ENCAPSULATION OF THE PROBIOTIC BIFIDOBACTERIUM ANIMALIS IN ALGINATE-SKIM GOAT MILK AND SURVIVAL IN SIMULATED GASTROINTESTINAL CONDITIONS, LOW-FAT YOGHURT, AND ORANGE JUICE

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Abstract

In this work, the survival of encapsulated probiotic cells of Bifidobacterium animalis subsp. lactis in alginate-skim goat milk (AGM) and alginate-skim cow milk (ASM) was evaluated under simulated gastrointestinal conditions, in acidic food systems including low-fat yoghurt and orange juice under refrigerated storage for 28 d. Free cells were used as the control. Encapsulation resulted in significantly higher cell concentration under all experimental conditions compared to free cells. The cells in ASM resulted in slightly higher survival in the simulated gastrointestinal conditions than the cells in AGM, but the values were not significantly different. No significant differences were observed in terms of cell concentration in low-fat yoghurt and orange juice with capsules while both products with free cells exhibited lower pH values than products containing encapsulated cells during the storage. Overall, the results indicated the potential application of AGM to encapsulate probiotic bifidobacteria to improve their survival in acidic food systems.

Keywords: *Bifidobacterium; Encapsulation; Orange juice; Refrigeration; Storage; Yoghurt*

INTRODUCTION

Probiotics have been incorporated into a wide range of food products, ranging from liquid (probiotic liquid milk, fruit juices) to solid products (cereals, milk powders) (Burgain, Gaiani, Linder, & Scher, 2011). They can exert many health benefits to humans by immuno-modulation, lowering of serum cholesterol, relief of inflammatory bowel disease, prevention of allergy, and modulation of gut microflora (Bajaj, Claes, & Lebeer, 2021; Saad, Delattre, Urdaci, Schmitter, & Bressollier, 2013). Nevertheless, they should be able to maintain their viability above 6 log cfu g^{-1}/ ml^{-1} in a particular product during processing and storage until the end of their shelf life, though there are no general agreements about this value (Etchepare et al., 2016; Prasanna, Grandison, & Charalampopoulos, 2013). In addition, these probiotics should be able to survive against adverse gastrointestinal conditions during digestion (Ranadheera, Evans, Adams, & Baines, 2012).

The most common probiotic bacteria used in food applications are from genera *Lactobacillus* and *Bifidobacterium*. Many species of genus *Bifidobacterium* are commonly used as probiotics in different food products including fermented dairy products (Agrawal et al., 2009; Bezerra, Araujo, Santos, & Correia, 2015; Gomi, Iino, Nonaka, Miyazaki, & Ishikawa, 2015), fruit juices (Bevilacqua, Campaniello, Corbo, Maddalena, & Sinigaglia, 2013; Saarela, Virkajärvi, Alakomi, Sigvart-Mattila, & Mättö, 2006; Wang, Korber, Low, & Nickerson, 2015) and infant powdered formulas (Abe, Miyauchi, Uchijima, Yaeshima, & Iwatsuki, 2009; Liu et al., 2015; Pérez-Conesa, López, Abellán, & Ros, 2006). Despite the health advantages associated

with bifidobacteria, they are very fastidious organisms, and therefore, they perform poorly in food applications and subsequent handling. In general, bifidobacteria are highly vulnerable to changes in acidity, temperature and O_2 content of the environment (Prasanna, Grandison, & Charalampopoulos, 2012, 2014; Ranadheera, Evans, Adams, & Baines, 2015; Ranadheera, Baines, & Adams, 2010).

In this context, encapsulation is effective in improving performances of bifidobacteria in different food products, storage conditions, and the simulated gastrointestinal conditions (Amine et al., 2014a; Fritzen-Freire, Prudêncio, Pinto, Muñoz, & Amboni, 2013; Hansen, Allan-Wojtas, Jin, & Paulson, 2002). There are different techniques, which have been used in the encapsulation of probiotic bacteria. These techniques include extrusion, emulsion, spray drying, freeze-drying, coacervation followed by fluidized bed coating and phase separation (Cocero, Martín, Mattea, & Varona, 2009; Reque, & Brandelli, 2021). Among these techniques, the extrusion technique is effective in the encapsulation of bifidobacteria with higher encapsulation yield and it is considered as a simple and mild technique (Amine et al., 2014b; Picot & Lacroix, 2004).

Sodium alginate is a naturally extracted polymer and it has been widely used as an encapsulating material for probiotics. However, this material yields porous capsules during the extrusion, which cannot protect the encapsulated probiotics from the external environment. In addition, capsules made of sodium alginate are highly vulnerable to extreme pH values (Amine et al., 2014b; Rajam, Karthik, Parthasarathi, Joseph, & Anandharamakrishnan, 2012). Many studies have shown that mixing alginate with other materials during encapsulation and coating of alginate capsules with some other materials could overcome the shortcomings associated with the pure alginate capsules (Etchepare et al., 2016). Some of these modified matrices include alginate-chitosan (Chávarri et al., 2010; Krasaekoopt, Bhandari, & Deeth, 2004), alginate-gelatin (Li, Chen, Cha, Park, & Liu, 2009), and alginate-starch (Mirzaei, Pourjafar, & Homayouni, 2012).

In addition, there are some published data that alginate-cow milk-based matrices could improve the performances of alginate capsules in the simulated gastrointestinal conditions and storage conditions (Gbassi, Vandamme, Ennahar, & Marchioni, 2009; Rajam et al., 2012; Shi et al., 2013). These dairy-based alginate matrices are effective to give protection for the encapsulated bacteria during handling and storage (Maciel, Chaves, Grosso, & Gigante, 2014). Milk and milk proteins have been shown to possess a higher buffering capacity, good emulsification properties, and network formation at low concentrations (Würth et al., 2015). Furthermore, Burgain et al. (2014) reported that bacteria encapsulated in capsules with dairy proteins could improve survival during digestion. Though, there are published works about the encapsulation of probiotics with alginate-cow milk-based matrices, to the best of authors' knowledge skim goat milk has barely been examined with alginate to encapsulate probiotic bacteria using the extrusion technique. Our previous study has shown that goat milk can modify the alginate matrix leading to better protection for *Bifidobacterium longum* subsp. *infantis* CCUG 52486 in cow milk and goat milk during refrigerated storage (Prasanna & Charalampopoulos, 2018). Therefore, this study aimed to encapsulate the probiotic *Bifidobacterium animalis* subsp. *lactis* BB-12 in alginate-skim goat milk (AGM) matrix, and to evaluate the effect of the encapsulating material on the viability of cells under the simulated gastrointestinal conditions and in the different acidic food systems including low-fat yoghurt and orange juice during storage at 4 ^{o}C for 28 days. The cells

encapsulated in alginate-skim cow milk (ASM) were used as the control matrix whereas free cells were also used in every experimental condition to compare the results.

