IJBPAS, March, 2017, 6(3): 521-545

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

COMPARATIVE EVALUATION OF THE MICROENCAPSULATION METHODS EFFICIENCY TO PROTECT PROBIOTIC STRAINS IN SIMULATED GASTRIC CONDITIONS

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Received 29th Sept. 2016; Revised 1st Nov. 2016; Accepted 24th Dec. 2016; Available online 1st March 2017

ABSTRACT

Three microencapsulation methods [alginate-milk microspheres, skimmed milk and Transglutaminase-induced caseinate gelation and rennet-gelation of milk proteins] were evaluated to protect *Lactobacillus gasseri* and *Lactobacillus acidophilus* during exposed to bile salt concentration, simulated gastric juice (SGJ) and simulated intestinal juice (SIJ). Results showed that encapsulated probiotics using alginate-milk microspheres, followed by skimmed milk, offered protection throughout the storage time when exposed to SGJ, SIJ and bile salt compared to free cultures of probiotic. Encapsulated strains using alginate-milk or skimmed milk were used to prepare functional yoghurt. Treatment samples and control were analyzed bacteriologically, chemically as well as sensory and rheological properties during storage for 15 days. The data indicated that the encapsulated probiotics in yoghurt more stable than free cultures during the storage when exposed to SIJ and SGJ for 90 minutes. as compared to the initial counts. Our results indicated that the yoghurt samples with encapsulated culture were more acceptability than control and free culture yoghurt samples. Also, highest efficiency in probiotic protection was observed with alginate-milk microspheres method.

Keywords: Microencapsulation- Simulated gastric conditions- *Lactobacillus gasseri* - *Lactobacillus acidophilus* – Yoghurt

INTRODUCTION

Probiotic bacteria should be steady, remain active in the product and able to transit through the upper digestive tract in large numbers. Presence of low pH in stomach with the bile salts in small intestine is the reason for decline in the viability of probiotic bacteria. Their viability can be ameliorating by using microencapsulating or addendum of cryoprotectant. Microencapsulation in which the cells are retained within an encapsulating matrix or membrane has emerged as an alternative for protection of probiotics, providing a particular and convenient micro-environment for the encapsulated microorganism, enhancing their viability, and enabling controlled release of cells in the intestinal tract. Encapsulation technology has been proved to be one of the most effective ways to protect probiotics during processing and subsequent storage **[1, 2].**

Milk proteins are very interesting as encapsulation material by their physico-chemical properties. Consequently, milk protein-based microcapsules are widely used for industrial applications **[3].** Whey protein, casein and milk fat globule membrane proteins were used as encapsulation carriers **[4-6].** Also, the immobilization of lactic acid bacteria by entrapment in calcium alginate beads was used **[7].**

Yoghurt is one of the important common fermented dairy products widely consumed all over the world. On the other side, yoghurt is more healthful than many other fermented milk products in order to it has a rise scale of milk solids in addition to nourishment advanced during the fermentation operation**[8].**

It is practically known that the survival of probiotic strains in yoghurt products has been manipulated by acid conditions and turnout of other cultures, such as *Lb. delbrueckii* ssp. *Bulgaricus* **[9, 10].** However, microencapsulation of acid sensitive strains increases their survival rate during the shelf life of the yoghurt and during their passage through the gastrointestinal tract **[11-13].**

The objective of this study is to evaluate the stability of microencapsulated probiotic bacteria by using different methods during exposed to bile salt concentration, simulated gastric juice (SGJ) and simulated intestinal juice

(SIJ) Likewise, manufacturing yoghurt product using probiotic strains encapsulated with more resistant method and estimate the microbiological and some chemical analysis as well as sensory and rheological properties during storage.

MATERIALS AND METHODS

Milk

Fresh buffaloes' milk was obtained from the herd of faculty of Agriculture, Cairo University.

