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Viability of Microencapsulated Probiotics Combined with Plant Extracts in Fermented Camel Milk under Simulated Gastrointestinal Conditions

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ABSTRACT

Extrusion technique was used to produce microencapsulated probiotic with and without Beetroot or Ginger aqueous extract. Microencapsulation efficiency and survival of probiotic after subjected to simulated gastrointestinal tract were evaluated. Fermented camel milk was produced using different chitosan-coated beads. Probiotic viability and acceptance of the fermented camel milk during storage at 4°C for 21 days were evaluated. Maximum probiotic counts were observed in the presence of 10% of beetroot aqueous extract or 1% ginger aqueous extract in comparison to other concentrations. The highest survival rates were found in synbiotic chitosan-coated beads after exposure to simulate gastric, small and large intestinal conditions. Moreover, significant differences were found concerning probiotic counts of different fermented camel milk. Regarding to the sensory evaluation, significant differences were observed among all fermented camel milk treatments. Fermented camel milk by synbiotic chitosan beads containing beetroot aqueous extract was the most favorable among all treatments.

Key words: Microencapsulation, synbiotic, probiotic, beetroot and ginger aqueous extract, fermented camel milk

Introduction

Probiotic are "live microorganisms which when administered in adequate amounts (10^7 CFU/g) confer health benefits to the host" (FAO/WHO, 2001). Probiotic bacteria provide health advantages including anticancer and antibacterial activities, reducing serum cholesterol and improved digestion. Lactobacillus and Bifidobacterium are the main genera of probiotic bacteria. Most of probiotic strains are sensitive to lower pH, bile salt, dissolved oxygen and digestive enzymes thus loss most of their viability during food processing, storage and passage through gastrointestinal tract (GIT) (Kailasapathy & Chin, 2000). Several techniques have been developed to improve the survival of probiotic bacteria. Also, additions of prebiotics improve the stability and stimulate the growth of probiotic.

Prebiotic defined as "a nonviable food component that confers a health benefit on the host associated with modulation of the microbiota"(FAO, 2007). A lot of studies approved the importance of prebiotics including inulin, resistance starch, pectin and fructo-oligosaccharide to supply needs of probiotic and enhancement their growth as well as increase resistance to harsh environment conditions. Plant extracts contain fibers, vitamins and minerals that make it a suitable material to promote bacterial growth. Natural extracts of vegetables, vegetable powders and pulps contain many bioactive components such as: phenolic substances, carotenoids, ascorbic acid, tocopherols and provide a variety/plenty of ingredients as nutrients, minerals, vitamins and dietary fiber (Tuorila & Gardello, 2002). It had used in the preparation of fermented dairy products (Srivastava *et al.,* 2015).

The benefits of probiotic in combination with prebiotic has given rise to the concept of synbiotic which described as "a combination of synergistically acting probiotics and prebiotics" (Gibson &

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Roberifroid, 1995). Also, it defined as "A selected component introduced to the gastrointestinal tract should selectively stimulate growth and/or activate the metabolism of a physiological intestinal microbiota, thus conferring beneficial effect to the host's health" (Skalkam *et al.*, 2016).

Microencapsulation is an effective technique to provide good protection to biological compounds as probiotic, antioxidants, vitamins and natural colors from undesirable environment conditions. Several coating materials had been used i.e. xanthan gum, gelatin, starch, alginate, whey protein, fat and glycerides derivatives (Oliveira *et al.,* 2007).

Alginate, is the most normally microencapsulation agent used as biopolymer for bacterial microencapsulation due to its non-toxicity, formation of gentle matrices with calcium chloride to trap sensitive material as microbial cells, simply, low cost, accepted as food additive and used in dairy preparations (Krasaekoopt *et al.*, 2006). Microencapsulation using alginate alone is limited because of its low stability in very low pH causing release of core material. Therefore, using chitosan in coating alginate beads could improve their stability in addition to increase the efficiency of microencapsulation process (Sabnis & Malavkar, 2016).

Beetroot is a common variety of vegetables. Aqueous extract of red beetroot consists mainly of betalaine pigments/dyes, are water soluble nitrogen compounds which contain of high concentration of betacyanine (red-violet) and a small amount of betaxanthines (yellow-orange). Betanine is the major component of betacyanine, it is a biologically active component and principle of beet root health benefits (Herbach *et al.,* 2004). In food industries it has used as raw substance for production of juices, pickles, natural colorants to dairy products, meat substitutes and beverages. Despite mentioned benefits, but uses are in a limited scale due to its low stability and high sensitively to heat, oxygen, changes pH and light (Singh & Hathan, 2014).

Ginger is a famous spicy and has a plenty of therapeutic properties so it has been used as a traditional medicine in many countries around the world. It contains a large number of nutrients, including: vitamins, starch, gingerol, shogaol, and amino acids (Salmon *et al.,* 2012).

Therefore, the present study examines the effect of microencapsulation of probiotic strains combined with plant aqueous extract on the viability of the probiotics under simulated gastrointestinal conditions as well as the properties of the resultant fermented camel milk.

Materials and Methods

Preparation of plant extracts

Fresh beetroot and dried ginger powder were purchased from the local market. To obtain beetroot aqueous extract (BAE), beetroot was cleaned, cut into small slices then blended in water in the ratio of 1:10 and strained then subjected to heat treatment at 80 ºC / 15 min (Damunupola *et al.*, 2014), while ginger aqueous extract (GAE) was prepared by mixing 10 g of ginger powder with 100 ml of distilled water then stirred and centrifugated at 4000 xg /10 min (Abd El-Khalek *et al.*, 2016).

Preparation of cultures

Lyophilized *Lactobacillus (Lb) plantarum* B-4496 and *Bifidobacterium (Bif.) animalis* B-41405 were provided by Northern Regional Research Laboratory (NRRL), Agriculture Research Service, National Center for Agriculture, Peoria, Illinois, USA. *Lb. plantarum* B-4496 was enumerated in MRS broth medium and incubated at 37°C/24 hrs under aerobic conditions. While *Bif. animalis* B-41405 was enumerated in MRS broth medium supplemented with 0.5% L-cystein HCL and 1% lithium chloride and incubated at 37°C/24 hrs using the Gas Pak system for anaerobic conditions.