MATERIALS AND METHODS

Microorganism and culture conditions

Bifidobacterium animalis subsp. *lactis* BB-12 was provided by Chr. Hansen Company (Horsholm, Denmark). The freeze-dried culture was activated in MRS broth (Oxoid, Hampshire, UK), under anaerobic conditions at 37 °C for 18 h. The culture was subcultured two times in MRS broth before every experiment. The cells were harvested by centrifugation at 10,000 rpm, 4 °C for 10 min, and washed twice with sterilized phosphate-buffered saline (PBS) (Oxoid, UK). The pellet was dissolved in 10 ml of sterilized PBS (Oxoid, UK) to prepare the concentrated cell suspension.

Encapsulation of *B. animalis subsp. lactis BB-12*

Sterilized skim cow milk and sterilized skim goat milk were purchased from a local supermarket. Sodium alginate solution (2%, (w/v), low viscosity, Sigma-Aldrich, Dorset, UK) was sterilized at 121 ^{o}C for 15 min. Alginate-skim cow milk (ASM), (alginate/skim cow milk = 1.5/1, (v/v)) and alginate-skim goat milk (AGM), (alginate/ skim goat milk = 1.5/1, (v/v)) based encapsulating materials were prepared under aseptic conditions. The concentrated bacterial cell suspension was encapsulated using ASM and AGM as described by Prasanna and Charalampopoulos (2018). The prepared capsules were hardened for 30 min and the separated capsules were washed with sterilized PBS. The capsules were stored in sterilized plastic containers at 4 ^{o}C until further used. In the case of control, 40 ml of PBS (Oxoid, UK) was mixed with 10 ml of the concentrated cell suspension.

Determination of viability of free and encapsulated *B. animalis subsp. lactis BB-12*

The encapsulated bacterial cells and the free cells were enumerated as described previously (Prasanna & Charalampopoulos, 2018).

Viability of free and encapsulated bacterial cells under simulated gastrointestinal conditions

Simulated gastric juice (SGJ) was prepared according to Sun and Griffiths (2000). SGJ was prepared by mixing 0.2% NaCl (w/v) in 0.08 M HCl. The free cells (1ml) or the fresh capsules (1g) were added to glass tubes containing 9 ml of SGJ and incubated in a water bath at 37 ^{o}C. Sampling was done at 0, 30, 60, 90, and 120 min. In the case of free cells, the samples were centrifuged at 10,000 rpm for 10 min, at 4 °C. The pellets were serially diluted and used for the enumeration of the cells, which is described in section 2.3. For the encapsulated bacterial cells, the samples were filtered and dissolved in sodium citrate (0.05 mM). The released cells were enumerated as described in section 2.3.

Simulated intestinal juice (SIJ) was prepared according to Chávarri et al. (2010) by dissolving 3 g of bile salt (Sigma-Aldrich, UK) in 1 l of intestinal solution (6.5 g/l NaCl, 0.835 g/l KCl, 0.22 g/l $CaCl_2$, and 1.386 g/l $NaHCO_3$), at pH 7.5. One milliliter of the free cell or one gram of fresh capsule was added to glass tubes containing 9 ml of SIJ and placed in a water bath at 37 ^{o}C. The sampling and enumeration of free and encapsulated bacteria were carried out according to the methods described in the above paragraph.

Low-fat yoghurt manufacturing with free and encapsulated *B. animalis subsp. lactis BB-12*

Skim cow milk was standardized to 15% (w/v) total solids with skim cows'milk

powder. The mixture was pasteurized at 85 ^{o}C for 30 min and cooled to 43 ^{o}C. The mixture 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 mixed thoroughly and 10 ml of the mixture was poured into sterilized centrifuge tubes (15 ml capacity, polypropylene, Fisher Scientific, UK); the tubes were incubated at 43 ^{o}C until the pH reached ~ 4.5. After the fermentation, 1 ml of the free cells or 1 g of the capsulated *B. animalis* subsp. *lactis* BB-12 was separately mixed with 10 ml of low-fat yoghurt and stored at 4 ^{o}C for 28 days. The samples were collected on 0, 7, 14, 21 and 28 d and the free and encapsulated cells were enumerated according to the methods described in section 2.3.

Storage of free and encapsulated bacterial cells in orange at 4 ^{o}C for 28 days

Commercial orange juice was purchased from a local supermarket and 10 ml of orange juice in sterilized centrifuge tubes (15 m) was separately mixed with 1 ml of the free cells or 1 g of the encapsulated cells. The inoculated samples were stored under refrigerated conditions (4 ^{o}C) for 28 days. The samples were collected on 0, 7, 14, 21, and 28 d and analysed for the viability of free and encapsulated bacteria as described in section 2.3.

Determination of pH

The pH of low-fat yoghurts and the samples of orange juice during the storage period was measured on 0, 7, 14, 21 and 28 d using a pH meter (Mettler Toledo, UK) at room temperature.

Statistical analysis

All the experiments were carried out in triplicate and results of viable counts from simulated gastrointestinal conditions and storage studies were analysed as a split-plot in time design using the General Linear Model (GLM) procedure of SAS, version 9.2 (SAS Institute Inc., Cary NC, USA).

RESULTS AND DISCUSSION

Survival of cells under simulated gastrointestinal conditions

The survival of free and encapsulated *B. animalis* subsp. *lactis* BB-12 in SGJ is shown in Figure 1. Encapsulation of bacterial cells significantly ($p<0.05$) improved cell viability in the simulated gastric conditions for 120 min compared to free cells. The addition of milk either skim goat milk or skim cow milk during the encapsulation increased the viability of *B. animalis* subsp. *lactis* BB-12 in SGJ. The number of bacterial cells encapsulated reduced by 0.85 and 0.98 log cfu g^{-1} for ASM and AGM respectively, after the incubation period. Nevertheless, there was a rapid loss of free bacterial cells during 120 min incubation in SGJ where free *B. animalis* subsp. *lactis* BB-12 cell count decreased by 3.90 log cfu mL^{-1}. Complementary results have been reported with the survival of encapsulated bacterial cells in the simulated gastrointestinal conditions; poor viability of encapsulated bacteria have been observed by some authors (Gbassi et al., 2009; Sultana et al., 2000) whereas, higher survival rates of encapsulated bacteria have been reported in other studies (Chávarri et al., 2010; Krasaekoopt et al., 2004; Sandoval-Castilla, Lobato-Calleros, García-Galindo, Alvarez-Ramírez, & Vernon-Carter, 2010). Similarly, the mixing of dairy-based matrices with alginate matrix was reported to improve the viability of encapsulated cells in SGJ. Rajam et al. (2012) showed the higher viability of *Lactobacillus plantarum*

encapsulated in alginate-whey proteins in SGJ than that of the free cells. In addition, the encapsulated *B. lactis* BB-12 in the casein-based matrix was shown to have better viability in SGJ compared to the free cells (Heidebach, Först, & Kulozik, 2009). Furthermore, higher survival of the encapsulated *Lactobacillus bulgaricus* (Shi et al., 2013) and *Enterococcus faecalis* (Shi, Zheng, Zhang, & Tang, 2016) in alginate-milk matrices was observed in SGJ compared to free cells.