Probiotic strains

Lactobacillus acidophilus was obtained from Chr. Hansen's Lab., Denmark and *Lactobacillus gasseri* was obtained from Dr. Nakamura [Northern Regional Research Laboratory "NRRL" Illinois, USA].

Starter cultures

Lactobacillus dulbueckii subsp. bulgaricus Lb-12 DRI-VAC, provided by Northern Regional Research Laboratory. Illinois, USA. *Streptococcus thermophilus* CH-1 obtained from Chr. Hansens's Lab., Denmark.

Microencapsulation of probiotic strains using different materials

1. Microencapsulation using skimmed milk and Transglutaminase-induced caseinate gelation

Microencapsulation using sodium caseinate was prepared by the method described according to .Heidebach *et al*. **[14] .**In briefly, the protein suspension was prepared by added 15g sodium caseinate to100 ml distilled water and stirring to 2h. Adjusted the pH of suspension to 7.0 and stirred by magnetic stirrer for one day. Ten g of each probiotic cells were mixed with 28 g of the suspension. Five U per g of Transglutaminase enzyme was added to mixture. After that 150 g sunflower oil was added to the mixture with stirred at 900 rpm using a magnetic stirrer for 120 min. During the stirring the beads will be formed.

2. Microencapsulation using rennet-gelation of milk proteins

Micro encapsulation using skimmed milk was prepared by the method described according to Heidebach *et al*. **[15].**Thirty five g of skim milk powder was added to 100 ml of distilled water and stirred over night. Ten g of each probiotic cells were mixed with 28.0 g of the skim milk. The 30 g of the suspension was incubated with 400 ml rennet solution at 5 ºC. After 60 min, 180 ml 10% $CaCl_2$ solution was added to the mixture and 15

g of the mixture was added to150 vegetable oil and stirred for 5 min at 500 rpm to emulsify the mixture into the oil. At temperature 20 °C the emulsified droplets turned into gel particles. The microcapsules were separated from the oil by centrifugation (500 rpm, 5 min).

3. Encapsulation of probiotics in alginate-milk microspheres

Pure milk (11%) and sodium alginate (4%) were sterilized for 15 min at 110 and 121 °C, respectively. The probiotics cells were added to the pure milk and sodium alginate to make mixture containing (1:1:1 w/w). After then, the mixture was added into 100 mM $CaCl_2$ while gently stirring (100 rpm), microspheres will form and solidified in $CaCl_2$ solution for 30 min according to Shi Lu *et al*. **[16].**

Simulated gastric juice (SGJ) tolerance of free and encapsulated strains

SGJ solution contains 9 g/L of NaCl, 3.0 g/L of pepsin and adjusted pH to 2.0 according to **[17].** 1.00 g of each encapsulated strains with different materials (*Lb. acidophilus* or *Lb. gasseri*) or 1.00 ml of free culture of the same strains were mixed in 10 ml of SGJ and incubated for 5, 30, 60 and 120 min at 37 °C. The viability of bacterial cells was detected using pour plate counts in MRS agar and incubated at 37 ° in anaerobic condition.

Simulated intestinal juice (SIJ) tolerance of free and encapsulated strains

SIJ solution prepared by added3.00 g bile salt to one liter of the intestinal solution (6.5 g NaCl, 0.835 g KCl, 0.22 g $CaCl_2$ and 1.386 g $NaHCO_3$) and pH 7.5.One g of each encapsulated strains with different materials (*Lb. acidophilus* or *Lb. gasseri*) or 1.00 ml of free culture of the same strains were mixed in 10 ml of SGJ and incubated for 60, 90, 120 min and 24 hr at 37 °C. The viability of bacteria strains were determined by pour plate counts in MRS agar and incubated at 37 °C in anaerobic condition.