Effect of beetroot or ginger aqueous extract on growth of probiotic strains

Lb. plantarum and *Bif. animalis* were separately activated twice in 20ml of MRS broth as above mentioned conditions. Then 1% incolum of each strain was transferred into MRS broth containing 0%, 1%, 5% and 10% of beetroot aqueous extract or ginger aqueous extract and incubated as previously mentioned. Pour plate count technique was performed using MRS agar to assess bacterial counts which were expressed as $\log_{10}$ CFU/mL.

Microencapsulation procedures

Extrusion microencapsulation technique was used to produce probiotic alginate beads coated with and without chitosan (Krasaekoopt *et al.*, 2006). Briefly, *Lb. plantarum* and *Bif. animalis* were enumerated separately as mentioned above then harvested cells were obtained by centrifugation at 4000 x g / 10 at 4°C and washed with sterile saline solution. The resultant cells was mixed with sodium alginate 2% then divided into 3 equal parts. The first part mixed with BAE while the second one mixed with GAE and compared with the third one (cells + alginate without plant extract (control). After mixing well, each solution was extruded separately into 0.1 M calcium chloride and stands for 30 min for gelification to get beads then rinsed. The obtained alginate beads were divided separately into two subdivisions the first subdivision was kept in sterile 0.1% peptone solution to serve as alginate beads. The second subdivision was coated with chitosan solution 0.4%. Finally, chitosan-coated beads were washed with 0.1 % peptone water and stored in sterile 0.1% peptone solution.

Encapsulation efficiency (EE)

The EE of the probiotic bacteria was calculated according to following equation (Gul & Dervisoglu, 2016)**:**

$$\text{EF } \% = \frac{\text{viable cells counts released from beads}}{\text{free cells counts added to the alginate matrix}} \times 100$$

In vitro studies

Survival of microencapsulated probiotic strains under simulated gastric juice (SGJ)

Encapsulated beads (1g) or cell suspensions (1mL) was mixed separately with 9 mL of SGJ (9 g/L of sodium chloride and 3.0 g/L of pepsin then adjusted the pH to 2.0 with HCL) and incubated for 1 and 2 hrs at 37 °C with agitation. Then pour plate count technique was carried out to determine survival bacterial counts and expressed as log CFU/mL (Chavarri *et al.,* 2010)**.**

Survival of microencapsulated probiotic strains under simulated small intestinal juice (SSIJ)

The resistance to the small intestinal conditions was evaluated by mixing 1g of encapsulated beads or 1 mL of cell suspensions separately with 9 mL of SSIJ (3.0 g/L bile salts, 6.5 g/L NaCl, 0.835 g/L KCl, 0.22 g/L $CaCl_2$ and 1.386 g/L $NaHCO_3$ then adjusted the pH to 7.5) and incubated at 37 °C with agitation for 1 and 2 hrs. Then survival bacteria were enumerated by pour plate counts technique (Chavarri *et al.,* 2010).

Survival rate after exposure to SGJ and SSIJ separately was calculated as:

$$\text{Survival } \% = \frac{\text{viable cells counts after exposure to gastrointestinal conditions}}{\text{viable cells counts before exposure to gastrointestinal conditions}} \times 100$$

Survival of microencapsulated probiotic strains under simulated large intestinal juice (SLIJ)

Simulated large intestinal juice was prepared (0.1M KH_2PO_4, then adjusted pH 7.4 ± 0.2) then 1 g of encapsulated beads or 1 mL of cell suspensions was mixed with 9 mL of SLIJ and incubated with agitation at 37 °C for 1 and 2 hrs. Then bacterial counts were determined by pour plate counts technique (Mandal *et al.,* 2006).

Production of fermented camel milk

Camel milk (CM) (3.5% fat, 3.2% protein, 11.5% total solids) was obtained from Animal Production Research Institute, Agriculture Research Center, Ministry of Agriculture. Fermented camel milk (FCM) was produced triplicate (Gassem *et al.,* 2016). Briefly, after heat treatment (80º C / 15 min), CM was divided into four parts; each part was inoculated with 3% of mixed starter cultures of yoghurt culture (YC), *Lb. plantarum* and *Bif. animalis* (either microencapsulated or free cell) in a ratio of 1:1:1 as follows:

First part (T1): YC + free cells of *Lb. plantarum* + free cells of *Bif.animalis*.

Second part (T2): YC + chitosan-coated beads of *Lb. plantarum* without plant extract + chitosan-coated beads of *Bif. animalis* without plant extract.
Third part (T3): YC + chitosan-coated beads of *Lb. plantarum* and 10% BAE+ chitosan-coated beads of *Bif. animalis* and 10% BAE.
Fourth part (T4): YC + chitosan-coated beads of *Lb. plantarum* and 1% GAE + chitosan-coated beads of *Bif. animalis* and 1% GAE.

All treatments were incubated until reached pH 4.6. The pH value of the resultant fermented camel milk was determined as well as the microbiological analysis and sensory evaluation during storage at 4° C for 1, 3, 7, 14 and 21 days.

Microbiological analysis

The counts of *Lb. bulgaricus* was determined using MRS agar acidified to pH 5.4 and the plates were incubated at 37º C/48 hrs (De Man *et al.,* 1960). *S. thermophilus* was enumerated using M17 agar; the plates were incubated at 37ºC/48 hrs (Terzaghi *et al.*, 1975). Counts of *Lb. plantarum* were determined using MRS agar, and then plates were incubated at 37°C/48 hrs (De Man *et al.,* 1960)**.** While, *Bif. animalis* counts were determined using MRS agar supplemented with 0.5% L-cystein HCL and 1% lithium chloride then plates were incubated at 37°C/72 hr using the Gas Pak system (Dave & Shah, 1996)**.**

Chemical analysis

The pH of the fermented camel milk samples was measured using a digital pH meter.

Sensory evaluation

The sensory attributes of the fermented camel milk samples were determined (flavor 60, consistency 30 and appearance 10) performed by 15 staff members of the Animal Production Research Institute, Cairo, Egypt (Keating & Randwhite, 1990) .

