

## Studies on microencapsulation of *Lactobacillus acidophilus* NCIM 5306 and evaluation of matrix material efficiency in pomegranate juice

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### Abstract

The present study was conducted on the microencapsulation of *Lactobacillus acidophilus* NCIM 5306 probiotic lactic acid bacteria. Sodium alginate, sodium alginate + 0.4% chitosan, sodium alginate with 2% inulin, and sodium alginate with 2% inulin + 0.4% chitosan coating were used as microencapsulated matrix material. The extrusion method was used for the formation of various micro encapsulates. The probiotic pomegranate juice was formulated using the obtained beads. The freshly prepared beads were first subjected to viability assay and then inoculated in pasteurized pomegranate juice at 1% quantity. The juice was stored at 4°C and further subjected to analysis for cell viability and sensory analysis. Response for cell viability was found discrete in various types of beads. The obtained results showed the use of 2% inulin as prebiotic in microencapsulation results in the prolonged period survival of the *L. acidophilus* NCIM 5306 up to six weeks.

## 1. Introduction

Probiotics are the 'live microorganisms that, when administered in adequate amounts, confer a health benefit to the host' (Hill *et al.*, 2014) These lactic acid bacteria are characterized by their ability to ferment carbohydrates into lactic acid, a weak acid conducive to the conservation and improvement of the organoleptic quality of food. Lactic acid bacteria are Gram-positive and non-spore-forming microorganisms. The ability to convert some sugars into lactate and so, acidify the surrounding environment is the main reason for the growing interest in the application of Lactic Acid bacteria (LAB) in the food industry. The lactic acid bacteria produced as commercial starter cultures are pure cultures or a mix belonging to the genera *Streptococcus*, *Lactobacillus*, *Enterococcus*, *Leuconostoc*, *Aerococcus*, *Lactococcus*, *Streptococcus*, *Pediococcus*, and *Bifidobacterium* (Luquet and Corrieu *et al.*, 2005). Extensive research in light of the selection of proper probiotic strains has revealed the use of the bacteria from genera *Bifidobacteria* and *Lactobacillus* as effective probiotic strains. *Bifidobacteria* are commonly used in the manufacture of fermented dairy products (Ranadheera *et al.*, 2010). Consumption of products containing probiotic *Bifidobacteria* has related to the numerous health benefits about lowering serum

cholesterol levels, enhancing immune function, alleviating diarrhoea, reducing lactose intolerance, modulation of the gut microbiota, and prevention of allergy (Prasanna *et al.*, 2014). However, a significant number of bacterial cells die during the processing and storage of the food products, and subsequently during passage through the gastrointestinal tract (Champagne *et al.*, 2005) survival of probiotic bacteria in food products used as delivery vehicles is of major concern. The consumption of the required quantity ( $10^8$ – $10^9$  cells per day) only may lead to confer their health benefits to the host (Champagne *et al.*, 2008).

Microencapsulation is one of the effective methods to enhance probiotic survival in the food systems process among the available techniques (Martin *et al.*, 2015). In this method, the live cells are entrapped within an encapsulating matrix. Microencapsulation of probiotics has been investigated for improving their viability in food products and the intestinal tract (Rao *et al.*, 1989). Sodium alginate is a commonly used material for probiotic encapsulation. However, this material is disintegrated very easily at low pH leading to the release of microorganisms entrapped in beads into the environment (Krasaekoopt *et al.*, 2004). Therefore, alginate is mixed with other materials to improve the stability of alginate capsules in food systems. Various

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polymers are reported, which can be used as encapsulation materials including alginate, pectin, k-carrageenan, xanthan gum, gellan gum, starch derivatives, cellulose acetate phthalate, casein, whey proteins, and gelatin; among these alginate is the most studied and commonly used encapsulation material. Alginate is an anionic copolymer of 1,4-linked-dmannuronic acid and l-guluronic acid residues which form a gel through the cross-linking of the guluronic acid blocks by the calcium ions, resulting in an “egg-box” structure (Gombotz and Wee, 1998). The concentration of alginate commonly used to form the gel ranges from 0.6% to 3%, whereas that of calcium chloride from 0.05 to 1.5 M (Krasaekoopt *et al.*, 2003). Chitosan, is another coating material that can be used with sodium alginate and which has proven to increase in survival of probiotics in simulated gastric and intestinal juices compared to uncoated alginate beads. Chitosan is the partly acetylated (1-4)-2-amino-2-deoxy-D-glucan obtained from chitin (Chavarri *et al.*, 2010; Muzzarelli *et al.*, 2012). The negatively charged alginate forms a semi-permeable membrane with the positively charged chitosan and as a result, the capsule has a smoother surface and is less permeable to water-soluble molecules (Krasaekoopt *et al.*, 2004).

The survival of probiotics, such as *Lactobacilli* and *Bifidobacteria*, has already been studied in a variety of fruit juices during refrigerated storage. It was shown that the free cells survived well during storage for up to 6 weeks (cell decrease <1 log) in certain fruit juices with high pH (orange, apple, grapefruit, blackcurrant, pineapple) (Champagne *et al.*, 2008; Nualkaekul *et al.*, 2011). In other juices, however, such as pomegranate, strawberry, and cranberry, the probiotic cells died very quickly, within 1–4 weeks. The reason for this was most likely the very low pH of these juices ( $\text{pH} \leq 3$ ) and the high total phenol concentration (Nualkaekul and Charalampopoulos, 2011). The pomegranate is acknowledged as a rich source of bioactive compounds such as phenols, (ellagic acid, punicalagin, punicalin, and flavonoids), anthocyanin, ascorbic acid, vitamins and minerals (potassium, calcium, phosphorous, magnesium, sodium) as well the complex polysaccharides and organic acids emphasized pomegranate to be the outstanding functional food (Mirdehghan and Rahemi 2007). To develop the pomegranate juice as effective functional food under the category of probiotic food, in the present study attempt was made to develop a suitable microencapsulation system (proper coating materials) using coating material along with prebiotic as an effective microencapsulation system which could deliver through the pomegranate juice. The present investigation was carried out aiming to investigate the potential protective effect of uncoated as

well as single and double chitosan, inulin and chitosan and inulin coated alginate beads on the survival of a model *L. acidophilus* strain in pomegranate juice, a highly acidic juice.