Bile salt tolerance of free and encapsulated strains

Each One ml suspension of free strain (1.00 ml) or 1.00 g of encapsulated strain with different materials were added to 9.00 ml of bile salt solution (1 or 2%, w/v) and incubated at 37 °C for 1 or 2 h. The viability of each free strain was determined by pouring on MRS agar. The viability of encapsulated strains (1.00 g) was dissolved in 9.00 ml, 3% sodium

citrate solution on a shaker to completely release of strains from capsules. The released strains was serially diluted with saline solution and plated on MRS agar and incubated at 37 °C for 48 h anaerobically according to Shi Lu *et al*. **[16].**

Survival rate in SGJ, SIJ and Bile salts concentration:

For evaluated the survival rate of each encapsulated probiotic with different materials, 1.0 ml of free culture or 1.0 g of encapsulated probiotics cells were inoculated into 9.0 ml of sterile SGJ, SIJ or bile salts solution (1% or 2%) and incubated at 37 C for 120 min or 24h. After incubation, the cultures or encapsulated cells removed from solutions and survival rate (%)was calculated by:

Survival rate (%) = (Log cfu N_1/ Log cfu N_0) X 100

Where N_1 is the number of viable cells in microcapsules after treatments by SGF, SIF or Bile salt solutions and N_0 is the number of viable cells in microcapsules before treatments**.**

Improvement the quality of yoghurt by microencapsulated strains

The selected microencapsulation material applied to manufacture yoghurt. Fresh buffalo`s milk, heated at 90 ºC for 30 min, then cooled and adjusted to 42 ºC according to **[18].** The milk was divided into seven portions as the following:

C: Yoghurt starter cultures (1:1 w/w).

T_1:Yoghurt starter cultures(1:1 w/w) with free *Lb. acidophilus*.

T_2:Yoghurt starter cultures(1:1 w/w) with *Lb. acidophilus* encapsulated with skim milk.

T_3:Yoghurt starter cultures (1:1 w/w)with *Lb. acidophilus* encapsulated with alginate-milk.

T_4:Yoghurt starter cultures (1:1 w/w) with free *Lb. gasseri* .

T_5:Yoghurt starter cultures (1:1 w/w) with *Lb.gasseri* encapsulated with skim milk.

T_6:Yoghurt starter cultures (1:1 w/w) with *Lb.gasseri* encapsulated with alginate-milk.

Then, samples were transferred into plastic cups and incubated at 42 ºC for 4h until coagulation. The cups were stored at 7 ºC for 15 days. The produced yoghurt treatments were analyzed chemically, microbiologically, organoleptically and rheological during storage period.

Microbiologically analysis

Lactobacillus bulgaricus counts were determined using MRS agar according to Shah **[19].** *Streptococcus thermophilus* counts were determined using M17 agar according to Terzaghi and Sandine **[20].** The plates were incubated at 35 °C for 48h.

Lactobacillus acidophilus and *Lactobacillus gasseri* counts were determined using modified deMan-Rogosa Sharpe agar containing bromophenol blue (mMRS-BPB) according to Lee and Lee **[21].** The plates were incubated at 37°C for 48h under anaerobic condition.

Chemical analysis

The pH was measured using a digital microprocessor pH meter (HANNA, Instrument, Portugal). The pH meter was standardized using reference pH 4.0 and 7.0 buffer solutions. Also, total solids (TS) were determined according to AOAC **[22].**

Texture profile analysis

In the recent past, instrumental texture analysis (TPA) has been applied as a useful method to evaluate mechanical properties in a wide range of foods. TPA compresses a piece product twice imitating the conditions in the mouth. **[23].**

Texture profile analysis of yoghurt was performed using the double compression test (Multi test 1d Memesin, Food Technology Corporation, Slinfold, W.Sussex, UK). Experiments were carried out by a compression test that generated a plot of force (N) versus time (s). A 25-mm-diameter perplex conical-shaped probe was used to perform the TPA analysis at five different points on the sample surface. In the 1st stage, the sample was compressed by 80% of their original depth at a speed of 2 cm/min during the pretest, compression and the relaxation of the sample. From the force–time curve, the hardness, cohesiveness, gumminess, springiness, and chewiness were determined according to the definition given by the International Dairy Federation **[24]** as follows:

Hardness, N = maximum force of the first compression

Cohesiveness =Area under 2nd compression (Area 2) /Area under 1st compression (Area1)

Springiness, mm = Length 2 / Length 1

Gumminess, N = Hardness × Cohesiveness

Chewiness, N. mm= Springiness × Gumminess

Sensorial Evaluation

The organoleptic properties of stirred yoghurt samples were

assessed by a regular taste panel of the staff- members of the dairy science department, National Research Center. Stirred yoghurt samples were evaluated for flavor (50 points), body and texture (40 points) and appearance (10 points)according to Bodyfelt *et al*., **[25]**.

Statistical analysis

The data were analyzed according to Statistical Analysis System Users Guide **[26]** SAS Institute, Inc, U.S.A. (1994). Separation among means in triplicates was carried out using Duncan multiple tests.

RESULTS AND DISCUSSION

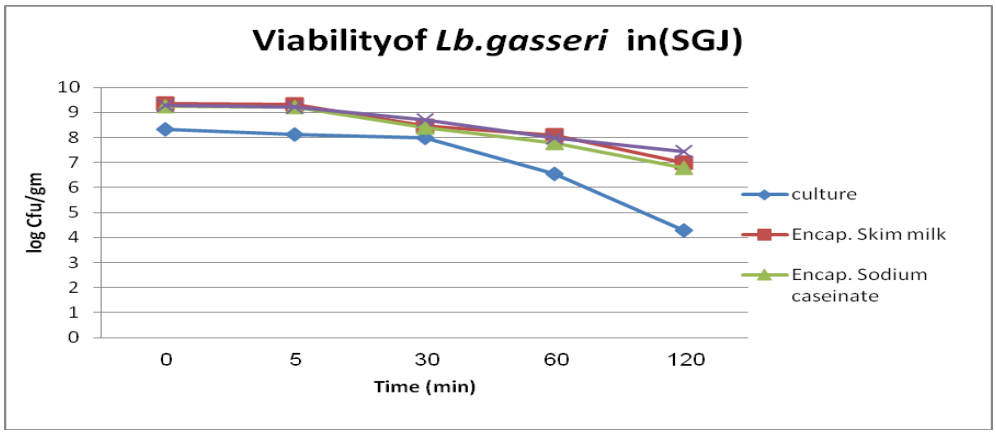
1. Effect of SGJ on encapsulated probiotics with different materials

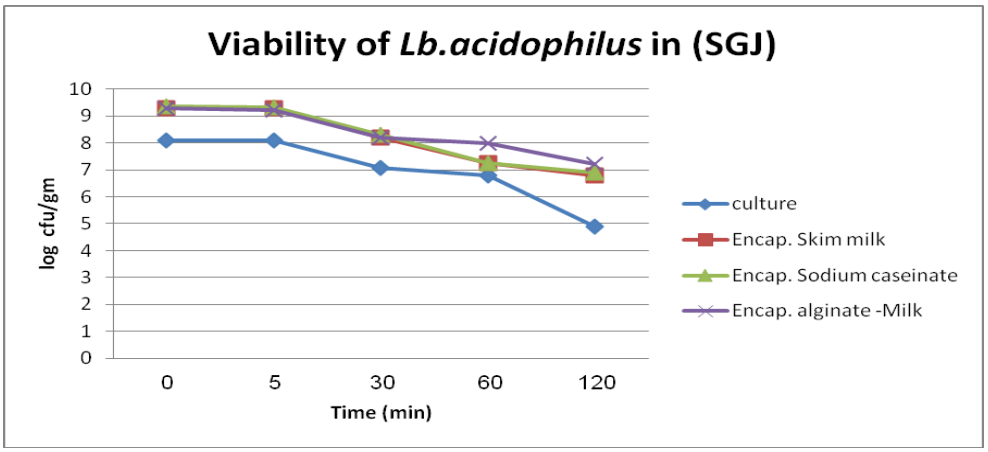
The stability of free and encapsulated probiotics with different materials were shown in Fig. (1). The viability of all free probiotics was lost when exposed to SGJ conditions. Which, the viable free culture count of *Lb. acidophilus* decreased from 8.11 to 4.90 log cfu/ml, and *Lb. gasseri* from 8.30 to 4.90 log cfu/ml after 120 min possessing the survival rate of 60.41%. & 51.68% respectively. Encapsulation in alginate-milk microspheres and skimmed milk improved the survival of probiotics when compared with free culture. Sohail *et al.* **[27]** reported that encapsulated *Lb.acidophilus* using milk-alginate decreased around 1.5 log cycle after 120 min, also encapsulated *Lb. gasseri* with the same material decreased around1.8 log cycle after 120 min. Also, viability of encapsulated *Lactobacillus paracasei* spp. *paracasei* F19 and *Bifidobacterium lactis* Bb 12 with casein may perhaps enhanced when exposed to SGF pH 2.5 **[14]** .