Statistical analysis

The experimental data were statistically analyzed using the general model program, Statistical Analysis System software at $p<0.05$ level of significance. The differences among means were tested using Duncan's multiple range tests. Also, the correlation coefficient analysis was done between the different parameters (SAS, 1999).

Results and Discussion

Evaluation of the effect of beetroot or ginger aqueous extract on the growth of tested probiotic strains

Data in Table (1) show that the addition of plant aqueous extract increased bacterial counts of probiotic strains compared to control. Concerning to beetroot aqueous extract (BAE), both counts of *Lb. plantarum* and *Bif. animalis* counts increased significantly at 10% and increased non-significantly at 1% of BAE compared to control. The ability of some lactic acid bacteria (*Lb. plantarum*, *S. thermophilus*, *Lb. acidophilus* and *Lb. delbrueckii ssp. bulgaricus)* to ferment beetroot extract were observed (Chwastek *et al.,* 2016). Also, synbiotic yoghurt was produced with *S. thermophilus*, *Lb. delbrueckii ssp. bulgaricus* and *Lb. acidophilus* La-5 using beetroot as a prebiotic (Khosravi-Darani *et al.,* 2015).

In the case of ginger aqueous extract (GAE), the highest bacterial counts were observed at 1% GAE. Moreover, the viable counts slightly decreased at 5% and 10% of GAE compared to 1%. It might be due to the presence of some antimicrobial components in ginger extract such as gingerols as (Sayeed *et al.,* 2016). Maximum bacterial counts of *Lb.acidophilus* and *Bif. bifidium* found in yoghurt fortified with 2% ginger extract compared to 0, 1, and 3% ginger extract (Marhamatizadeh *et al.,* 2017). Significant reduction trend of viable counts of *Lb. bulgaricus* and *S. thermophilus* found with increasing of ginger juice concentration to ≥4% (Yang *et al.,* 2012).

Obtained data indicated that 10% beetroot or 1% aqueous extract of ginger can be used as a prebiotic. Therefore, *Lb. plantarum and* was encapsulated with 10% BAE or 1% GAE with different

coated materials by extrusion method to get synbiotic beads; the same procedure was performed with *Bif. Animalis.*

Table 1: Effect of different concentrations of plant aqueous extract on the growth of tested probiotic strains (log_{10} CFU/ml)

Probiotic strains	Beetroot aqueous extract %			
	0%	1%	5%	10%
Lb. plantarum B-4496	9.02 ^{C}	9.19 ^{BC}	9.34 AB	9.53 ^{A}
Bif. animalis B-41405	8.46 ^{B}	8.62 ^{AB}	8.82 AB	9.00 ^{A}
	Ginger aqueous extract %			
	0%	1%	5%	10%
Lb. plantarum B-4496	9.02 ^{B}	9.43 ^{A}	9.41 ^{A}	9.36 ^{A}
Bif. animalis B-41405	8.46 ^{B}	9.07 ^{A}	8.80 ^{AB}	8.75 ^{AB}

Different superscripts capital letters within the same raw (A, B……) are significantly different (P<0.05)

Encapsulation efficiency (EE)

The results in Table (2) illustrate the encapsulation efficiency of the probiotic strains. Generally, higher EE was observed in all treatments, it ranged from 95.02% to 97.71% for microencapsulated *Lb. plantarum*, while it ranged from 87.77% to 92.49% for *Bif. animalis*. This might be due to microencapsulation procedure conditions, which were performed at room temperature without using solvents. Also, the viscosity of alginate solution resulting in increased diameter of formed beads thus reserved more probiotic counts inside the beads (Kailasapathy & Chin, 2000). High values of EE% ranged from 95.92 to 99.75% for microencapsulated of *Lb. casei* by extrusion method (Gul & Dervisoglu, 2016).

Table 2: Encapsulation efficiency of microencapsulated probiotic strains with different material agents and different plant aqueous extract

Microencapsulated probiotic		Encapsulation efficiency %
Alginate beads of *Lb. plantarum* B-4496	Without plant extract	95.02^{C}
	With 10% BAE	97.17^{AB}
	With 1% GAE	95.78^{BC}
Chitosan- coated beads of *Lb. plantarum* B-4496	Without plant extract	95.42^{BC}
	With 10% BAE	97.71^{A}
	With 1% GAE	96.21A^{BC}
Alginate beads of *Bif. animalis* B-41405	Without plant extract	87.77 ^{a}
	With 10% BAE	89.30 ^{a}
	With 1% GAE	89.04 ^{a}
Chitosan- coated beads of *Bif. animalis* B-41405	Without plant extract	88.81 ^{a}
	With 10% BAE	92.49 ^{a}
	With 1% GAE	90.81 ^{a}

Different superscripts capital letters within microencapsulated Lb. plantarum B-4496 (A, B……) are significantly different (P<0.05)
Different superscripts small letters within microencapsulated Bif. animalis B-41405 (a, b……) are significantly different (P<0.05)

It was clear that EE % of synbiotic alginate beads with or without chitosan was higher than microencapsulated probiotic without plant extract which might be due to the presence of different essential ingredients of plant extract that enhancement the growth of probiotic inside the capsules and act as a prebiotic. Moreover, the encapsulation efficiency slightly increased when beetroot extract was used compared to ginger extract. EE ranged from 96.20% to 96.71% for alginate beads without prebiotic while, it ranged from 99.19% to 99.59% for chitosan beads with prebiotic for microencapsulated *Lb. casei* and *Bif. adolescents* (Zanjani *et al.,* 2017).

In vitro studies

Survival of microencapsulated probiotic strains under simulated gastric juice (SGJ)

Data of Fig (1) show that alginate beads with or without chitosan led to higher survival rate which increased in the presence of either BAE or GAE in comparison to free cells. Counts of free cell either *Lb. plantarum* or *Bif. animalis* declined significantly and rapidly more than 4.0 log cycles after 2 hr. Microencapsulated probiotics only were able to maintain viability after exposure to SGJ in comparison to non-encapsulated probiotic (Chandramouli *et al.,* 2004) .