## 2. Materials and methods

### 2.1 Preparation of the bacterial suspension

A volume of 100  $\mu\text{L}$  of *Lactobacillus acidophilus* NCIM 5306 was inoculated in 10 mL of sterile MRS broth and incubated at 37°C for 24 hrs under aerobic conditions. The probiotic cells were harvested by centrifugation at 3000 rpm and 4°C for 5 mins. The cells were washed with sterile 0.1% peptone solution and then successively dispensed to microencapsulation (Krasaekoopt *et al.*, 2004).

### 2.2 Microencapsulation using sodium alginate and inulin

For the preparation of sodium alginate beads, the extrusion technique was used (Krasaekoopt *et al.*, 2004). The 3% sodium alginate, 2% prebiotic inulin, bacterial cell culture (approximately  $10^{12}$  CFU/mL) were kept for 30 mins hardening in 0.15 M calcium chloride solution. The cell suspension/sodium alginate and cell suspension/sodium alginate and inulin mixture were extruded through a nozzle having a diameter of 200 microns into the 0.15 M calcium chloride. The droplets formed gel spheres. The distance between the nozzle and calcium chloride ( $\text{CaCl}_2$ ) solution was 25 cm (Mirzaei *et al.*, 2012). For hardening beads were allowed to stand for 30 mins then beads were harvested using Whatman filter paper no. 4. The whole procedure was carried out under aseptic conditions by using a horizontal laminar airflow cabinet (Darjani *et al.*, 2016)

### 2.3 Coating with chitosan

Two-stage methods were used for the coating of sodium alginate beads with chitosan (Krasaekoopt *et al.*, 2004). For encapsulation low molecular weight chitosan was used. 0.4 g of chitosan was dissolved in 90 mL of distilled water and acidified with 0.4 mL of glacial acetic acid (0.1M) to achieve 0.4% final chitosan concentration. After dissolution, pH was adjusted to 5.5 – 6.0 by adding 1 M NaOH. The solution was filtered through a Whatman filter paper no.4 and then the volume was adjusted to 100 mL. About 25 g of Alginate and alginate inulin beads were washed with the distilled water placed in 100mL of chitosan solution and kept on an orbital shaker at 100 rpm for 20 mins. The chitosan-coated beads were harvested using Whatman filter paper no. 4 and washed with sterile peptone solution (Darjani *et al.*, 2016).

#### 2.4 Assessment of viability of free and encapsulated bacteria

Determination of cell viability of microencapsulated strains of probiotic in the juice was done by releasing the entrapped cells from the capsules. The released culture was enumerated using the MRS Agar media at 37°C for 24-48 hrs (Hruyia *et al.*, 2018).

#### 2.5 Determination of encapsulation yield of capsules

The encapsulation yield (EY) of the encapsulated cell with different matrices were determined as described previously using the following formula (Prasanna and Charalampopoulos, 2018).

$$EY = \frac{\text{(Number of cells released from microcapsules)}}{\text{(Number of cells added to the respective alginate-based microsphere formulation)}} \times 100.$$

#### 2.6 Scanning electron microscopic (SEM) analysis of microcapsules

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 CPD030, Liechtenstein, Germany) with liquid carbon dioxide was used to dry capsules. Coating of samples and morphology of microcapsules were measured using a scanning electron microscope (FEI, Quanta 600 F, Thermo Scientific, Oregon USA) were carried out as described earlier (Prasanna and Charalampopoulos 2018).

#### 2.7 Effect of probiotic microencapsulation on bioactive compounds

Total phenolic compounds were determined by Folin-Ciocalteu (FC) colourimetric method at 765 nm as described by Singleton and Rossi (1965). The results were expressed as mg of Gallic acid equivalent (GAE) per litre of the sample. Total anthocyanin contents were estimated by a pH-differential method using two buffer systems, 0.025 M potassium chloride buffer at pH 1.0 and 0.4 M sodium acetate buffer at pH 4.5 using a spectrophotometric method at 510 and 700 nm wavelengths (Wrolstad *et al.*, 2005). The antioxidant activity was determined by performing FRAP assay reported by (Benzie and Strain 1996) with slight modifications. Free radical scavenging activity using DPPH assay was determined as like (Siddhuraju *et al.*, 2002) and DPPH% inhibition was calculated.

#### 2.8 Statistical analysis

Results were presented as a mean  $\pm$  standard deviation of three independent determinations.

Experimental data were analysed for analysis of variance (ANOVA) and differences between means were assessed by Duncan's new multiple range tests at the significance defined  $p \leq 0.001$  using SAS 9.3 software.

### 3. Results

#### 3.1 Effect of different coating materials on cell survival

The freshly prepared beads with different combinations of coating materials as mentioned in Table 1 are first subjected to viability assay and then inoculated in pasteurized pomegranate juice at 1% quantity. The juice was stored at 4°C and further subject to analysis for cell viability the different patterns in the cell survival were obtained with respect to different types of the bead. The obtained values of bacterial survival are presented in Table 1.