2. *Effect of SIJ on encapsulated probiotics with different materials*

Data in Fig. (2) Shows that encapsulation in alginate-milk microspheres improved the survival of probiotics when compared with free culture. The data indicated that the viability of free culture decreased more than 5 log cycles for *Lb. acidophilus* and more than 5.80 log cycles for *Lb. gasseri* after 24hr, which have survival rate 38.50 and 34.55 % for both strains respectively. On the other hand, the viability of encapsulated probiotics with alginate-milk decreased only around 2 log cycle after 24hr for both strains possessing the survival rate of 74.60 and 77.15 % for *Lb acidophilus* and *Lb. gasseri,* respectively.

Also, encapsulation of probiotics with skim milk decreased only around 3 log cycle compared with free cultures**.** Our results are in agreement with those obtained by Kailasapathy **[28]** and Shi *et al.* **[16].**





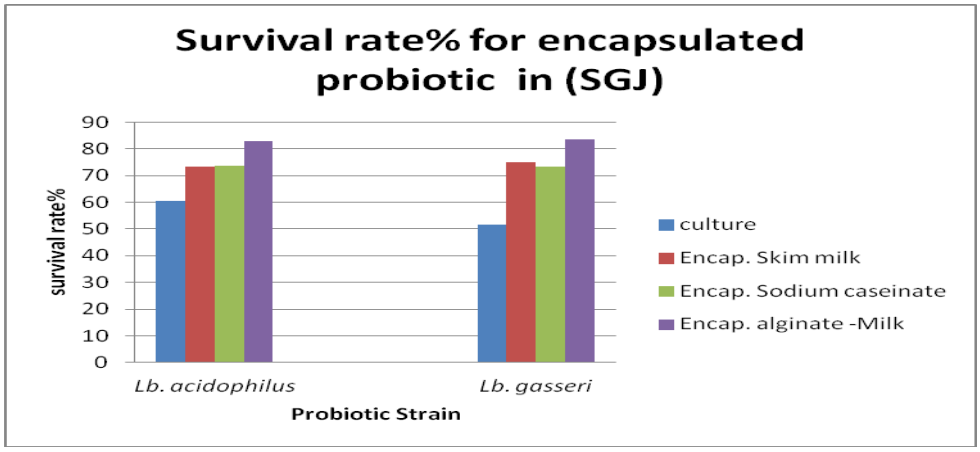
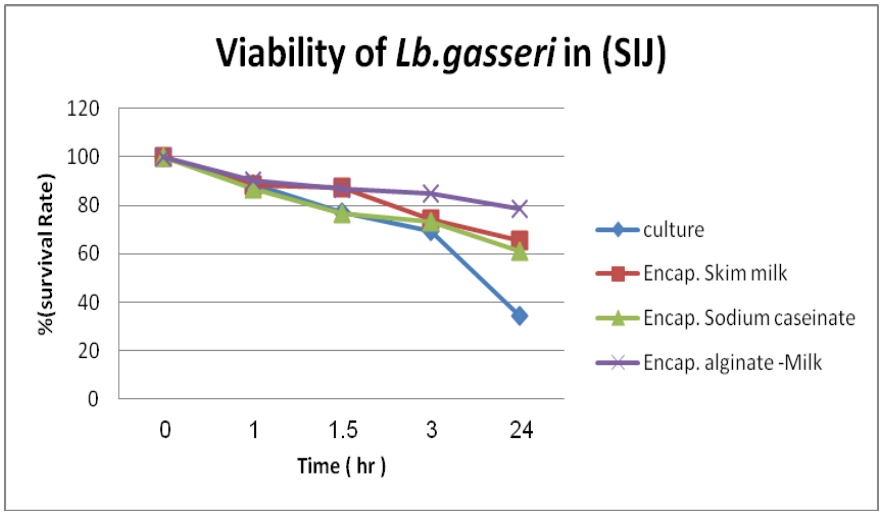
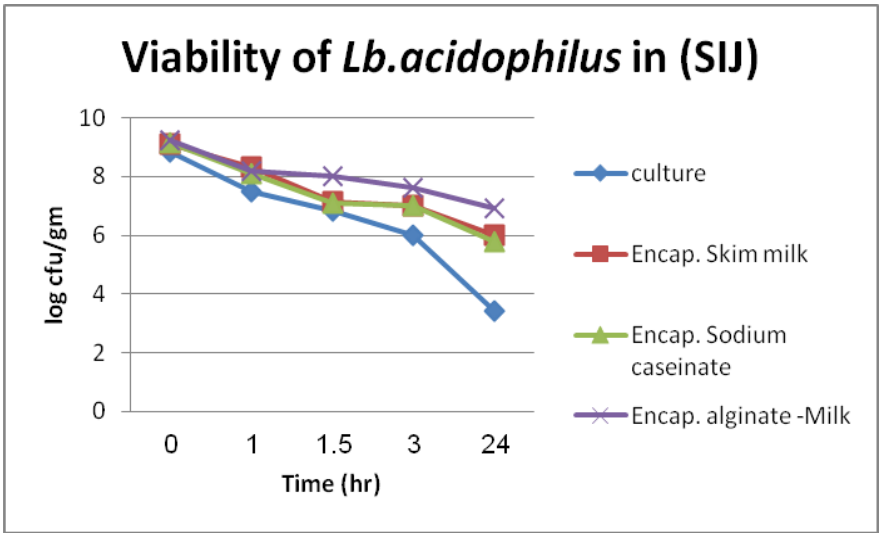


Fig 1: Viability of free and encapsulated *Lb. acidophilus* or *Lb. gasseri* in SGJ





