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

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Abstract

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

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

1. Introduction

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

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

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

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

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

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

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

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

2. Materials and methods

2.1. Activation of microorganism

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

2.2. Preparation of capsules

Capsules were prepared using sterilized goat milk and sodium alginate (2%, w/v, low viscosity, Sigma-Aldrich, UK). Five treatments were prepared by mixing sodium alginate and inulin at 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

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

2.6. Preparation of probiotic goat milk yoghurt

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

2.7. Determination of pH of yoghurt during storage

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

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

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

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

2.9. Statistical analysis

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

3. Results and discussion

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

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

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

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

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

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

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

Furthermore, it was reported that milk fat can play a role in protecting probiotics from the acidic environment since fat can reduce diffusion of H^+, organic acid and O_2 (Picot and Lacroix, 2004). As it was observed in this study using SEM micrographs [Fig.1 (b), (c), (d) and (e)], inulin modified the capsule structure by interacting with proteins leading a compact alginate-goat milk matrix which may limit exposure of bacterial cells to the external environment and diffusion of chemical substances. Furthermore, the addition of inulin may improve the strength of the matrix and reduce the dissolution of capsules, consequently protecting the probiotic cells within the matrix.

3.3. Changes of survival rate of encapsulated and free bacterial cells in probiotic goat milk yoghurt at 4 ℃

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

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

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

4. Conclusions

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

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

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Figure captions

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

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

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

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

Tables

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

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

[ab]Mean values (±standard deviation) within the same column not sharing a common superscript differ significantly ($P < 0.05$).

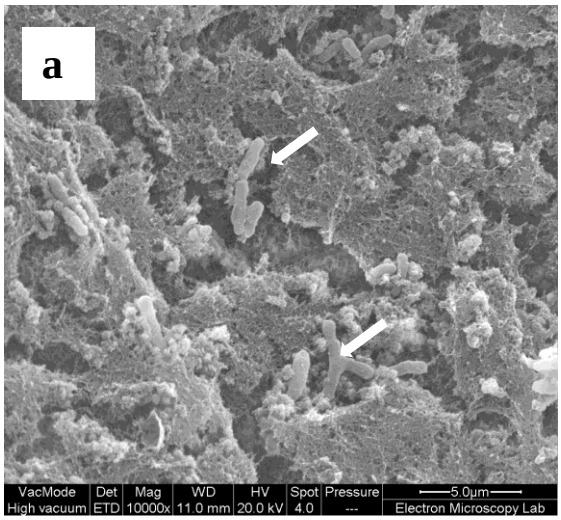
Table 2. Survival of free and encapsulated *B. animalis* subsp. *lactis* BB-12 after incubation in simulated gastric juice (pH 2) at 37 °C for 30, 60, 90 and 120 min and in simulated intestinal juice pH (7.5) at 37 °C for 2h (Value represents both after gastric and intestinal digestion *in vitro*)

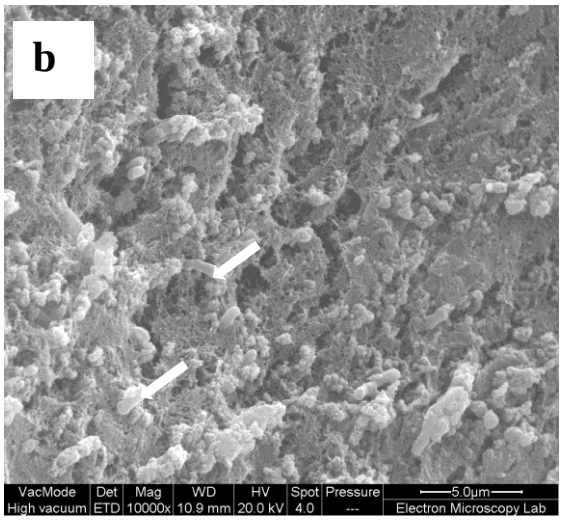
Type of cells	Concentration of inulin (%)	Number of viable cells (log cfu mL^{-1}/ log cfu g^{-1})				
		0	30	60	90	120
Free		$9.43 \pm 0.08^{A\,a}$	$7.37 \pm 0.32^{B\,b}$	$3.49 \pm 0.09^{C\,c}$	ND	ND
Encapsulated	0	$9.49 \pm 0.12^{A\,a}$	$8.22 \pm 0.10^{B\,b}$	$8.11 \pm 0.11^{B\,b}$	$8.09 \pm 0.09^{B\,b}$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