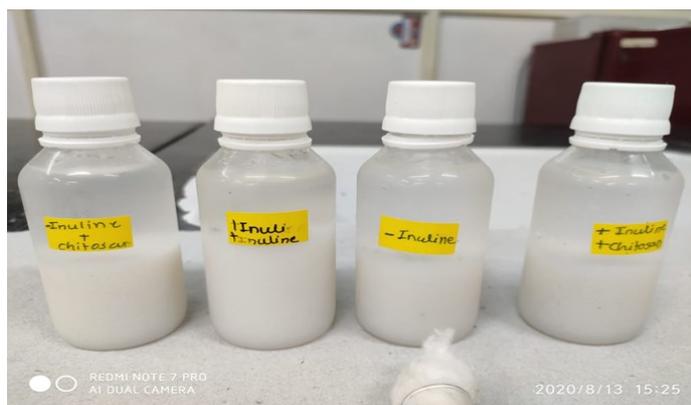


Figure 1. Microencapsulated beads with different coating material

The cell viability in the sample inoculated with live probiotic bacteria was found to be decreasing till at the end of 5 weeks, while no growth was detected at the end of six weeks. Our results are in agreement with the studies of Yoon *et al.* (2004), in their study, the viable cell population of *L. plantarum* and *L. delbrueckii* remained at an acceptable level ( $10^6$  CFU/mL) after 1 week of cold storage, but their microbial population decreased below the minimum accepted after 2 weeks (Yoon *et al.* 2005). These results are in accordance with the previous studies performed by Mousavi *et al.*, (2013) they used *L. plantarum*, *L. delbrueckii*, *L. paracasei* and *L. acidophilus* for the fermentation of pomegranate juice. Their results demonstrated that *L. plantarum* and *L. delbrueckii* had higher viability levels during fermentation and storage time compared to the other LAB ( $2.8 \times 10^5$  CFU/mL and  $1.5 \times 10^5$  CFU/mL after the second week of storage, respectively). The probiotic viability was obtained for up to two weeks and then cell viability decreases thereafter.

The effect of 2% inulin addition during bead formation was studied with chitosan coating and uncoating. The obtained results were very promising, no

Table 1. Effect of free and immobilized *L. acidophilus* cells on the cell viability in pomegranate juice

Sample	0 Day (CFU/g)	7 Days (CFU/g)	14 Days (CFU/g)	21 Days (CFU/g)	28 Days (CFU/g)	35 Days (CFU/g)	42 Days (CFU/g)
Culture	$3.366 \times 10^{11}$	$4 \times 10^8$	$1 \times 10^8$	$3 \times 10^5$	$3.3 \times 10^3$	$2 \times 10^1$	0
Alginate Beads	$22.09 \times 10^{11}$	$1 \times 10^8$	$0.3 \times 10^8$	$26 \times 10^7$	$1.5 \times 10^6$	$1 \times 10^6$	$7 \times 10^5$
Alginate beads with 0.4%	$3.33 \times 10^9$	$4 \times 10^7$	$3 \times 10^7$	$0.5 \times 10^6$	$1 \times 10^6$	0	0
Alginate beads +Inulin2% with	$0.33 \times 10^8$	$0.35 \times 10^8$	$0.3 \times 10^8$	$0.33 \times 10^8$	$1 \times 10^4$	$1 \times 10^4$	0
Alginate beads +Inulin 2%	$0.33 \times 10^8$	$0.9 \times 10^8$	$1 \times 10^8$	$2 \times 10^8$	$2 \times 10^8$	$2 \times 10^8$	$2 \times 10^8$

reduction in bacteria numbers in both the sample was detected at the end of 3<sup>rd</sup> week when juice was stored at 4°C. After 3<sup>rd</sup> week till the end of six weeks, the inulin containing beads were shown the highest cell viability as compared to inulin containing chitosan-coated beads. In fact, the uncoated beads containing inulin and chitosan-coated beads containing inulin was the first time studied for the survival of *Lactobacillus acidophilus* in pomegranate juice in the present study.

### 3.2 Scanning electron micrographs

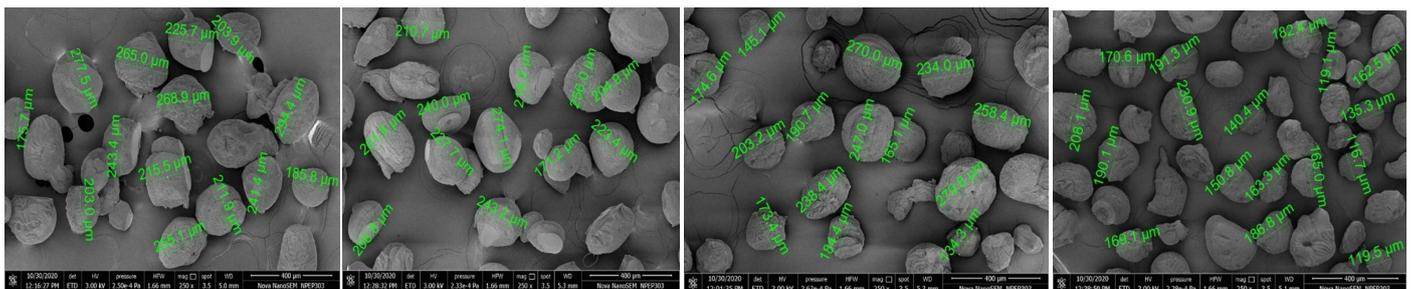
The surface morphology of the microcapsules was investigated using SEM micrographs. Figure 2 shows the surface of different microcapsules at a magnification of 10000. Porous microcapsules were observed with sodium alginate Figure 2(A). Furthermore, Sodium alginate microcapsules had cracks on their surface and could not protect entrapped cells from acidity, P<sup>H</sup> and other adverse effects on cell survival. Similarly, Li *et al.* (2009) also noted the porous structure of alginate microcapsules in their study. Modification of alginate with the addition of 2% inulin resulted in the microcapsules with denser surface morphology Figure 2 (B). In addition, these microcapsules did not have cracks that could ensure high protection for encapsulated cells from adverse conditions. Chitosan coated microcapsules (Figure 2(C) and 2(D) showed irregular surface morphology with porous nature which could not give better protection for entrapped cells than that of alginate and inulin containing alginate beads.