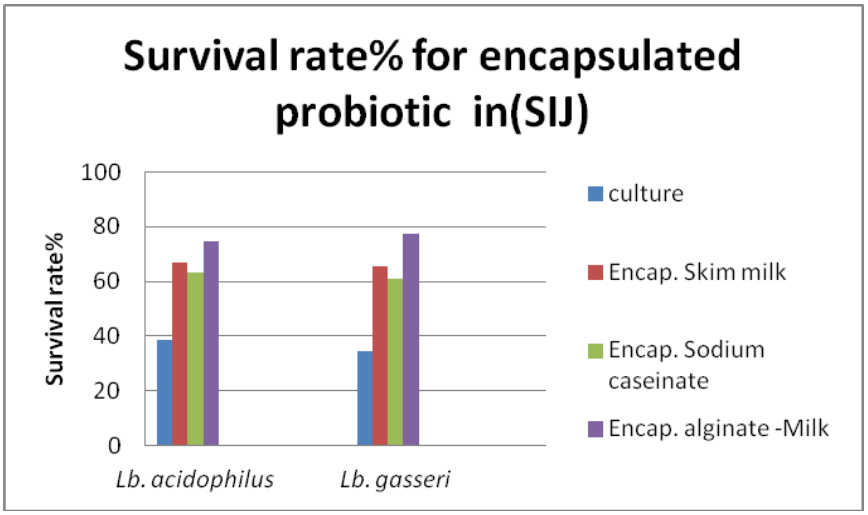
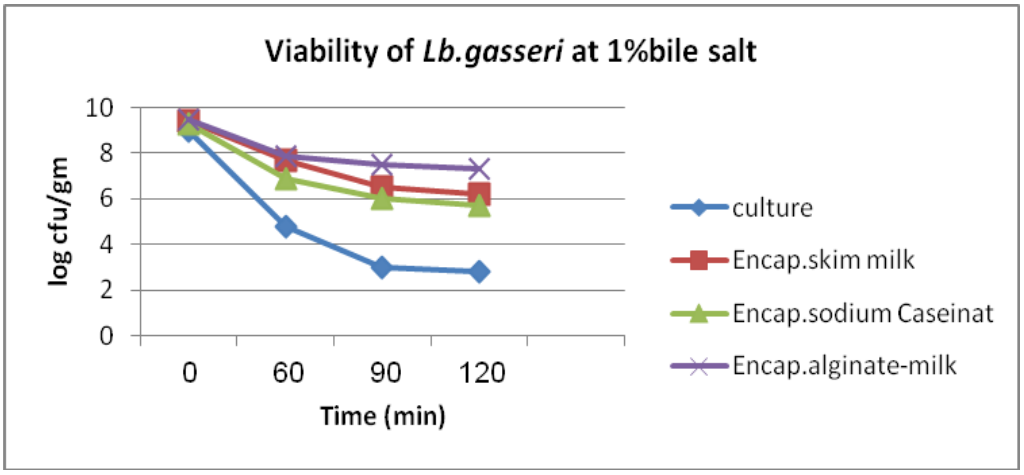
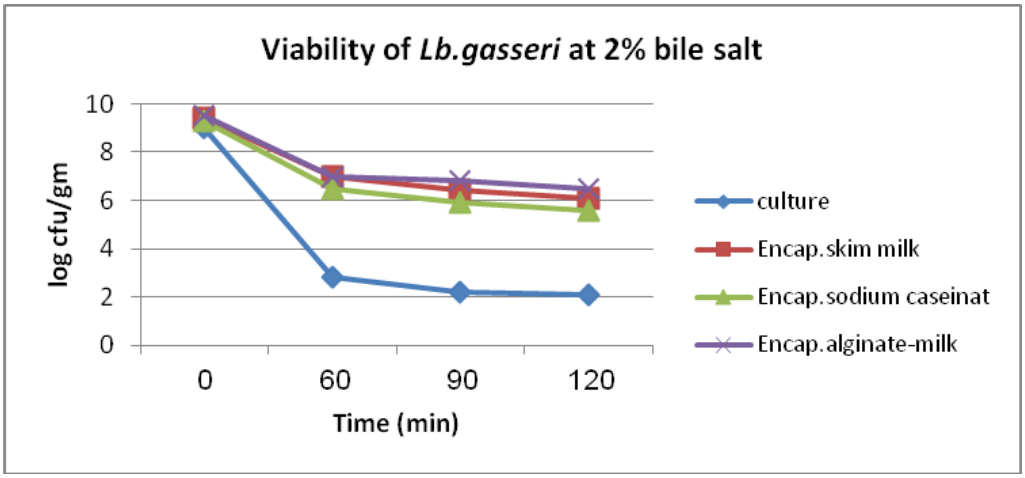