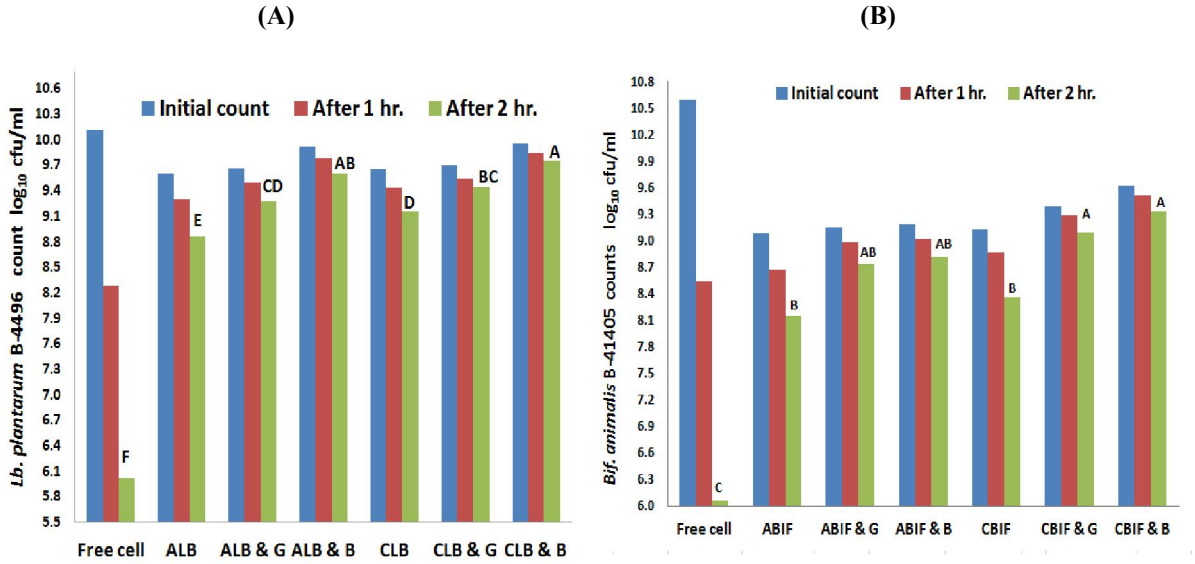

Fig 1: Counts of *Lb. plantarum* B-4496 and *Bif. animalis* B-41405 under simulated gastric juice.

(A) Free cell: free cells of *Lb. plantarum* B-4496, ALB: alginate beads of *Lb. plantarum* B-4496, ALB & G: alginate beads of *Lb. plantarum* B-4496 + 1% GAE, ALB&B: alginate beads of *Lb. plantarum* B-4496 + 10% BAE, CLB: chitosan-coated beads of *Lb. plantarum* B-4496, CLB&G: chitosan-coated beads of *Lb. plantarum* B-4496 + 1% GAE, CLB&B: chitosan-coated beads of *Lb. plantarum* B-4496 + 10% BAE.

(B) Free cell: free cells of *Bif. animalis* B-41405, ABIF: alginate beads of *Bif. animalis* B-41405, ABIF&G: alginate beads of *Bif. animalis* B-41405 + 1% GAE, ABIF&B: alginate beads of *Bif. animalis* B-41405 + 10% BAE, CBIF: chitosan-coated beads of *Bif. animalis* B-41405, CBIF&G: chitosan-coated beads of *Bif. animalis* B-41405 + 1% GAE, CBIF&B: chitosan-coated beads of *Bif. animalis* B-41405 + 10% BAE.

Bacterial count of microencapsulated *Lb. plantarum* without prebiotic significantly decreased about 1.0 and 0.5 log CFU/g in alginate beads and chitosan-coated beads, respectively. Also, *Bif. animalis* significantly decreased about 1.0 and 0.7 log CFU /g in alginate beads and chitosan-coated beads respectively after 2hr in SGJ. The viable counts of encapsulated *Lb. bulgaricus* decreased 1.0 log CFU/g after incubation in SGJ for 2 hrs (Chen *et al.,* 2014). Previous studies showed the efficiency of using double layer in microencapsulation to protect viable cells and increased survival of probiotic. Microencapsulation using alginate in addition to chitosan as a wall material have presented more protection to probiotic cells from harsh conditions of SGJ compared to free cells (Kailasapathy & Chin, 2000; Chavarri *et al.,* 2010).

The survival rate of probiotic bacteria in chitosan coated beads containing prebiotic were more than microencapsulated probiotic without prebiotic (Sajad *et al.,* 2017; Etchepare *et al.,* 2016). Survival rate of synbiotic chitosan beads containing either ginger or beetroot aqueous extract were more than microencapsulated probiotic without prebiotic. Moreover, survival rate in synbiotic chitosan-coated beads was 96.70, 96.99, 97.32, and 97.89 % for *Bif. animalis* + GAE, *Bif. animalis* + BAE, *Lb. plantarum* + GAE and *Lb. plantarum* + BAE while it was 57.32 and 59.55% for *Bif. animalis* free cells and *Lb. plantarum* free cells respectively. *Lb. plantarum* counts were significantly higher in synbiotic

beads containing BAE than their counterpart containing GAE; while *Bif. animalis* counts were non-significant higher in synbiotic beads containing BAE than their counterpart containing GAE.

Using chitosan as a double layer had less pore size thus limiting the leakage of SGJ into the beads, thus decrease the strict effect of SGJ on probiotic (Kailasapathy & Chin, 2000)**.** Double layers wall material increase microencapsulated probiotic beads rigidly that provide more resistance to SGJ and SIJ and improve probiotic survivals (Sajad *et al.,* 2017). Highest survival rate of encapsulated probiotic with resistance starch as a prebiotic was observed due to more protection presented by chitosan (Etchepare *et al.,* 2016). Additionally, encapsulated probiotic bacteria with Hi-maize as prebiotic survived better than the encapsulated bacteria without prebiotic (Iyer & Kailasapathy, 2005).

Survival of microencapsulated probiotic strains under simulated small intestinal juice (SSIJ)

Data in Fig (2) show highest reduction rate in free cells that decreased significantly more than 3.70 and 3.91 log cycles for *Lb. plantarum* and *Bif. animalis* respectively after 2 hr. These findings are in agreement with other studies that used similar concentrations of bile salts, which stated that encapsulated probiotic bacteria can survive better than free probiotic cells (Chandramouli *et al.,* 2004; Kailasapathy, 2005). Probiotic bacteria lost their viability during pass through the gastro-intestinal tract (Marteau, *et al.,* 1997).