Figure 2 shows the viability of the free and the encapsulated bacterial cells in SIJ at 37 ^{o}C for 120 min. The free cells showed the highest loss of viability when exposed to SIJ; the cell concentration was reduced by 2.82 log cfu mL^{-1} within 120 min. The encapsulated bacterial cells showed higher viability in SIJ; viability of ASM capsules was reduced by 0.66 log cfu g^{-1} while the reduction of cell viability for AGM capsules was 0.65 log cfu g^{-1} after the incubation period. Similarly, encapsulated cells of *Bifidobacterium longum* subsp. *infantis* CCUG 52486 in alginate milk-based matrices were shown to have better survival in SGJ and SIJ (Prasanna & Charalampopoulos, 2018). The results indicate that the encapsulated *B. animalis* subsp. *lactis* BB-12 in ASM and AGM could give a better protection against harmful environmental conditions in the digestive tract. This can be explained by

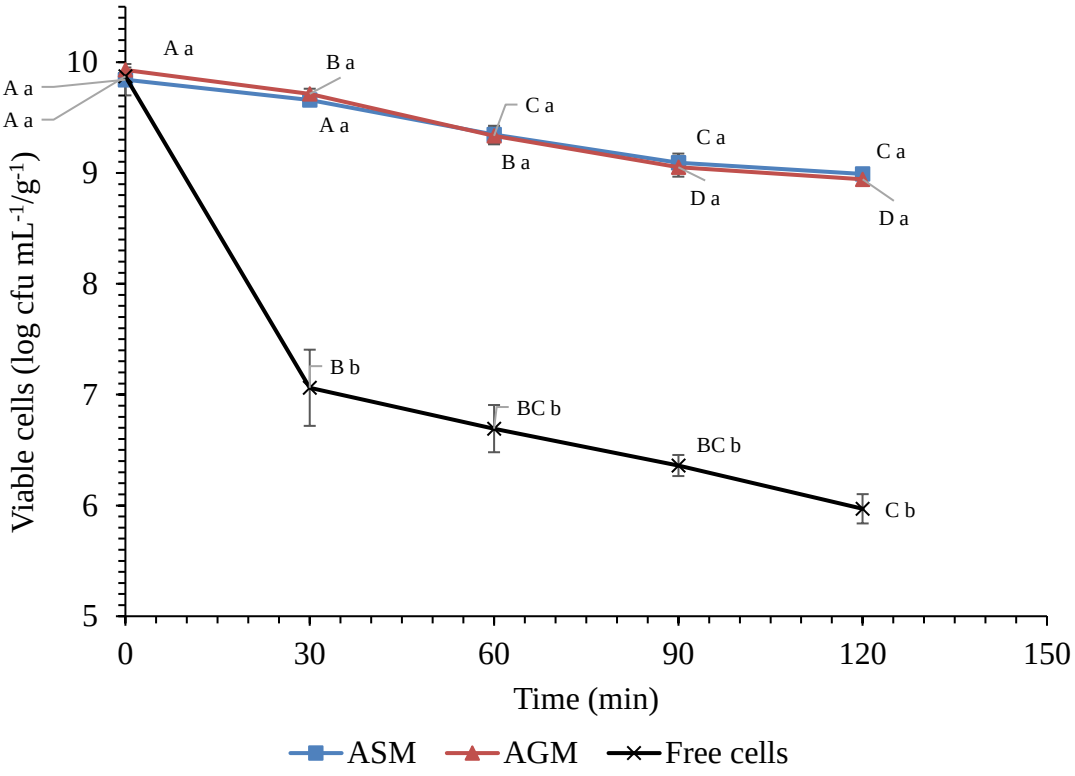

Figure 1: Survival of free and encapsulated *B. animalis subsp. lactis BB-12* in simulated gastric juice (pH 2) at 37 ^{o}C for 120 min.

Vertical lines represent standard deviations.

^{ABCD}Means with different uppercase are significantly different ($p<0.05$) between each time, for each type of alginate-dairy based capsule during the period of the analysis.

^{ab}Means with different lowercase are significantly different ($p<0.05$) between each type of alginate-dairy based capsule, for a particular time of the analysis.

ASM: capsules were produced using alginate and skim cow milk at a ratio of 1.5:1 (v/v).

AGM: capsules were produced using alginate and skim goat milk at a ratio of 1.5:1 (v/v).

that milk ingredients could improve the textural properties of alginate-based capsules, which can limit the diffusion of acid and bile into the beads (Gbassi et al., 2009) which was further observed in a previous study (Prasanna & Charalampopoulos, 2018).

bacteria in orange juice, and at the end of the storage period of 4 weeks, the count of bacterial cells was 9.56 log cfu g^{-1} and 9.57 log cfu g^{-1} respectively. Similarly, Ding and Shah (2008) reported that encapsulation of *B. longum*, *B. lactis* type Bi-04 and *B. lactis* type Bi-07 in alginate resulted in higher survival rate in orange juice during storage