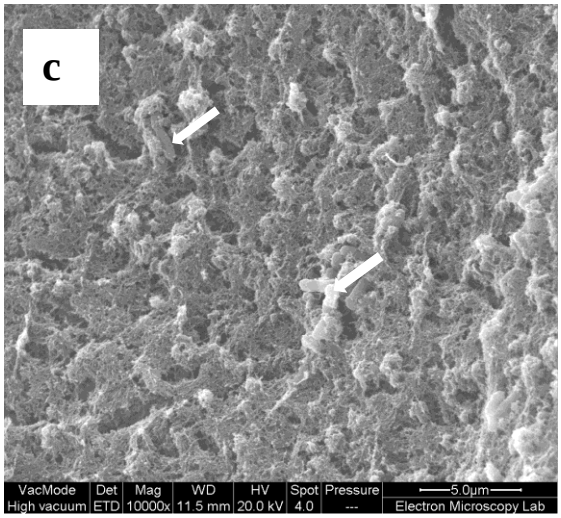
[ABCD]Means in the same row without common letter differ significantly ($p<0.05$) for each type of capsules. [abcde]Means in the same column for each type of capsule without common letter differ significantly ($p<0.05$) for a particular time. Data are expressed as mean ± standard deviation. ND: Not detected.

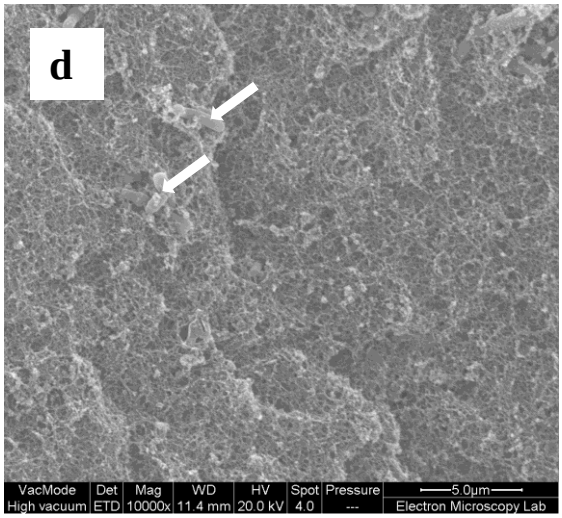
627 **Figures**

628









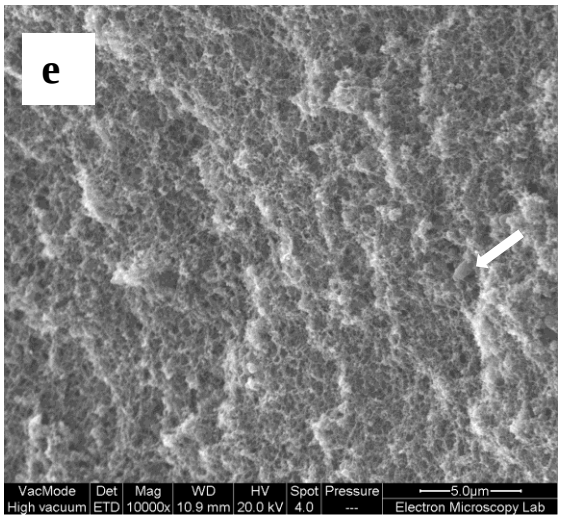


Fig.1.