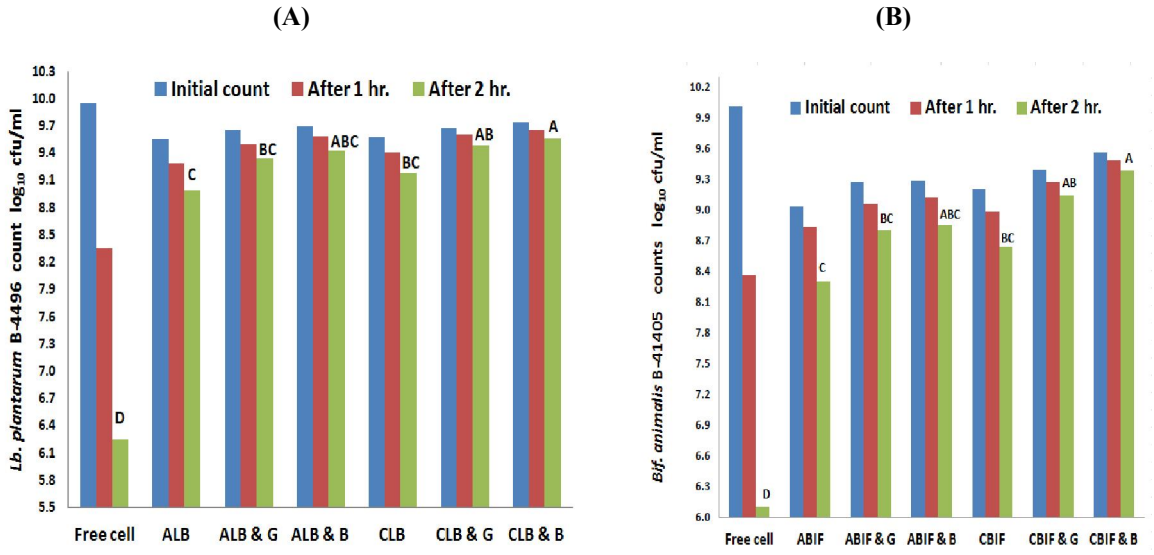

Fig 2: Counts of *Lb. plantarum* B-4496 and *Bif.animalis* B-41405 under simulated small intestinal juice.

(A) Free cell: free cells of *Lb. plantarum* B-4496, ALB: alginate beads of *Lb. plantarum* B-4496, ALB & G: alginate beads of *Lb. plantarum* B-4496 + 1% GAE, ALB&B: alginate beads of *Lb. plantarum* B-4496 + 10% BAE, CLB: chitosan-coated beads of *Lb. plantarum* B-4496, CLB&G: chitosan-coated beads of *Lb. plantarum* B-4496 + 1% GAE, CLB&B: chitosan-coated beads of *Lb. plantarum* B-4496 + 10% BAE.

(B) Free cell: free cells of *Bif. animalis* B-41405, ABIF: alginate beads of *Bif. animalis* B-41405, ABIF&G: alginate beads of *Bif. animalis* B-41405 + 1% GAE, ABIF&B: alginate beads of *Bif. animalis* B-41405 + 10% BAE, CBIF: chitosan-coated beads of *Bif. animalis* B-41405, CBIF&G: chitosan-coated beads of *Bif. animalis* B-41405 + 1% GAE, CBIF&B: chitosan-coated beads of *Bif. animalis* B-41405 + 10% BAE.

The counts of microencapsulated *Lb. plantarum* or *Bif. animalis* in chitosan-coated beads without prebiotic decreased significantly less than 1.0 log cycle in comparison with synbiotic chitosan-coated beads which decreased less than 0.5 log cycles after 2 hrs in the presence of SIJ. Microencapsulated probiotic with prebiotic inulin was more resistance to gastrointestinal condition than microencapsulated probiotic without prebiotic (Pinto *et al.,* 2015).

As well as the highest survival rate was found in synbiotic chitosan-coated beads followed by synbiotic alginate beads in comparison to free cells. Survival rate ranged from 96.79% to 98.15% for *Lb. plantarum* in synbiotic chitosan-coated beads while ranged from 94.94% to 98.12% for *Bif. animalis*

in synbiotic chitosan-coated beads in comparison to almost 62.81 and 60.98% for free cells of *Lb. plantarum* and *Bif. animalis* respectively after exposure to SSIJ for 2 hr.

Survival of microencapsulated probiotic strains under simulated large intestinal juice (SLIJ)

Initial bacterial counts were higher in chitosan-coated beads as compared to its similar in alginate beads (Fig. 3). Released probiotic counts after the first hour were less than the second hour after undergoing of simulated large intestinal conditions. Counts of released bacteria increased during incubation time in SLIJ and the complete release occurred after 3 hrs (Mandal *et al.,* 2006; Chen *et al.,* 2014).