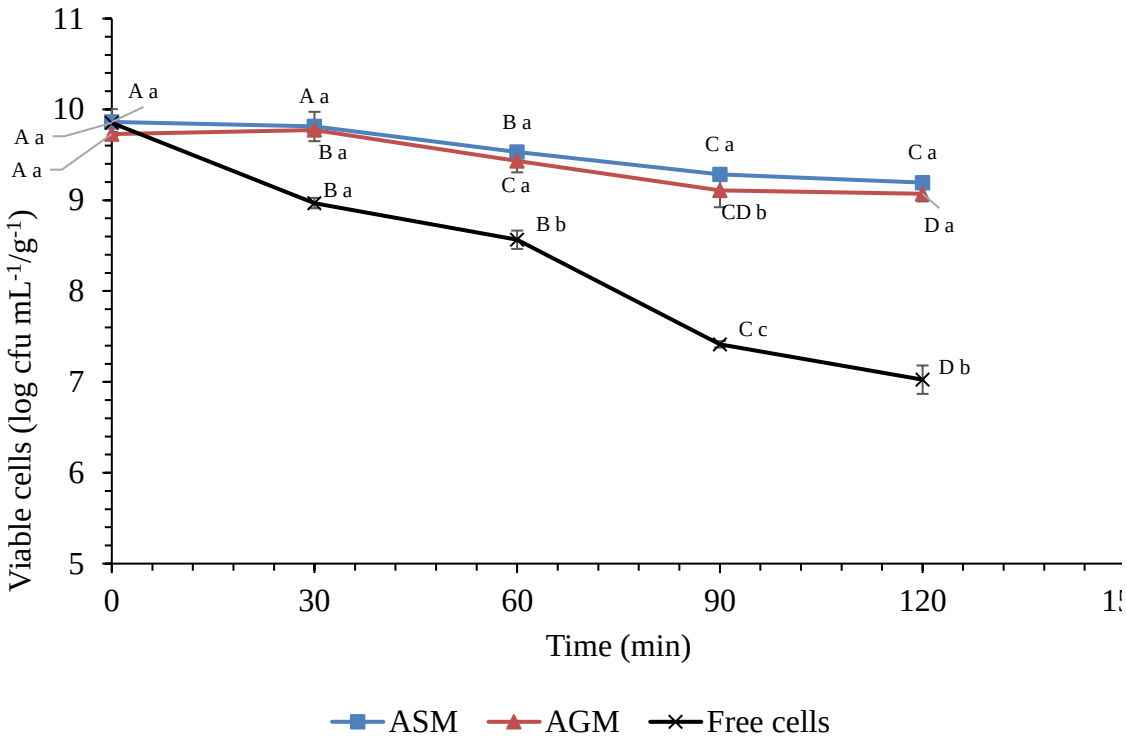

Figure 2: Changes in viable cell count of free and encapsulated *B. animalis subsp. lactis BB-12* in simulated intestinal juice (pH 7.5) at 37 ºC for 120 min.

Vertical lines represent standard deviations.
[ABCD]Means with different uppercase are significantly different ($p<0.05$) between each time, for each type of alginate-dairy based capsule during the period of the analysis.
[abc]Means with different lowercase are significantly different ($p<0.05$) between each type of alginate-dairy based capsule, for a particular time of the analysis.
For legend explanations see Figure 1.

Survival of free and encapsulated bacteria in orange juice during storage

The changes of cell numbers of free and encapsulated bacteria in orange juice during the storage period are shown in Figure 3. There was a significant ($p<0.05$) loss of free bacterial cells in orange juice within a four-week storage period at 4 ºC. The encapsulated bacterial cells showed higher survival in orange juice during the storage. ASM and AGM improved the viability of

than free cells. In addition, the improved viability of encapsulated *Lactobacillus paracasei* L26 with alginate in orange juice at 5 ºC was observed by Rodrigues et al. (2012). Furthermore, Nualkaekul, Lenton, Cook, Khutoryanskiy, and Charalampopoulos (2012) reported better survival of encapsulated *Lactobacillus plantarum* with alginate in pomegranate

juice under the refrigerated storage than that of the free cells.

significantly ($p>0.05$) different. The lower pH changes of orange juice containing the encapsulated bacterial cells may be due to

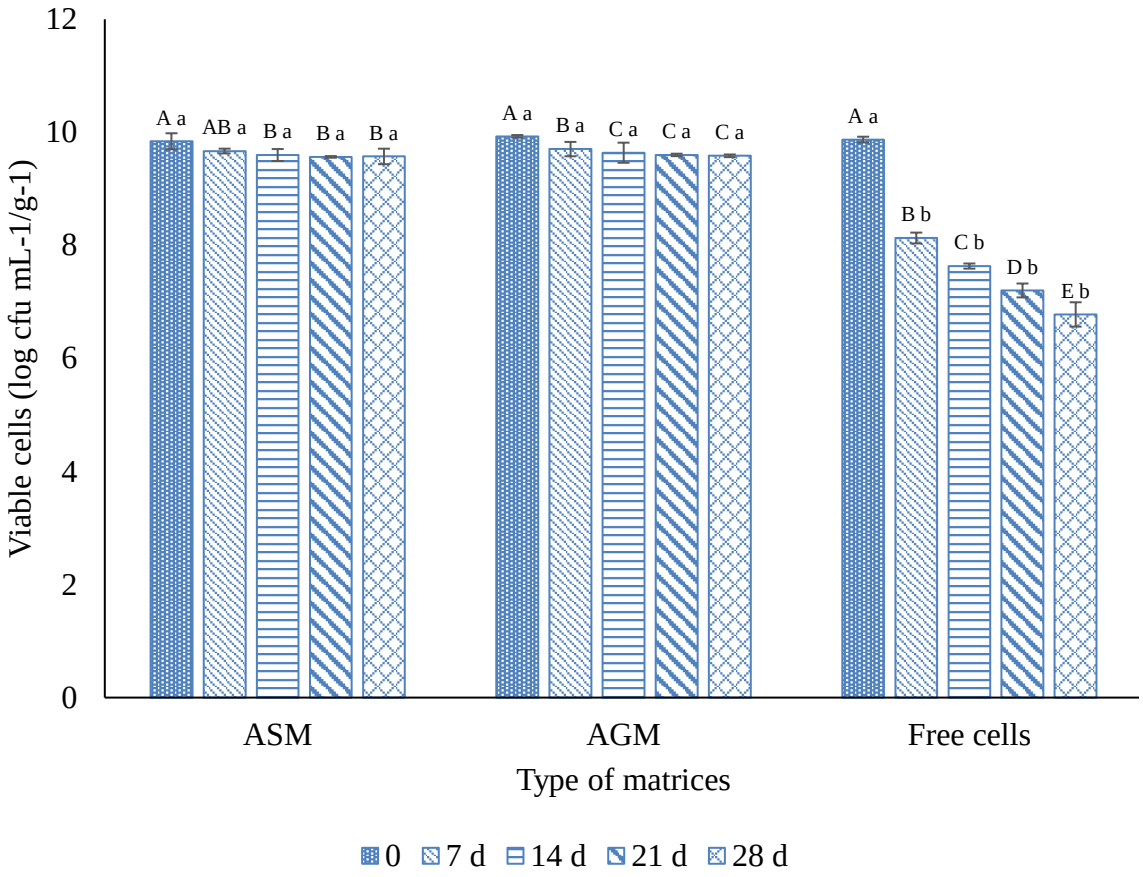

Figure 3: Survival of free and the encapsulated *B. animalis subsp. lactis BB-12* in orange juice at 4 ºC for 28 days.