Scanning electron microscopy showed that the shape of all microcapsules was generally spherical and uniform and starch granules were present on the surface of the capsules without coating (Figure 2). Chitosan coating changed the size of the alginate capsules increased with the addition of chitosan coating. The diameter of chitosan-coated microcapsules was reported significantly higher than that of uncoated microcapsules (Table 2). Obtained results are in agreement with chitosan coating with alginate beads and their findings with respect to an increase in size and viability with Nualkaekul *et al.* (2012). This study also reveals the addition of 2% inulin in sodium alginate also increases the size of capsules than that of normal alginate beads. The encapsulation efficiency of each type of bead was also calculated and the average size was presented in Table 2 The type of encapsulating matrices had no significant ( $p > 0.05$ ) effect on the encapsulation yield (Table 2).

Table 2. Influence of different combinations of encapsulated material on the size and encapsulation yield

Sr. no.	Encapsulated material details	Size of capsule ( $\mu\text{m}$ )	Encapsulation yield (%)
1	Sodium alginate beads	$197.92 \pm 0.53^c$	93
2	Sodium alginate+1% Inulin containing beads	$209.04 \pm 0.62^b$	94.3
3	Chitosan coated sodium alginate beads	$258.64 \pm 0.71^a$	96.1
4	Sodium alginate + 1% inulin and chitosan	$218.34 \pm 0.47^b$	95.8

Values are presented as mean $\pm$ SD. Values with different superscripts are significantly each other at  $P \leq 0.05$  according to Duncan's Multiple Range Test.



(A) Average of Sodium alginate beads - 197.92 $\mu\text{m}$

(B) Average of inulin beads - 209.04  $\mu\text{m}$

(C) Average of chitosan beads - 258.64  $\mu\text{m}$

(D) Average of Inulin + chitosan beads - 218.34  $\mu\text{m}$

Figure 2. Scanning electron micrographs showing the diameter and surface of different capsules. (A) Capsules were prepared using sodium alginate with 2% inulin addition (B) Capsules were prepared using alginate and inulin. (C) Capsules were prepared using sodium alginate and coated with chitosan (D) Capsules were prepared using sodium alginate with 2% inulin addition and chitosan coating.

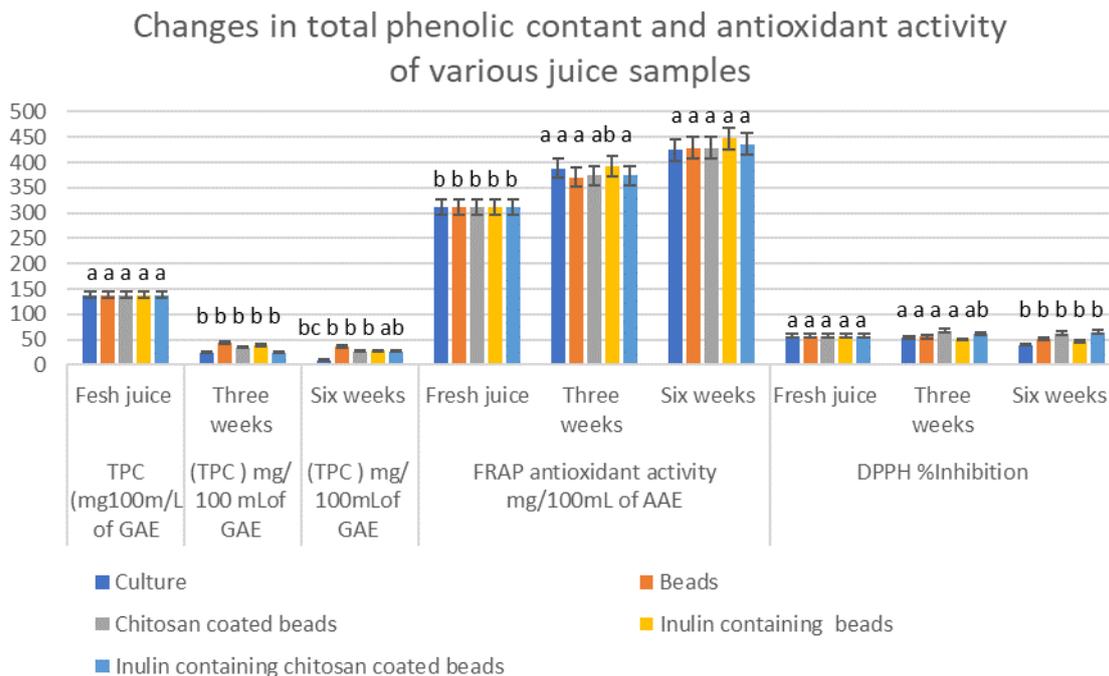


Figure 3. Effect on TPC and antioxidant activity of pomegranate juice inoculated with microencapsulated probiotic beads formulated using different encapsulated and coating material. Vertical bars indicate %S.E. The values are mean of triplicates and vertical bars indicates S.E Means with different letters are significantly different from each other at  $P < 0.05$  according to Duncan's Multiple range Test.

### 3.3 Effect of different beads on bioactive compounds of pomegranate juice

The microencapsulated beads of *Lactobacillus acidophilus* formed using different encapsulated and coating material was inoculated in the pasteurized juice and preserved at 4°C for six weeks. Effect on the major bioactive compounds of juices such as total phenolic content and antioxidant activity were evaluated and the obtained results were presented in Figure 3. The total phenolic content of fresh pomegranate juice was found to be high as compared to the few pomegranate varieties studied by Gözlekçi *et al.* (2011). In other studies, the total phenolic contents of various pomegranate cultivars showed significant differences and were 208–344 mg/100 g (Çam *et al.*, 2009), 2015–5186 mg/L (Fischer *et al.*, 2011). When the TPC content of free and immobilized *L. acidophilus* inoculated in pomegranate juice at different storage times was compared the 17.4%, 31%, 25.19%, 28.23% and 17.11% TPC content were found in juice sample of free culture, sodium alginate beads, chitosan-coated sodium alginate beads, inulin containing beads and inulin containing chitosan-coated beads respectively at the end of six weeks storage. This indicates the highest loss of TPC in the free cell samples while less TPC reduction in all immobilized cells except inulin containing chitosan beads.

