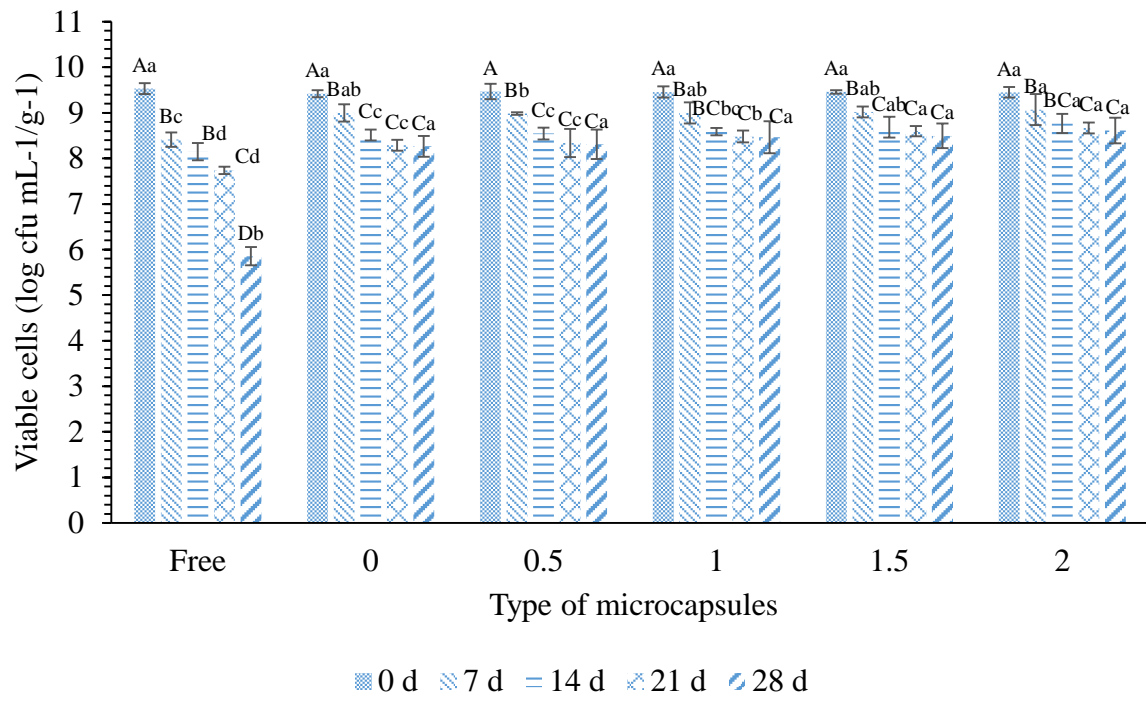


Fig. 2.

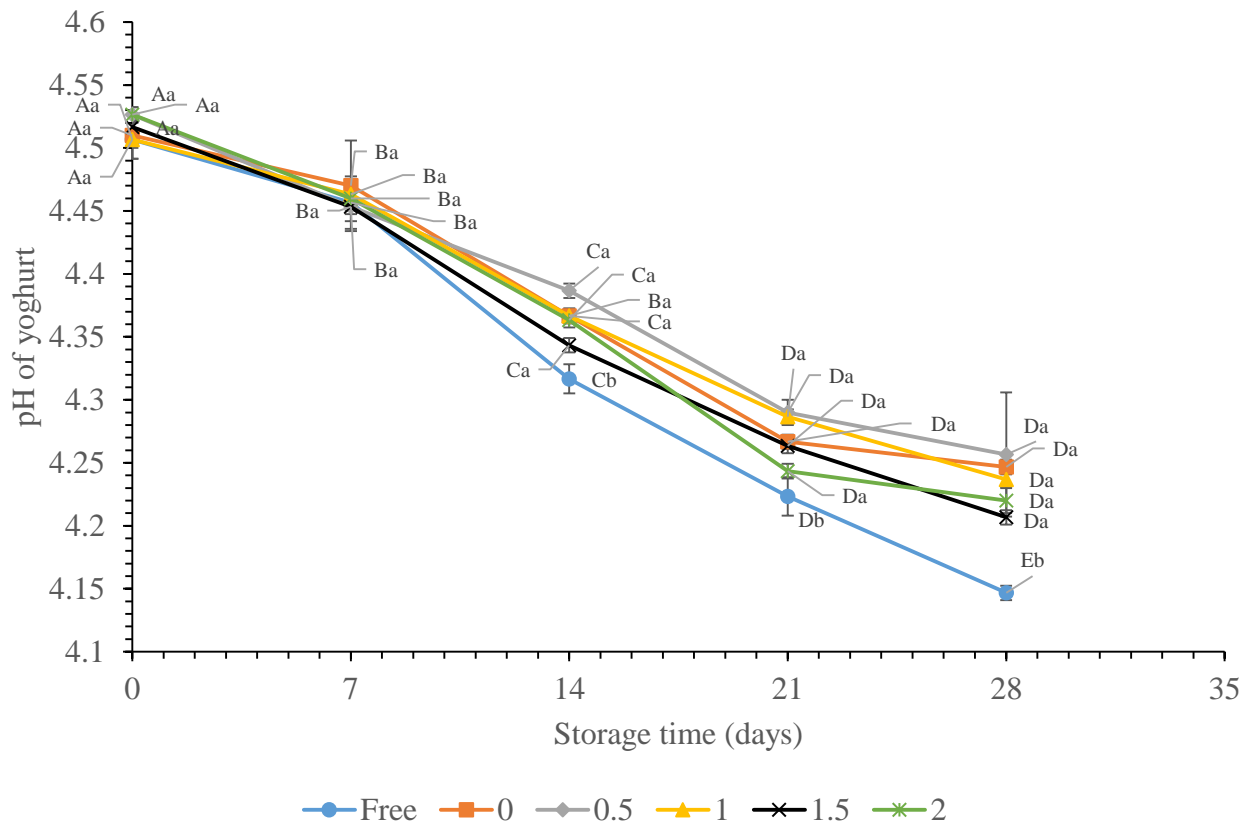


Fig.3.