Vertical lines represent standard deviations.

[ABCDE]Means with different uppercase are significantly different ($p<0.05$) between each time, for each type of alginate-dairy based capsule during the storage.

[ab]Means with different lowercase are significantly different ($p<0.05$) between each type of alginate-dairy based capsule, for a particular day of the storage period.

For legend explanations see Figure 1.

Changes in pH values of orange juice during storage

The changes of pH of free and encapsulated bacteria containing orange juice at 4 ºC for 4 weeks are shown in Figure 4. An only slight reduction of pH of orange juice was observed despite whether the bacterial cells were introduced in the form of free or encapsulated cells. However, orange juice containing the encapsulated bacteria in ASM and AGM resulted in higher pH values than that of free cells at the end of the storage period, the values were not

limited diffusion of sugars into capsules which limit the production of organic acids by bacteria. The slightly lower pH observed in orange juice containing free cells may be due to the production of the small amount of organic acids by free bacterial cells in fruit juices by utilizing available carbohydrates in orange juice during the storage as explained by Ding and Shah (2008). In addition, dead bacterial cells during the storage could release enzymes, which can hydrolyze carbohydrates in fruit juice leading to lowering pH value. Furthermore, these results are in

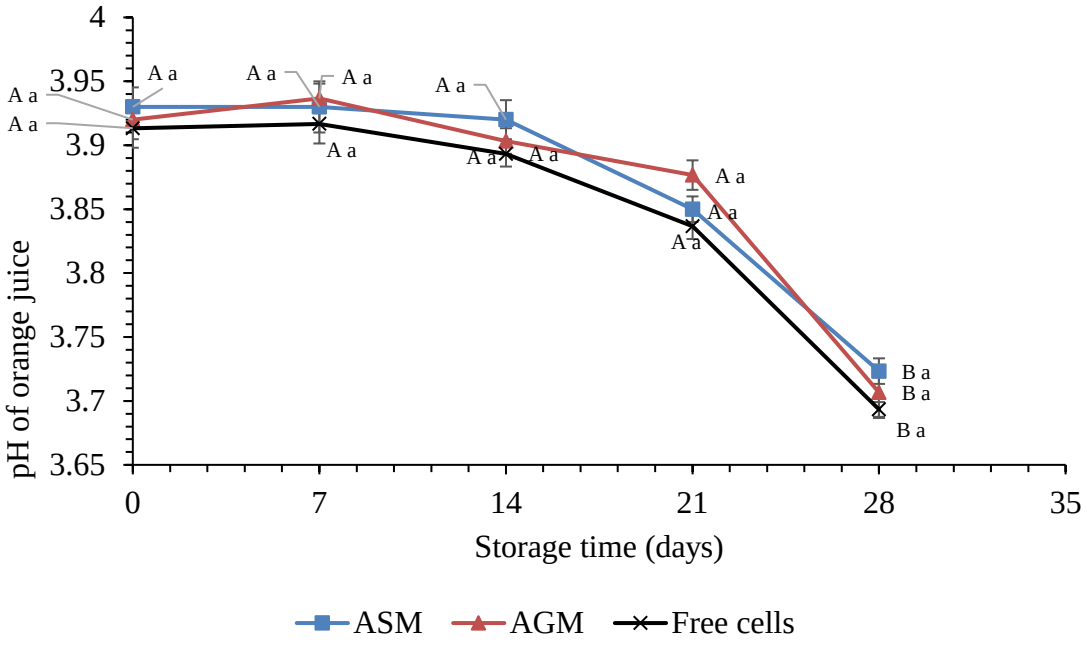

Figure 4: Changes in pH of orange juice at 4 ºC for 28 days. Vertical lines represent standard deviations.

[AB]Means with different uppercase are significantly different ($p<0.05$) between each time, for each type of alginate-dairy based capsule during the storage.

[a]Means with different lowercase are significantly different ($p<0.05$) between each type of alginate-dairy based capsule, for a particular day of the storage period.

For legend explanations see Figure 1.

accordance with the findings of Rodrigues et al. (2012) who observed a similar pattern of pH variation of the encapsulated and the free *Lactobacillus paracasei* L26 in orange juice during the storage.

Performances of free and encapsulated bacteria in low-fat yoghurt during storage

The changes in the viable counts of free and encapsulated *B. animalis* subsp. *lactis* BB-12 in low-fat yoghurts during the refrigerated storage are shown in Table 1. The results showed that there was a significant ($p<0.05$) loss of the cell numbers of free bacteria over a period of 4 weeks. There was 2.25 log cfu mL^{-1} loss in

Table 1: Survival of free and encapsulated *B. animalis subsp. lactis BB-12* in low-fat yoghurt at 4 ºC for 28 days

Type of capsule	Storage period (days)				
	0	**7**	**14**	**21**	**28**
ASM (log cfu g^{-1})	9.86 ± 0.21^{Aa}	9.75 ± 0.04^{AB} a	9.60 ± 0.12^{Ba}	9.57 ± 0.13^{Ba}	9.57 ± 0.14^{B}
AGM (log cfu g^{-1})	9.87 ± 0.03^{Aa}	9.67 ± 0.13^{Ba}	9.62 ± 0.05^{Ba}	9.60 ± 0.07^{Ba}	9.54 ± 0.02^{B}
Free Cells (log cfu mL^{-1})	9.88 ± 0.07^{Aa}	8.28 ± 0.10^{Bb}	8.05 ± 0.14^{Cb}	7.92 ± 0.03^{Db}	7.63 ± 0.22^{E}

[ABCDE]Means in the same row without common letter differ significantly ($p<0.05$) for each type of capsules.

[ab]Means in the same column for each type of capsule without common letter differ significantly ($p<0.05$) for a particular day of storage.

Data are expressed as mean ± standard deviation. For legend explanations see Fig.1.

viable counts of free bacterial cells during the storage. However, the encapsulation with dairy-based matrices significantly improved the viability of bacterial cells in low-fat yoghurt during the storage; encapsulated cells in ASM and AGM resulted in approximately 0.28 and 0.33 log cfu g^{-1} losses in viable counts, respectively.