When the antioxidant content of free and immobilized *L. acidophilus* inoculated in pomegranate juice at different storage time was compared the rise in FRAP antioxidant activity at the end of six weeks

storage was found to be 36%, 37%, 43% and 39% in the juice sample of free culture, sodium alginate beads, chitosan-coated sodium alginate beads, inulin containing beads and inulin containing chitosan-coated beads respectively, which indicate high amount of antioxidant formation in pomegranate juice containing immobilized cells as compare to free cells. However, DPPH% inhibition was found to be increasing by the end of three weeks storage period while it is depleted at the end of six weeks storage. In the present study, the reduced TPC did not show a positive correlation with the enhanced antioxidant activity (FRAP and DPPH% inhibition) of microencapsulated pomegranate juice.

## 4. Discussion

### 4.1 Effect of different coating materials on cell survival

The decrease in cell viability of inoculated probiotic culture might be due to the high acidity (pH 3.5) of the pomegranate juice (Nualkaekul and Charalampopoulos 2011). Moreover, studies by Srisukchayakula *et al.* (2018) on the survival of probiotics in acidic juices including pomegranate, cranberry, lemon and lime have revealed less survival in pomegranate juice as compared to lime and lemon may be because of high phenolic compounds (Gil *et al.*, 2000). The reason could be addressed to the lack of their ability to survive in the stressful condition of low pH and high acidity of the pomegranate juice and also the relatively low temperature of the environment (4°C). As Sheehan *et al.* (2007) reported, low pH fruit juices, with a range of pH

typically between 2.5 and 3.7, causes the bacteria more sensitive towards stressful conditions.

The prebiotic effect of inulin could be the reason for getting the survival constant for 6 weeks. A similar protective effect of inulin on probiotic viability was recorded by Haghshenas *et al.* (2015) when studying the effect of the addition of inulin and fenugreek on the survival of microencapsulated *Enterococcus durans* 39<sup>o</sup> C in alginate-psyllium polymeric blends in simulated digestive system and yoghurt. In the present study addition of inulin may improve the strength of the matrix and reduce the dissolution of capsules, consequently protecting the probiotic cells within the matrix. Better protection for bacterial survival is provided due to the more compact structure of inulin beads as shown in scanning electron micrographs Figure 2B than other types of beads (Pradeep Prasanna, and Charalampopoulos, 2019). Further addition of inulin causes the limited potential of growth-inhibiting substances passing the capsule wall, resulting during the fermentation process, which includes acids and hydrogen peroxide, as reported by Krasaekoopt and Watcharapoka (2014). Furthermore, it was observed that capsules containing prebiotics could provide the carbon and nitrogen sources for encapsulated probiotics leading to higher survival rates for *Bifidobacterium* sp. and *Lactobacillus* sp. in milk in the study of Chen *et al.* (2005).

Microencapsulation using sodium alginate without the coating of chitosan was already studied by so many researchers, in the present study the survival rate is more than free cell survival and chitosan coating beads but less than inulin containing beads. At the end of the 3<sup>rd</sup> week, 4 log reductions of probiotic cells were observed in pomegranate juice and the cell viability was reported of the cell at the end of six weeks of juice preservation. This is because sodium alginate is capable of forming a highly versatile matrix, biocompatible and non-toxic for the protection of cells, as probiotic microorganisms are sensible to heat, pH, oxygen, and other processing and storage factors (Goh *et al.*, 2012). Due to ease of handling, non-toxic nature and low cost, besides increasing the viability of probiotic bacteria when exposed to different conditions and when compared with non-encapsulated bacteria the polymer is mainly preferred for encapsulation study (Burgain *et al.*, 2011). Similar findings were reported by Ozer *et al.* (2009) they have added *L. acidophilus* microencapsulated in 2% of alginate gel by the extrusion technique in white cheese and analysed it throughout 90 days of storage, at 4°C and obtained the counting above 10<sup>6</sup> CFU/mL. Sodium alginate gel presents porosity and sensibility to extreme pH, which can interfere both with the release and

protection of the compounds (Mortazavian *et al.*, 2007). Hence in order to improve the stability of microorganisms coating the particles with ionic gelling with biopolymers through electrostatic interactions (Patil *et al.* 2010) the addition of prebiotics in the capsule formulation (Chen *et al.*, 2005) are the preferred way. Therefore, in the present study, the attempt was made to quote the sodium alginate beads with chitosan and also the addition of inulin in beads and its coating with chitosan were attempted to select the better combination of encapsulated matrix on survival of probiotic strain. The effect of chitosan coating on bacterial survival is also reported positive as compared to uncoated sodium alginate beads. 3 log reductions in chitosan-coated beads at the end of 3<sup>rd</sup> week when juice is stored at 4°C, while 4 log reductions in uncoated beads of sodium alginate under similar storage conditions were reported in the present study. This might be due to that coating alginate beads with chitosan develops a complexation of chitosan with alginate resulting in several important properties, such as alginate beads with reduced porosity, reduced linkage of encapsulated bacteria and stability at various P<sup>H</sup> ranges. Moreover, the protective effect could be due to the fact that the polysaccharides might be acting as a buffer against acids as they penetrate the bead by binding to the protons, thus raising the pH inside the matrix to a level that is less harmful to the bacteria. The development of a semipermeable membrane occurs when negatively charged alginate is in contact with positively charged chitosan resulting in a capsule with a smoother surface with reduced permeability properties (Krasaekoopt *et al.*, 2004). Moreover, protection in simulated gastrointestinal conditions was also reported with the chitosan-coated beads (Morales and Ruiz 2016). In the study by Varankovich *et al.* (2017) they used extrusion technique was used for the development of novel pea protein–alginate microcapsules coated with chitosan. These microcapsules when tested for immobilization and survivability of *L. rhamnosus* R0011 and *L. helveticus* R0052 during storage and exposure to in vitro GI conditions increased cell viability was reported. Four weeks of storage at room temperature, significantly improved the microcapsule performance when compared to non-chitosan coated microcapsules. Similar findings were reported by Nualkaekul *et al.* (2012), who investigated the potential protective effect of uncoated as well as single and double chitosan-coated alginate beads on the survival of a model *L. plantarum* strain in simulated gastric solution and pomegranate juice. In their study, the enhanced protection to probiotics in the case of the single and double coated beads with chitosan compared to the uncoated beads was most likely due to the even increased ability of the polyelectrolyte matrix to buffer acidic compounds as

they penetrate the beads, which was probably reflected by the thicker membrane observed in the images of their beads. Surprisingly, in the present study after 4 weeks till the end of six weeks, the results show a decrease in cell viability in chitosan-coated beads as compared to uncoated beads. This indicates the matrix material combination needs to be optimized to achieve better survival