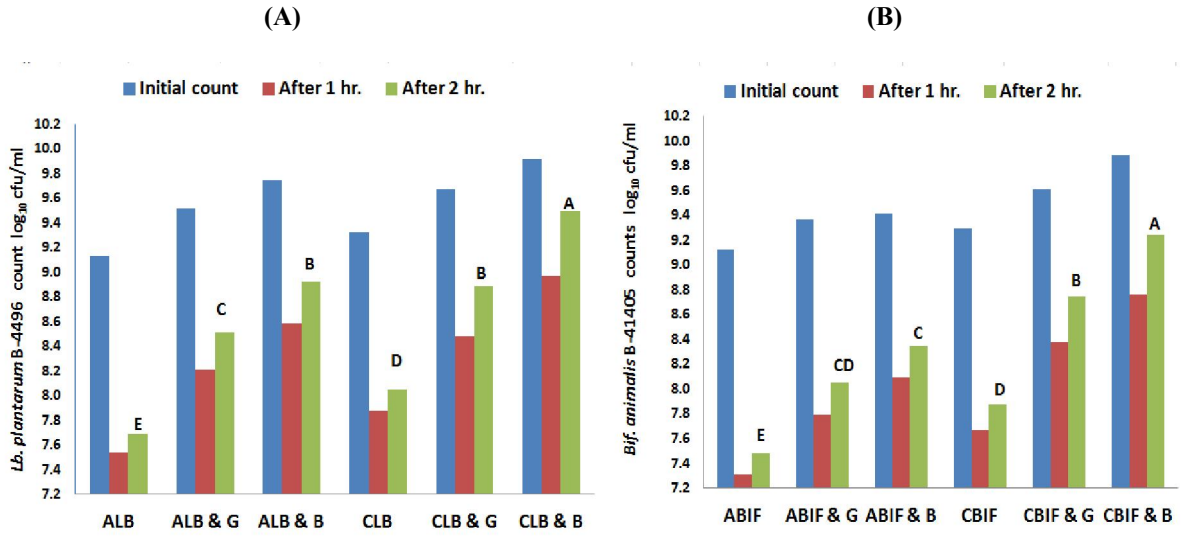

Fig. 3: Counts of *Lb. plantarum* B-4496 and *Bif.animalis* B-41405 under simulated large intestinal juice

(A) ALB: alginate beads of *Lb. plantarum* B-4496, ALB & G: alginate beads of *Lb. plantarum* B-4496 + 1% GAE, ALB&B: alginate beads of *Lb. plantarum* B-4496 + 10% BAE, CLB: chitosan-coated beads of *Lb. plantarum* B-4496, CLB&G: chitosan-coated beads of *Lb. plantarum* B-4496 + 1% GAE, CLB&B: chitosan-coated beads of *Lb. plantarum* B-4496 + 10% BAE.

(B) ABIF: alginate beads of *Bif. animalis* B-41405, ABIF&G: alginate beads of *Bif. animalis* B-41405 + 1% GAE, ABIF&B: alginate beads of *Bif. animalis* B-41405 + 10% BAE, CBIF: chitosan-coated beads of *Bif. animalis* B-41405, CBIF&G: chitosan-coated beads of *Bif. animalis* B-41405 + 1% GAE, CBIF&B: chitosan-coated beads of *Bif. animalis* B-41405 + 10% BAE

Maximum bacterial counts were significantly released from synbiotic chitosan-coated beads after 2 hours (Fig. 3). The microencapsulation of different probiotic bacteria with Hi-maize as prebiotic and coating with chitosan significantly improve the survival of encapsulated probiotic bacteria (Iyer & Kailasapathy, 2005). Using chitosan improved stability of microencapsulated alginate in strict conditions (Chavarri *et al.,* 2010). Also, it significantly enhanced the survival of bacteria in simulated gastrointestinal tract, thus viable cells can reach a beneficial desirable level as probiotic.

Results also indicate that both of alginate and chitosan together provide excellent wall materials for probiotic and plant extract due to good film-forming properties in addition to the low permeability to gases, especially oxygen that affect on bifidobacteria growth. Survival rate of bifidobacteria in alginate beads coated with chitosan was higher than in alginate beads (Yu *et al.,* 2001).

From our data, it is clear that highest survival rate was found in synbiotic chitosan-coated beads in comparison to other treatments; it might be due to using chitosan as a double layer thus formed a complex of chitosan and alginate causing reduction in porosity of alginate beads and limited the diffusion of simulated gastrointestinal juices through probiotic beads. In addition, the presence of aqueous plant extracts ingredients provided nutrients to probiotic inside the beads. Consequently, incorporation of synbiotic chitosan-coated beads in camel milk to prepare a synbiotic camel milk was performed.

Stability and survival of microencapsulated probiotic strains in fermented camel milk during cold storage

pH of fermented camel milk

Results in Table (3) indicate that chitosan-coated beads with or without prebiotics had a significant effect on pH values of fermented camel milk in comparison with control (T1). A significant decreasing of pH in probiotic fortified yoghurt with beet during refrigerated storage (Damunupola *et al.*, 2014; Januario *et al.*, 2017).

During storage period, pH of T2, T3 and T4 were significantly higher than control. Moreover, no significant differences were observed among all fermented camel milk containing chitosan coated beads. It might be due to double layer coating resulting in less absorption of nutrients as well as less release of metabolites within coating wall [30]. Lower pH values in probiotic yoghurt than counterpart containing microencapsulated probiotic (Ribeiro *et al.,* 2014). Also, higher pH values of encapsulated *Lb. rhamnosus* than non-encapsulated strains (Abbaszadeh *et al.,* 2014). Yoghurt contained microencapsulated probiotic with prebiotic inulin was recorded higher pH values in compared to control (Pinto *et al.,* 2017).

Table 3: Changes in pH values of fermented Camel milk by synbiotic chitosan-coated beads containing different plant aqueous extract:

Storage period (days)	pH of fermented Camel milk treatments			
	T1	**T2**	**T3**	**T4**
1	4.57 ^{Ba}	4.80 ^{Aa}	4.77 ^{Aa}	4.73 ^{Aa}
3	4.30 ^{Bb}	4.63 ^{Ab}	4.60 ^{Ab}	4.53 ^{Ab}
7	4.17 ^{Bc}	4.50 ^{Ac}	4.47 ^{Ac}	4.43 ^{Abc}
14	4.03 ^{Cd}	4.43 ^{Acd}	4.40 ^{ABcd}	4.33 ^{Bcd}
21	3.93 ^{Bd}	4.37 ^{Ad}	4.33 ^{Ad}	4.27 ^{Ad}

Different superscripts capital letters within the same raw (A, B……) are significantly different (P<0.05)
Different superscripts small letters within the same column (a, b……) are significantly different (P<0.05)
(T1): Fermented camel milk by YC + free cells of Lb. plantarum B-4496 + free cells of Bif.animalis B-41405.
(T2): Fermented camel milk by YC + chitosan-coated beads of Lb. plantarum B-4496 without plant extract + chitosan-coated beads of Bif. animalis B-41405 without plant extract.
(T3): Fermented camel milk by YC + chitosan-coated beads of Lb. plantarum B-4496 and 10% BAE+ chitosan-coated beads of Bif. animalis B-41405 and 10% BAE.
(T4): Fermented camel milk by YC + chitosan-coated beads of Lb. plantarum B-4496 and 1% GAE + chitosan-coated beads of Bif. animalis B-41405 and 1% GAE.