Bifidobacteria is considered as very fastidious microorganisms which are highly vulnerable to the acidic environment leading to poor viability during prolong storage. However, the higher cell concentration observed with the encapsulated bacteria cells in low-fat yoghurt shows that these matrices can effectively protect bifidobacterial cells from the acidic environment in the yoghurt. Similarly, a higher survival rate of the encapsulated bifidobacteria in different yoghurt types has been reported. Picot and Lacroix (2004) showed that encapsulated *B. breve* R070 and *B. longum* R023 in whey protein-based matrices resulted in higher survival rate in yoghurt during the storage at 4 ^{o}C for 28 days than that of free cells. In addition, the encapsulated *B. lactis* in alginate-starch was shown to have a higher survival rate in yoghurt during the refrigerated storage compared to the free cells (Kailasapathy, 2006).

Changes in pH of yoghurt samples during refrigerated storage

A significant ($p<0.05$) decrease in pH for all low-fat yoghurt samples was recorded throughout the storage period of 4 weeks (Figure 5). However, free cells resulted in the lowest pH value after storage for 4 weeks. Similarly, a continuous decrease in pH was reported with yoghurt containing encapsulated *B. breve* R070 (Picot &

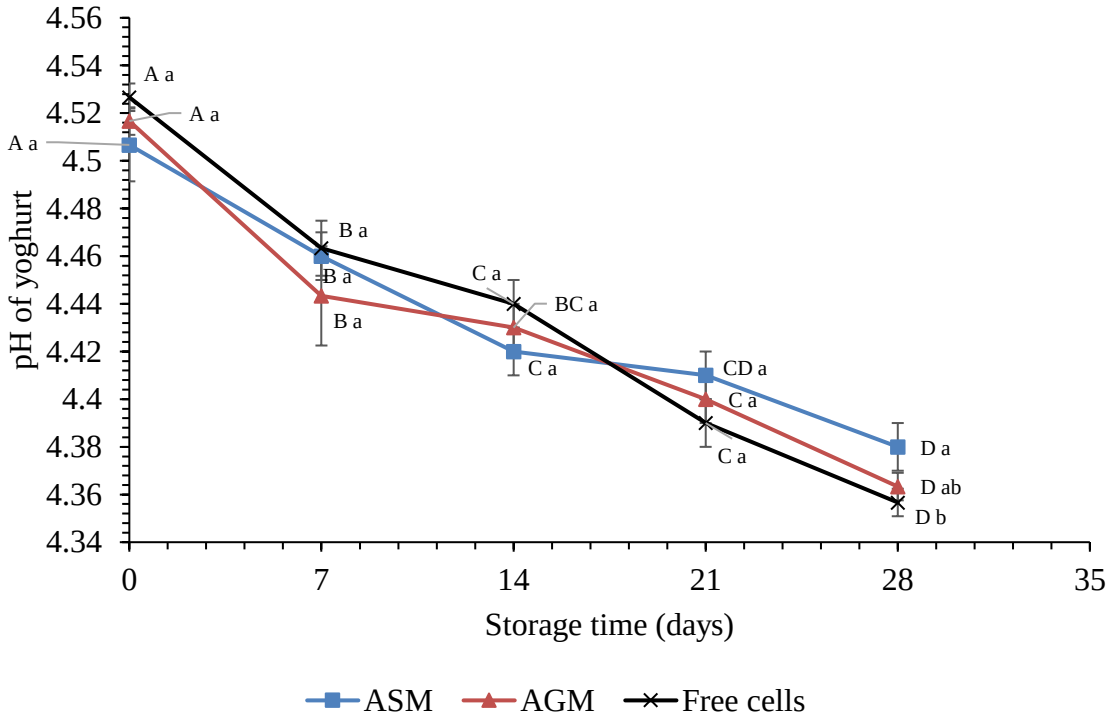

Figure 5: Changes in pH of low-fat yoghurt containing free and encapsulated bacterial cells at 4 ^{o}C for 28 days.

Vertical lines represent standard deviations.

[AB]Means with different uppercase are significantly different ($p<0.05$) between each time, for each type of alginate-dairy based capsule during the storage.

[a]Means with different lowercase are significantly different ($p<0.05$) between each type of alginate-dairy based capsule, for a particular day of the storage period.

For legend explanations see Figure 1

Lacroix, 2004), *B. lactis* (Kailasapathy, 2006), *B. longum* R023 (Picot & Lacroix, 2004), and *B. longum* (Adhikari, Mustapha, & Grün, 2003). *Streptococcus thermophiles* and *Lactobacillus delbrueckii* subsp. *bulgaricus* which are yoghurt starter cultures; can produce lactic acid even at the refrigerated storage at a slow rate which is mainly responsible for the reduction of pH in yoghurt (Shah, Lankaputhra, Britz, & Kyle, 1995). In addition, free bifidobacteria can produce both lactic and acetic acids with yoghurt starter cultures under refrigerated storage which can further reduce pH in yoghurt containing free cells than encapsulated bacterial cells (Samona, Robinson, & Marakis, 1996).

CONCLUSIONS

The present study showed that encapsulation of the probiotic *B. animalis* subsp. *lactis* BB-12 in AGM improved survival of bacterial cells similarly to the cells encapsulated in ASM in the simulated gastrointestinal conditions compared to free cells. The encapsulation of the bifidobacterial cells in ASM and AGM improved the survival of bacterial cells in acidic food systems namely orange juice and low-fat yoghurt during the refrigerated storage compared to the free cells over 28 days. Addition of encapsulated bifidobacteria showed the minimum pH changes in low-fat yoghurt and orange juice during the storage compared to the free cells. Overall, this study showed that both alginate-skim goat milk and alginate-skim cow milk, similarly have a potential to be used as encapsulating materials to encapsulate probiotic bifidobacteria which could be used in acidic food systems such as orange juice and fermented dairy products including low-fat yoghurt with improved viability during refrigerated storage.

REFERENCES

Abe, F., Miyauchi, H., Uchijima, A., Yaeshima, T., & Iwatsuki, K. (2009). Effects of storage temperature and water activity on the survival of bifidobacteria in powder form. *International Journal of Dairy Technology, 62*(2), 234-239.

Adhikari, K., Mustapha, A., & Grün, I. (2003). Survival and metabolic activity of microencapsulated *Bifidobacterium longum* in stirred yogurt. *Journal of Food Science, 68*(1), 275-280.

Agrawal, A., Houghton, L. A., Morris, J., Reilly, B., Guyonnet, D., Goupil Feuillerat, N., Schlumberger, A., Jakob, S., & Whorwell, P. J. (2009). Clinical trial: the effects of a fermented milk product containing *Bifidobacterium lactis* DN-173 010 on abdominal distension and gastrointestinal transit in irritable bowel syndrome with constipation. *Alimentary pharmacology & therapeutics, 29*(1), 104-114.