#### 4.2 Scanning electron micrograph

The results clearly showed that there was a very low loss of cell viability during the encapsulation which was due to the mild conditions used. In conclusion, the extrusion method of microencapsulation is when used with hydrocolloids offers higher encapsulation yield (Krasaekoopt *et al.*, 2003). In this technique, the capsules are formed in micron range size furthermore, several reports have stated the capsules with micron range size could deliver soft texture when they are added to food products (Fahimdanesh *et al.*, 2012).

#### 4.3 Bioactive compounds

Different amounts of total phenolic content in various studies can be attributed to analytical methods, cultivar, maturity stage, and environmental conditions and also due to overestimation of the total individual phenolic compounds by Folin-Ciocalteu reagent due to interference with other reducing factors (Fischer *et al.*, 2011). TPC decrease might be due to conversion of simple phenolic compounds and the depolymerization of high molecular weight phenolic compounds due to the slow metabolic activity of microencapsulated probiotics in pomegranate juice (Mousavi *et al.*, 2013). The decrease in soluble phenolic content could also result from the polymerization of phenolic compounds. LAB has a range of enzymes such as  $\beta$ -glucosidase, p-coumaric acid decarboxylase, decarboxylase, which may help in degrading certain phenolic compounds (Towo *et al.*, 2006). The obtained results are in accordance with Li *et al.* (2009). These results are highlighting the significance of microencapsulation in preserving the health beneficial properties of pomegranate juice. Because the slower metabolic activity of lactic acid bacteria in microencapsulated beads might have depleted the available glucose molecule in the phenolic compounds, resulting in the production of free aglycones with a higher number of hydroxyl groups or lower steric hindrance to hydroxyl groups (Cai *et al.*, 2006). Which led to some metabolites with higher antioxidant activity being produced during the storage of pomegranate juice containing microencapsulated probiotics (Mousavi *et al.*, 2013). The studies of Ankolekar *et al.* (2012) based on *L. acidophilus* fermentation of pear juice also concluded the decrease in total phenolic with the increase in DPPH

linked antioxidant activity (Figure 3).

## 5. Conclusion

In the present research, the efficiency of different matrix materials for microencapsulation of *L. acidophilus* NCIM 5306 was evaluated in pomegranate juice. Among the different matrix materials used for the microencapsulation of *L. acidophilus* NCIM 5306, inulin containing beads showed a significant survival rate. Inulin being prebiotic for the *L. acidophilus* NCIM 5306 could have favoured the long-term growth of bacteria. The study also provided information on a gradual increase in bead size because of inulin incorporation in sodium alginate. Scanning electron micrographs studies revealed compact interior structural characteristics of inulin containing beads. The present investigation also underlined the pomegranate juice as a suitable medium for the development of functional food/ juice using microencapsulated *L. acidophilus* NCIM 5306. However no significant effect was determined on the efficiency of microencapsulation with various matrix material beads. Furthermore, bioavailability and inaccessibility of the transformed phenolic compounds, antioxidants etc. due to free and immobilized *L. acidophilus* NCIM 5306 cells needs to be deliberate to quote the health benefits of probiotic pomegranate juice.

## Conflict of interest

The author declares no conflict of interest

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## References

- Ankolekar, C., Pinto, M., Greene, D. and Shetty, K. (2012). *In vitro* bioassay based screening of antihyperglycemia and antihypertensive activities of *Lactobacillus acidophilus* fermented pear juice. *Innovative Food Science and Emerging Technologies* 13, 221-230. <https://doi.org/10.1016/j.ifset.2011.10.008>
- Benzie, I.F. and Strain, J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical Biochemistry*, 239(1), 70-76. <https://doi.org/10.1006/abio.1996.0292>
- Burgain, J., Gaiani, C., Linder, M. and Scher, J. (2011). Encapsulation of probiotic living cells: From laboratory scale to industrial applications. *Journal of*