Microbiological analysis of fermented Camel milk

Data in Fig (4) show that *Lb. plantarum* counts were significantly higher in fermented camel milk containing synbiotic chitosan-coated beads compared to fermented camel milk containing free cells. Although *Lb. plantarum* increased until 7th day in all treatments, but it decreased 0.94, 0.44 and 0.34 log cycle in T2, T4 and T3 respectively compared to T1 that decreased 2.41 log cycles at the end of storage**.** Similarly, counts of microencapsulated *Lactobacillus acidophilus* with resistant starch increased during at the beginning of storage then it decreased gradually (Etchepare *et al.,* 2016).

During storage, it was noticed that *Bif. animalis* counts were significantly higher in T3 and T4 compared to T1 or T2. This exhibited that encapsulated probiotic and prebiotic in double layer has a more effective protection and promotion of the bacterial growth compared to free cells or encapsulated without prebiotic. Counts of *Bif. animalis* increased slightly during storage till 7th day then it declined gradually in all treatments. *Bif. animalis* reduced only less than 0.5 $\log_{10}$ cycle in T3 and T4 compared to T1 which reduced about 3 log cycles. This indicated that utilization of chitosan and prebiotics improved survival of probiotics compared to other treatments (Januario *et al.,* 2017)**.**

Additionally, no significant differences of *Str. thermophilus* population were observed between treatments at the beginning of storage. Counts of *Str. thermophilus* declined significantly during the storage period in all treatments as a result of lactic acid production.

Regarding to *Lb. bulgaricus* counts, it increased gradually during storage until 7th day then counts slightly decreased up to 21 days in all treatments. Moreover, it was observable that yoghurt culture (*Lb.*

bulgaricus & *Str. thermophilus*) counts were slightly higher in fermented camel milk containing synbiotic beads (T3 and T4) compared to T1 and T2. Bacterial counts of yoghurt culture were higher in fortified beetroot yoghurt than control (Damunupola *et al.,* 2014).

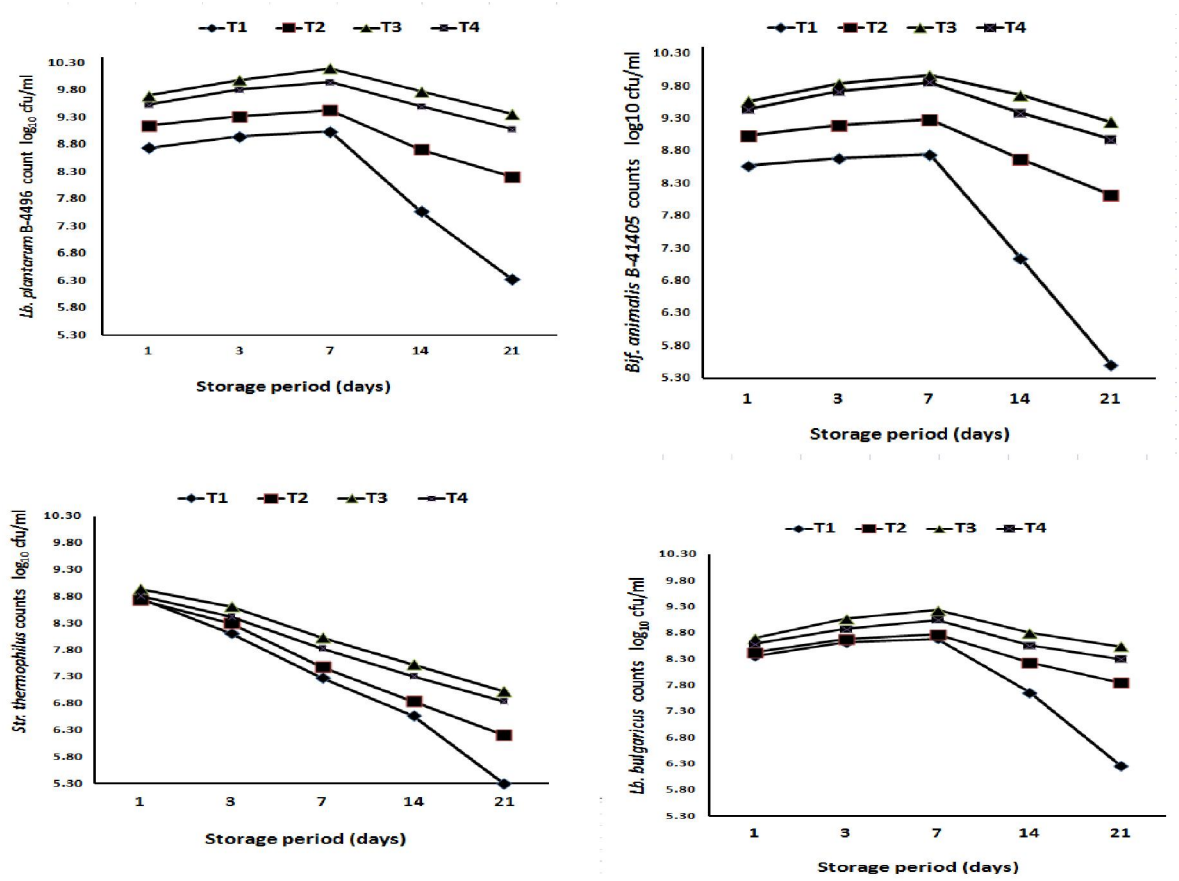

Fig. 4: Bacterial counts of fermented Camel milk (log_{10} cfu/ml).

(T1): Fermented camel milk by YC + free cells of *Lb. plantarum* B-4496 + free cells of *Bif.animalis* B-41405.
(T2): Fermented camel milk by YC + chitosan-coated beads of *Lb. plantarum* B-4496 without plant extract + chitosan-coated beads of *Bif. animalis* B-41405 without plant extract.
(T3): Fermented camel milk by YC + chitosan-coated beads of *Lb. plantarum* B-4496 and 10% BAE+ chitosan-coated beads of *Bif. animalis* B-41405 and 10% BAE.
(T4): Fermented camel milk by YC + chitosan-coated beads of *Lb. plantarum* B-4496 and 1% GAE + chitosan-coated beads of *Bif. animalis* B-41405 and 1% GAE.