Amine, K. M., Champagne, C. P., Raymond, Y., St-Gelais, D., Britten, M., Fustier, P., Salmieri, S., & Lacroix, M. (2014a). Survival of microencapsulated *Bifidobacterium longum* in Cheddar cheese during production and storage. *Food Control, 37*, 193-199.

Amine, K. M., Champagne, C. P., Salmieri, S., Britten, M., St-Gelais, D., Fustier, P., & Lacroix, M. (2014b). Effect of palmitoylated alginate microencapsulation on viability of *Bifidobacterium longum* during freeze-drying. *LWT-Food Science and Technology, 56*(1), 111-117.

Bajaj, B. K., Claes, I. J., & Lebeer, S. (2021). Functional mechanisms of probiotics. *Journal of microbiology, Biotechnology and Food Sciences*, 2021, 321-327.

Bevilacqua, A., Campaniello, D., Corbo, M. R., Maddalena, L., & Sinigaglia, M. (2013). Suitability of *Bifidobacterium* spp. and *Lactobacillus plantarum* as probiotics intended for fruit juices containing citrus extracts. *Journal of Food Science, 78*(11), M1764-M1771.

Bezerra, M., Araujo, A., Santos, K., & Correia, R. (2015). Caprine frozen yoghurt produced with fresh and spray dried jambolan fruit pulp (*Eugenia jambolana* Lam) and *Bifidobacterium animalis* subsp. *lactis* BI-07. *LWT-Food Science and Technology, 62*(2), 1099-1104.

Burgain, J., Gaiani, C., Linder, M., & Scher, J. (2011). Encapsulation of probiotic living cells: From laboratory scale to industrial applications. *Journal of Food Engineering, 104*(4), 467-483.

Burgain, J., Scher, J., Lebeer, S., Vanderleyden, J., Cailliez-Grimal, C., Corgneau, M., Francius, G., & Gaiani, C. (2014). Significance of bacterial surface molecules interactions with milk proteins to enhance microencapsulation of *Lactobacillus rhamnosus* GG. *Food Hydrocolloids, 41*, 60-70.

Chávarri, M., Marañón, I., Ares, R., Ibáñez, F. C., Marzo, F., & del Carmen Villarán, M. (2010). Microencapsulation of a probiotic and prebiotic in alginate-chitosan capsules improves survival in simulated gastro-intestinal conditions. *International Journal of Food Microbiology, 142*(1), 185-189.

Cocero, M. J., Martín, Á., Mattea, F., & Varona, S. (2009). Encapsulation and co-precipitation processes with supercritical fluids: fundamentals and applications. *The Journal of Supercritical Fluids, 47*(3), 546-555.

Ding, W. K., & Shah, N. P. (2008). Survival of free and microencapsulated probiotic bacteria in orange and apple juices. *International Food Research Journal, 15*(2), 219-232.

Etchepare, M. A., Raddatz, G. C., Cichoski, A. J., Flores, É. M. M., Barin, J. S., Queiroz Zepka, L., Jacob-Lopes, E., Grosso, C. R. F., & de Menezes, C. R. (2016). Effect of resistant starch (Hi-maize) on the survival of *Lactobacillus acidophilus* microencapsulated with sodium alginate. *Journal of Functional Foods, 21*, 321-329.

Fritzen-Freire, C. B., Prudêncio, E. S., Pinto, S. S., Muñoz, I. B., & Amboni, R. D. M. C. (2013). Effect of microencapsulation on survival of *Bifidobacterium* BB-12 exposed to simulated gastrointestinal conditions and heat treatments. *LWT-Food Science and Technology, 50*(1), 39-44.

Gbassi, G. K., Vandamme, T., Ennahar, S., & Marchioni, E. (2009). Microencapsulation of *Lactobacillus plantarum* spp in an alginate matrix coated with whey proteins. *International Journal of Food Microbiology, 129*(1), 103-105.

Gomi, A., Iino, T., Nonaka, C., Miyazaki, K., & Ishikawa, F. (2015). Health benefits of fermented milk containing *Bifidobacterium bifidum* YIT 10347 on gastric symptoms in adults. *Journal of Dairy Science, 98*(4), 2277-2283.

Hansen, L. T., Allan-Wojtas, P. M., Jin, Y. L., & Paulson, A. T. (2002). Survival of Ca-alginate microencapsulated *Bifidobacterium* spp. in milk and simulated gastrointestinal conditions. *Food Microbiology, 19*(1), 35-45.

Heidebach, T., Först, P., & Kulozik, U. (2009). Microencapsulation of probiotic cells by means of rennet-

gelation of milk proteins. *Food Hydrocolloids, 23*(7), 1670-1677.

Kailasapathy, K. (2006). Survival of free and encapsulated probiotic bacteria and their effect on the sensory properties of yoghurt. *LWT-Food Science and Technology, 39*(10), 1221-1227.

Krasaekoopt, W., Bhandari, B., & Deeth, H. (2004). The influence of coating materials on some properties of alginate beads and survivability of microencapsulated probiotic bacteria. *International Dairy Journal, 14*(8), 737-743.

Li, X. Y., Chen, X. G., Cha, D. S., Park, H. J., & Liu, C. S. (2009). Microencapsulation of a probiotic bacteria with alginate-gelatin and its properties. *Journal of Microencapsulation, 26*(4), 315-324.

Liu, Y., Yu, Y., Duan, W., Qu, Q., Zhang, Q., Zhao, M., & Zhu, Q. (2015). Home storage significantly impairs bifidobacteria survival in powered formula for infants and young children in the Chinese market. *International Journal of Dairy Technology, 68*(4), 495-502.

Maciel, G. M., Chaves, K. S., Grosso, C. R. F., & Gigante, M. L. (2014). Microencapsulation of *Lactobacillus acidophilus* La-5 by spray-drying using sweet whey and skim milk as encapsulating materials. *Journal of Dairy Science, 97*(4), 1991-1998.

Mirzaei, H., Pourjafar, H., & Homayouni, A. (2012). Effect of calcium alginate and resistant starch microencapsulation on the survival rate of *Lactobacillus acidophilus* La5 and sensory properties in Iranian white brined cheese. *Food Chemistry, 132*(4), 1966-1970.

Nualkaekul, S., Lenton, D., Cook, M. T., Khutoryanskiy, V. V., & Charalampopoulos, D. (2012). Chitosan coated alginate beads for the survival of microencapsulated *Lactobacillus plantarum* in pomegranate juice. *Carbohydrate Polymers, 90*(3), 1281-1287.