- Food Engineering*, 104(4), 467-483. <https://doi.org/10.1016/j.foodeng.2010.12.031>
- Cai, Y.Z., Sun, M., Xing J., Luo, Q. and Corke, H. (2006). Structure-radical scavenging activity relationships of phenolic compounds from traditional Chinese medicinal plants. *Life Science*, 78(25), 2872–2888. <https://doi.org/10.1016/j.lfs.2005.11.004>
- Champagne, C.P., Gardner, N.J. and Roy, D. (2005). Challenges in the addition of probiotic cultures to foods. *Critical Reviews in Food Science and Nutrition*, 45(1), 61–84. <https://doi.org/10.1080/10408690590900144>
- Champagne, C.P., Raymond, Y. and Gagnon, R. (2008). Viability of *Lactobacillus rhamnosus* R0011 in an apple-based fruit juice under simulated storage conditions at the consumer level. *Journal of Food Science*, 73(5), M221–M226. <https://doi.org/10.1111/j.1750-3841.2008.00775.x>
- Chavarri, M., Maranon, I., Ares, R., Ibanez, F.C., Marzo, F. and Villaran, M.D. (2010). Microencapsulation of a probiotic and prebiotic in alginate–chitosan capsules improves survival in simulated gastrointestinal conditions. *International Journal of Food Microbiology*, 142(1-2), 185–189. <https://doi.org/10.1016/j.ijfoodmicro.2010.06.022>
- Chen, K.N., Chen, M.J., Liu, J.R., Lin, C.W. and Chiu, H.Y. (2005). Optimization of incorporated prebiotics as coating materials for probiotic microencapsulation. *Journal of Food Science*, 70(5), 260-266. <https://doi.org/10.1111/j.1365-2621.2005.tb09981.x>
- Darjani, P., Hosseini Nezhad, M., Kadkhodae, R. and Milani, E. (2016). Influence of prebiotic and coating materials on morphology and survival of probiotic strain of *Lactobacillus casei* exposed to simulated gastrointestinal conditions. *LWT*, 73, 162-167. <https://doi.org/10.1016/j.lwt.2016.05.032>
- Fahimdanesh, M., Mohammadi, N., Ahari, H., Zanjani, M.A.K., Hargalani, F.Z. and Behrouz Nasab, K. (2012). Effect of microencapsulation plus resistant starch on survival of *Lactobacillus casei* and *Bifidobacterium bifidum* in mayonnaise sauce. *Africa Journal Microbiology Research*, 6(40), 6853-6858. <https://doi.org/10.5897/AJMR12.1240>
- Fischer, U.A., Carle, R. and Kammerer, D. (2011). Identification and quantification of phenolic compounds from pomegranate (*Punica granatum* L.) peel, mesocarp, aril and differently produced juices by HPLC-DAD-ESI/MSn. *Food Chemistry*, 127(2), 807–821. <https://doi.org/10.1016/j.foodchem.2010.12.156>
- Gil, M.I., Tomas-Barberan, F.A., Hess-Pierce, B., Holcroft, D.M. and Kader, A.A. (2000). Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *Journal Agriculture Food Chemistry*, 48(10), 4581–4589. <https://doi.org/10.1021/jf000404a>
- Goh, C.H., Heng, P.W.S. and Chan, L.W. (2012). Alginates as a useful natural polymer for microencapsulation and therapeutic applications. *Carbohydrate Polymers*, 88(1), 1-12. <https://doi.org/10.1016/j.carbpol.2011.11.012>
- Gombotz, W.R. and Wee, S. (1998). Protein release from alginate matrices. *Advanced Drug Delivery Reviews*, 31(3), 267-285. [https://doi.org/10.1016/S0169-409X\(97\)00124-5](https://doi.org/10.1016/S0169-409X(97)00124-5)
- Gözlekçi, Ş., Saraçoğlu, O., Onursal, E. and Özgen, M. (2011). Total phenolic distribution of juice, peel, and seed extracts of four pomegranate cultivars. *Pharmacognosy Magazine*, 7(26), 161. <https://doi.org/10.4103/0973-1296.80681>
- Haghshenas, B., Nami, Y., Haghshenas, M., Barzegari, A., Sharifi, S., Radiah, D. and Abdullah, N. (2015). Effect of addition of inulin and fenugreek on the survival of microencapsulated *Enterococcus durans* 39C in alginate-psyllium polymeric blends in simulated digestive system and yogurt. *Asian Journal of Pharmaceutical Sciences*, 10(4), 350-361. <https://doi.org/10.1016/j.ajps.2015.04.001>
- Hill, C., Guarner, F. and Reid, G. (2014). Expert consensus document: the international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology and Hepatology*, 11(8), 506–514. <https://doi.org/10.1038/nrgastro.2014.66>
- Hruyia, L., Deshpande H.W. and Bhate M.A. (2018). Probiotication of sweet orange juice using *Lactobacillus* strains. *International Journal of Food and Nutritional Science*, 7(1), 71-77.
- Krasaekoopt, W. and Watcharapoka, S. (2014). Effect of addition of inulin and galacto oligosaccharide on the survival of microencapsulated probiotics in alginate beads coated with chitosan in simulated digestive system, yogurt and fruit juice. *LWT-Food Science and Technology*, 57(2), 761-766. <https://doi.org/10.1016/j.lwt.2014.01.037>
- Krasaekoopt, W., Bhandari, B. and Deeth, H. (2003). Evaluation of encapsulation techniques of probiotics for yoghurt: a review. *International Dairy Journal*, 13(1), 3–13. [https://doi.org/10.1016/S0958-6946\(02\)00155-3](https://doi.org/10.1016/S0958-6946(02)00155-3)
- Krasaekoopt, W., Bhandari, B. and 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. <https://doi.org/10.1016/j.idairyj.2004.01.004>
- Li, X.Y., Chen, X.G., Cha, D.S., Park, H.J. and Liu, C.S. (2009). Microencapsulation of a probiotic bacteria with alginate-gelatin and its properties. *Journal of Microencapsulation*, 26(4), 315-324. <https://doi.org/10.1080/02652040802328685>
- Luquet, F. and Corrieu, M.G. (2005). Lactic Acid Bacteria and Probiotics. 8C ed., p. 307. Paris, France: Tec Doc Lavoisier.
- Mirdehghan S.H. and Rahemi, M. (2007). Seasonal changes of mineral nutrients and phenolics in pomegranate (*Punica granatum* L.) fruit. *Science Horticulture*, 111(2), 120-127. <https://doi.org/10.1016/j.scienta.2006.10.001>
- Mirzaei, H., Pourjafar, H. and 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), 1966-970. <https://doi.org/10.1016/j.foodchem.2011.12.033>
- Morales, M.E. and Ruiz, M.A. (2016). 16—Microencapsulation of probiotic cells: Applications in nutraceutical and food industry. In Grumezescu, A.M., (Ed.) *Nutraceuticals. Nanotechnology in the Agri-Food Industry Volume 4.*, p. 627-668. USA: Academic Press. <https://doi.org/10.1016/B978-0-12-804305-9.00016-6>
- Mortazavian, A.M. and Sohrabvandi, S. (2007). Probiotics and food probiotic products: based on dairy probiotic products, p. 131-169. Iran: Eta Publication.
- Mousavi, Z.E., Mousavi, S.M., Razavi, S.H., Hadinejad, M., Emam-Djomeh, Z. and Mirzapour, M. (2013). Effect of Fermentation of Pomegranate Juice by *Lactobacillus plantarum* and *Lactobacillus acidophilus* on the Antioxidant Activity and Metabolism of Sugars, Organic Acids and Phenolic Compounds. *Food Biotechnology*, 27(1), 1-13. <https://doi.org/10.1080/08905436.2012.724037>
- Muzzarelli, R.A.A., Boudrant, J., Meyer, D., Manno, N., DeMarchis, M. and Paoletti, M.G. (2012). Current views on fungal chitin/chitosan, human chitinases, food preservation, glucans, pectins and inulin: Attribute to Henri braconnot, precursor of the carbohydrate polymers science, on the chitin bicentennial. *Carbohydrate Polymers*, 87(2), 995-1012. <https://doi.org/10.1016/j.carbpol.2011.09.063>
- Nualkaekul, S. and Charalampopoulos, D. (2011). Survival of *Lactobacillus plantarum* in model solutions and fruit juices. *International Journal of Food Microbiology*, 146(2), 111-117. <https://doi.org/10.1016/j.ijfoodmicro.2011.01.040>
- Nualkaekul, S., Lenton, D., Cook, M.T., Khutoryanskiy, V.V. and Charalampopoulos, D. (2012). Chitosan coated alginate beads for the survival of microencapsulated *Lactobacillus plantarum* in pomegranate juice. *Carbohydrate Polymers*, 90(3), 1281-1287. <https://doi.org/10.1016/j.carbpol.2012.06.073>
- Nualkaekul, S., Salmeron, I. and Charalampopoulos, D. (2011). Investigation of the factors influencing the survival of *Bifidobacterium longum* in model acidic solutions and fruit juices. *Food Chemistry*, 129(3), 1037-1044. <https://doi.org/10.1016/j.foodchem.2011.05.071>
- Patil, J.S., Kamalapur, M.V., Marapur, S.C. and Kadam, D.V. (2010). Ionotropic gelation and polyelectrolyte complexation: the novel techniques to design hydrogel particulate sustained, modulated drug delivery system: a review. *Digest Journal of Nano materials and Bio Structures*, 5(1), 241-248.
- Pradeep Prasanna, P.H. and Charalampopoulos, D. (2019). Encapsulation in an alginate-goats' milk-inulin matrix improves survival of probiotic *Bifidobacterium* in simulated gastrointestinal conditions and goats' milk yoghurt. *International Journal of Dairy Technology*, 72(1), 132-141. <https://doi.org/10.1111/1471-0307.12568>
- Prasanna, P.H.P. and 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. <https://doi.org/10.1016/j.fbio.2017.12.002>
- Prasanna, P.H.P., Grandison, A.S. and Charalampopoulos, D. (2014). Bifidobacteria in milk products: an overview of physiological and biochemical properties, exo-polysaccharide production, selection criteria of milk products and health benefits. *Food Research International*, 55, 247-262. <https://doi.org/10.1016/j.foodres.2013.11.013>
- Ranadheera, C.S., Baines, S.K. and Adams, M.C. (2010). Importance of food in probiotic efficacy. *Food Research International Journal*, 43(1), 1-7. <https://doi.org/10.1016/j.foodres.2009.09.009>
- Rao, A.V., Shiwnarain, N. and Maharaj, I. (1989). Survival of microencapsulated *Bifidobacterium pseudolongum* in simulated gastric and intestinal juices. *Canadian Institute of Food Science and Technology Journal*, 22(4), 345-349. <https://doi.org/10.1016/j.ijfoodmicro.2011.01.040>