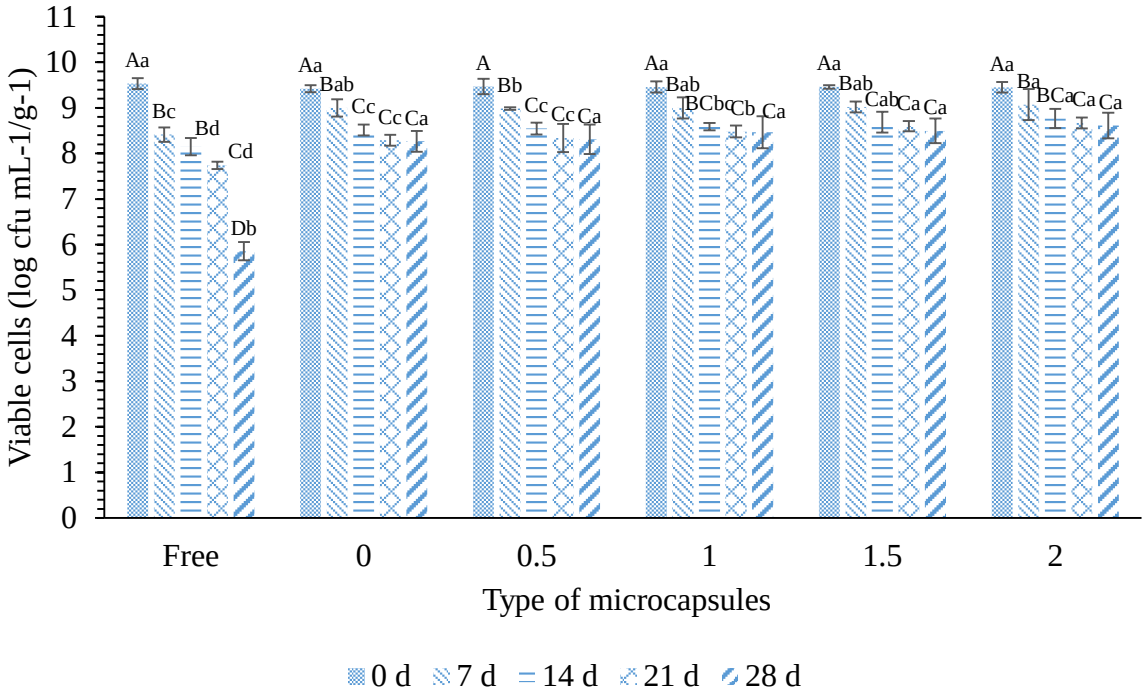

Viable cells (log cfu mL-1/g-1)
11
10
9
8
7
6
5
4
3
2
1
0
Aa
Bc
Bd
Cd
Db
Aa
Bab
Cc
Cc
Ca
A
Bb
Cc
Cc
Ca
Aa
Bab
BCbc
Cb
Ca
Aa
Bab
Cab
Ca
Ca
Aa
Ba
BCa
Ca
Ca
Free
0
0.5
1
1.5
2
Type of microcapsules
0 d
7 d
14 d
21 d
28 d

Fig. 2.

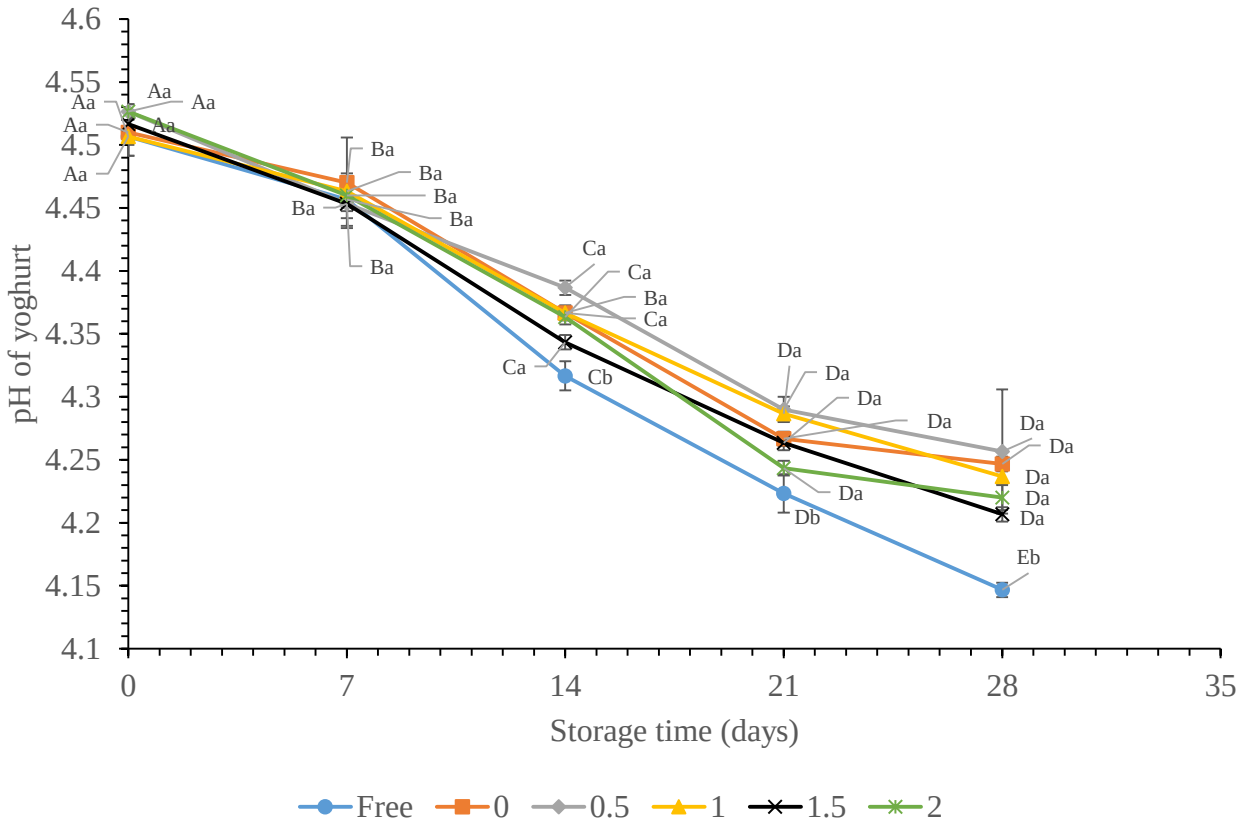

4.6
4.55
4.5
4.45
4.4
4.35
4.3
4.25
4.2
4.15
4.1
pH of yoghurt
0
7
14
21
28
35
Storage time (days)
Aa
Ba
Ca
Cb
Da
Db
Eb
Free
0
0.5
1
1.5
2

Fig.3.