Pérez-Conesa, D., López, G., Abellán, P., & Ros, G. (2006). Bioavailability of calcium, magnesium and phosphorus in rats fed probiotic, prebiotic and synbiotic powder follow-up infant formulas and their effect on physiological and nutritional parameters. *Journal of the Science of Food and Agriculture, 86*(14), 2327-2336.

Picot, A., & Lacroix, C. (2004). Encapsulation of bifidobacteria in whey protein-based microcapsules and survival in simulated gastrointestinal conditions and in yoghurt. *International Dairy Journal, 14*(6), 505-515.

Prasanna, P. H. P., & Charalampopoulos, D. (2018). Encapsulation of *Bifidobacterium longum* in alginate-dairy matrices and survival in simulated gastrointestinal conditions, refrigeration, cow milk and goat milk. *Food Bioscience, 21*, 72-79.

Prasanna, P. H. P., Grandison, A. S., & Charalampopoulos, D. (2012). Effect of dairy-based protein sources and temperature on growth, acidification and exopolysaccharide production of *Bifidobacterium* strains in skim milk. *Food Research International, 47*(1), 6-12.

Prasanna, P. H. P., Grandison, A. S., & Charalampopoulos, D. (2013). Microbiological, chemical and rheological properties of low fat set yoghurt produced with exopolysaccharide (EPS) producing *Bifidobacterium* strains. *Food Research International, 51*(1), 15-22.

Prasanna, P. H. P., Grandison, A. S., & Charalampopoulos, D. (2014). Bifidobacteria in milk products: An overview of physiological and

biochemical properties, exopolysaccharide production, selection criteria of milk products and health benefits. *Food Research International, 55*, 247-262.

Rajam, R., Karthik, P., Parthasarathi, S., Joseph, G., & Anandharamakrishnan, C. (2012). Effect of whey protein–alginate wall systems on survival of microencapsulated *Lactobacillus plantarum* in simulated gastrointestinal conditions. *Journal of Functional Foods, 4*(4), 891-898.

Ranadheera, C. S., Evans, C., Adams, M., & Baines, S. (2012). In vitro analysis of gastrointestinal tolerance and intestinal cell adhesion of probiotics in goat's milk ice cream and yogurt. *Food research international, 49*(2), 619-625.

Ranadheera, C. S., Evans, C. A., Adams, M. C., & Baines, S. K. (2015). Microencapsulation of *Lactobacillus acidophilus* LA-5, *Bifidobacterium animalis* subsp. *lactis* BB-12 and *Propionibacterium jensenii* 702 by spray drying in goat's milk. *Small Ruminant Research, 123*(1), 155-159.

Ranadheera, R. D. C. S., Baines, S. K., & Adams, M. C. (2010). Importance of food in probiotic efficacy. *Food Research International, 43*(1), 1-7.

Reque, P. M., & Brandelli, A. (2021). Encapsulation of probiotics and nutraceuticals: Applications in functional food industry. *Trends in Food Science & Technology*, 114, 1-10.

Rodrigues, D., Sousa, S., Gomes, A. M., Pintado, M. M., Silva, J. P., Costa, P., Amaral, M. H., Rocha-Santos, T., & Freitas, A. C. (2012). Storage stability of *Lactobacillus paracasei* as free cells or encapsulated in alginate-based microcapsules in low pH fruit juices. *Food and Bioprocess Technology, 5*(7), 2748-2757.

Saad, N., Delattre, C., Urdaci, M., Schmitter, J. M., & Bressollier, P. (2013). An overview of the last advances in probiotic and prebiotic field. *LWT - Food Science and Technology, 50*(1), 1-16.

Saarela, M., Virkajärvi, I., Alakomi, H. L., Sigvart-Mattila, P., & Mättö, J. (2006). Stability and functionality of freeze-dried probiotic *Bifidobacterium* cells during storage in juice and milk. *International Dairy Journal, 16*(12), 1477-1482.

Samona, A., Robinson, R. K., & Marakis, S. (1996). Acid production by bifidobacteria and yoghurt bacteria during fermentation and storage of milk. *Food Microbiology, 13*(4), 275-280.

Sandoval-Castilla, O., Lobato-Calleros, C., García-Galindo, H., Alvarez-Ramírez, J., & Vernon-Carter, E. (2010). Textural properties of alginate–pectin beads and survivability of entrapped *Lb. casei* in simulated gastrointestinal conditions and in yoghurt. *Food Research International, 43*(1), 111-117.

Shah, N. P., Lankaputhra, W. E. V., Britz, M. L., & Kyle, W. S. A. (1995). Survival of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in commercial yoghurt during refrigerated storage. *International Dairy Journal, 5*(5), 515-521.

Shi, L. E., Li, Z. H., Li, D. T., Xu, M., Chen, H. Y., Zhang, Z. L., & Tang, Z. X. (2013). Encapsulation of probiotic *Lactobacillus bulgaricus* in alginate–milk microspheres and evaluation of the survival in simulated gastrointestinal conditions. *Journal of Food Engineering, 117*(1), 99-104.

Shi, L. E., Zheng, W., Zhang, Y., & Tang, Z. X. (2016). Milk-alginate microspheres: Protection and delivery of *Enterococcus faecalis* HZNU P2. *LWT-Food Science and Technology, 65*, 840-844.

Sultana, K., Godward, G., Reynolds, N., Arumugaswamy, R., Peiris, P., & Kailasapathy, K. (2000). Encapsulation of probiotic bacteria with alginate–starch and evaluation of survival in simulated gastrointestinal conditions and in yoghurt. *International Journal of Food Microbiology, 62*(1–2), 47-55.

Sun, W., & Griffiths, M. W. (2000). Survival of bifidobacteria in yogurt and simulated gastric juice following immobilization in gellan–xanthan beads. *International Journal of Food Microbiology, 61*(1), 17-25.

Wang, J., Korber, D. R., Low, N. H., & Nickerson, M. T. (2015). Encapsulation of *Bifidobacterium adolescentis* cells with legume proteins and survival under stimulated gastric conditions and during storage in commercial fruit juices. *Food Science and Biotechnology, 24*(2), 383-391.

Würth, R., Hörmannsperger, G., Wilke, J., Foerst, P., Haller, D., & Kulozik, U. (2015). Protective effect of milk protein based microencapsulation on bacterial survival in simulated gastric juice versus the murine gastrointestinal system. *Journal of Functional Foods, 15*, 116-125.