doi.org/10.1016/S0315-5463(89)70426-0

- Siddhuraju, P., Mohan, P.S. and Becker, K. (2002). Studies on the antioxidant activity of Indian Laburnum (*Cassia fistula* L.): a preliminary assessment of crude extracts from stem bark, leaves, flowers and fruit pulp. *Food Chemistry*, 79(1), 61-67. [https://doi.org/10.1016/S0308-8146\(02\)00179-6](https://doi.org/10.1016/S0308-8146(02)00179-6)
- Singleton, V.L. and Rossi, J.A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American journal of Enology and Viticulture*, 16(3), 144-158.
- Srisukchayakula, P., Charalampopoulos, D. and Karatzas, K.A. (2018). Study on the effect of citric acid adaptation toward the subsequent survival of *Lactobacillus plantarum* NCIMB 8826 in low pH fruit juices during refrigerated storage. *Food Research International*, 111, 198–204. <https://doi.org/10.1016/j.foodres.2018.05.018>
- Towo, E., Matuschek, E. and Svanberg, U. (2006). Fermentation and enzyme treatment of tannin sorghum gruels: effects on phenolic compounds, phytate and in vitro accessible iron. *Food Chemistry*, 94(3), 369-376. <https://doi.org/10.1016/j.foodchem.2004.11.027>
- Varankovich, N., Martinez, M.F., Nickerson, M.T. and Korber, D.R. (2017). Survival of probiotics in pea protein-alginate microcapsules with or without chitosan coating during storage and in a simulated gastrointestinal environment. *Food Science Biotechnology*, 26(1), 189–194. <https://doi.org/10.1007/s10068-017-0025-2>
- Wrolstad, R.E., Durst, R.W. and Lee, J. (2005). Tracking color and pigment changes in anthocyanin products. *Trends in Food Science and Technology*, 16(9), 423-428. <https://doi.org/10.1016/j.tifs.2005.03.019>
- Yoon K.Y., Woodams, E.E. and Hang, Y.D. (2005). Fermentation of beet juice by beneficial lactic acid bacteria. *Lebensm Wiss Technology*, 38(1), 73–75. <https://doi.org/10.1016/j.lwt.2004.04.008>
- Yoon, K.Y., Woodams, E.E. and Hang, Y.D. (2004). Probiotication of tomato juice by lactic acid bacteria. *Journal of Microbiology*, 42(4), 315-318.