Sensory evaluation of fermented camel milk

Data in Table (4) indicated that T3 gained significantly the highest total scores among all treatments during different intervals of storage period. It might be due to the presence of synbiotic chitosan-coated beads which contained beetroot with favorable taste. In addition to, it's unique favorable pink color and the smooth texture. Fortified yoghurt with 4% beetroot was more preferred than 0%, 6% and 8% (Damunupola *et al.,* 2014). Fermented dairy products by encapsulated bacteria caused a mild sourness more favorable compared to free cells due to higher pH and lower acidity produced by encapsulated bacteria (Abbaszadeh *et al.*, 2014). No significant differences of probiotic yoghurt by encapsulated bacteria during 3 weeks of storage at 4º C in the sensory attributes (Krasaekoopt *et al.,* 2006).

Table 4: Sensory evaluation of fermented Camel milk by synbiotic chitosan-coated beads containing different plant aqueous extract:

	T1	T2	T3	T4
		Flavour (60)		
1	45.33^{Db}	47.33^{Cb}	56.50^{Aa}	51.50^{Bb}
3	47.00^{Cab}	48.33^{Cb}	57.17^{Aa}	53.33^{Bab}
7	47.83^{Cab}	49.17^{Cab}	57.83^{Aa}	54.17^{Bab}
14	48.33^{Ca}	50.00^{Cab}	58.50^{Aa}	55.00^{Bab}
21	48.67^{Ca}	50.33^{Ca}	58.83^{Aa}	55.50^{Ba}
		Consistency (30)		
1	16.00^{Cb}	21.33^{Bc}	24.83^{Ab}	25.83^{Ac}
3	17.33^{Ca}	22.17^{Bbc}	26.00^{Aab}	26.83^{Ab}
7	15.67^{Cbc}	23.50^{Bb}	26.83^{Aa}	27.50^{Aab}
14	14.83^{Ccd}	24.33^{Ba}	27.33^{Aa}	28.00^{Aa}
21	14.50^{Cd}	24.93^{Ba}	27.83^{Aa}	28.33^{Aa}
		Appearance (10)		
1	9.00^{Ba}	9.00^{Ba}	10.00^{Aa}	10.00^{Aa}
3	6.67^{Cb}	9.00^{Ba}	10.00^{Aa}	10.00^{Aa}
7	5.33^{Cc}	9.00^{Ba}	10.00^{Aa}	10.00^{Aa}
14	5.00^{Cc}	9.00^{Ba}	10.00^{Aa}	10.00^{Aa}
21	4.67^{Cc}	9.00^{Ba}	10.00^{Aa}	10.00^{Aa}
		Total score (100)		
1	70.33^{Da}	77.67^{Cc}	91.33^{Ac}	87.33^{Bd}
3	71.00^{Da}	79.50^{Cc}	93.17^{Abc}	90.17^{Bc}
7	68.83^{Da}	81.67^{Cb}	94.67^{Aab}	91.67^{Bbc}
14	68.17^{Da}	83.33^{Cab}	95.83^{Aa}	93.00^{Bab}
21	67.83^{Da}	84.26^{Ca}	96.67^{Aa}	93.83^{Ba}

Different superscripts capital letters within the same raw (A, B…….) are significantly different (P<0.05)
Different superscripts small letters within the same column (a, b…….) are significantly different (P<0.05)
(T1): Fermented camel milk by YC + free cells of Lb. plantarum B-4496 + free cells of Bif.animalis B-41405.
(T2): Fermented camel milk by YC + chitosan-coated beads of Lb. plantarum B-4496 without plant extract + chitosan-coated beads of Bif. animalis B-41405 without plant extract.
(T3): Fermented camel milk by YC + chitosan-coated beads of Lb. plantarum B-4496 and 10% BAE+ chitosan-coated beads of Bif. animalis B-41405 and 10% BAE.
(T4): Fermented camel milk by YC + chitosan-coated beads of Lb. plantarum B-4496 and 1% GAE + chitosan-coated beads of Bif. animalis B-41405 and 1% GAE.

Additionally, T4 was also preferable due to desirable spicy flavor and a light yellow color of synbiotic chitosan-coated beads containing ginger in addition to soft and smooth texture. Fortified yoghurt with ginger was more acceptable than control (Yang *et al.,* 2012).

Although, flavour scores of T1 increased significantly during storage period, it had the lowest values of total scores because of watery texture and precipitation appearance throughout storage compared to other treatments owing to the absent of curd formation in all fermented camel milk treatments produced by different lactic acid bacteria in addition to heterogeneous, poor and fragile structure **(**Abdel Rahman *et al.,* 2009; Barlowska *et al.,* 2011).

Storage at 4ºC resulting in improving texture scores of T2, T3 and T4 throughout the 21 days; it might be due to the increase of the viscosity of fermented camel milk containing alginate beads thus enhancement the texture. Fermented milk products properties such as consistency, odor, taste and appearance were improved during preserving samples at low storage temperature (Memisi *et al.*, 2014).

In brief, the microencapsulated synbiotic chitosan-coated beads improved significantly sensory attributes (flavor, consistency and appearance) of fermented camel milk compared to counterpart in the free form or in chitosan-coated beads without prebiotic. Fermented camel milk by synbiotic chitosan beads containing beetroot aqueous extract was the most favorable among all treatments.

Conclusion

The obtained results revealed the efficiency of microencapsulation technique to protect and increase survival of probiotics through simulated gastrointestinal tract as well as processing and

refrigerator storage of fermented camel milk. Among the free cells, alginate beads and chitosan-coated beads with or without prebiotic, maximum survival bacterial counts were found in synbiotic chitosan-coated beads containing 1% ginger or 10% beet root aqueous extract. Furthermore, synbiotic chitosan-coated beads enhanced probiotic stability during processing, storage and acceptability of fermented camel milk during refrigerated storage for 21 days. Additionally, it contained probiotic counts more than 10^7 cfu/ml as recommended level to get healthy benefits. Fermented camel milk by synbiotic chitosan beads containing beetroot aqueous extract was the most favorable among all treatments.

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