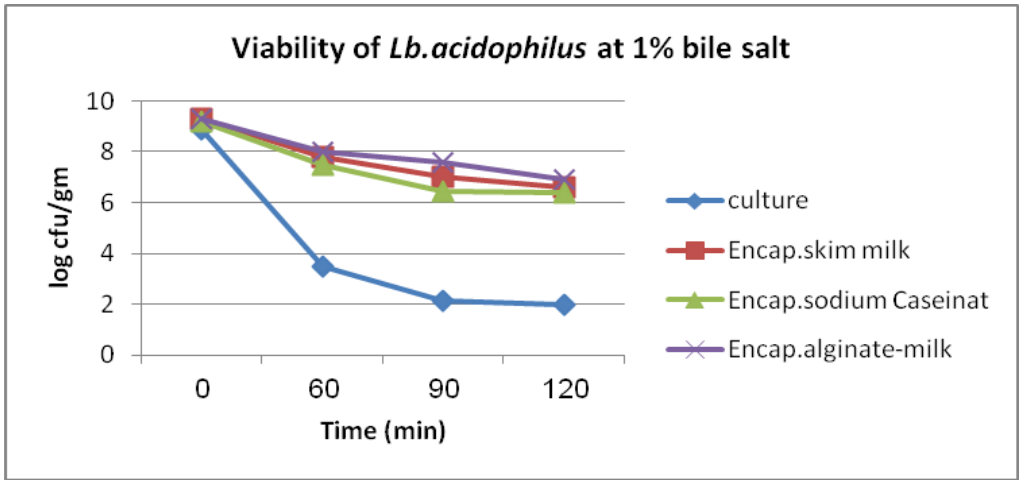
Fig. 2: Viability of free and encapsulated *Lb. acidophilus* or *Lb. gasseri* in SIJ

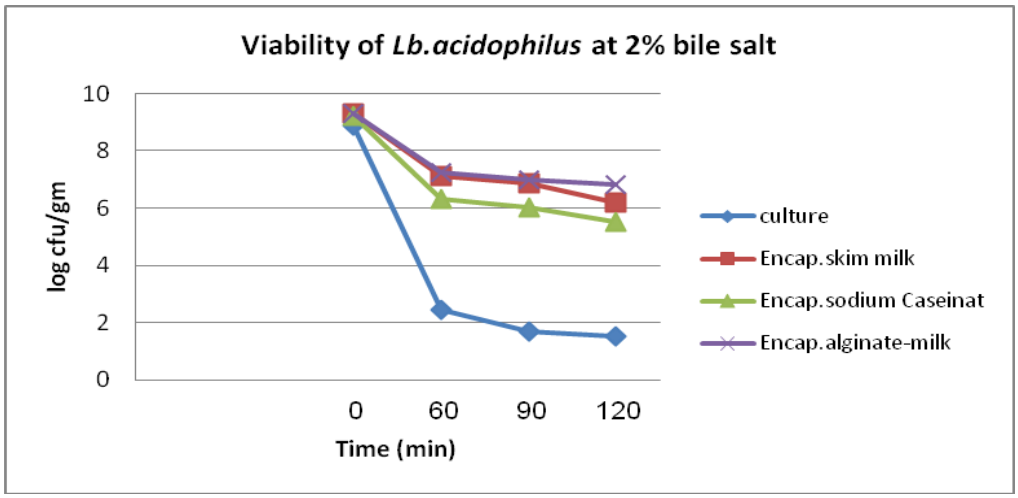
3. *Effect of Bile salts concentration on encapsulated probiotics with different materials*

In this study, free culture of *Lb. acidophilus* viability have survival rate of 19.10% when exposure to 2% bile salt (Fig. 3**)**. The same trend of results was observed with free culture of *Lb. gasseri*. This result might be due to the loss of cell wall integrity as a result of action of the bile salts. These results are in harmony with those obtained by **[29].** Moreover, the results shown in Fig. (3) Indicated that alginate-milk microspheres could offer a good protection from the effect of the bile salt compared to free cultures of probiotics. Our results confirmed by Sohail *et al***.[27]** and Shi *et al***.[16].**









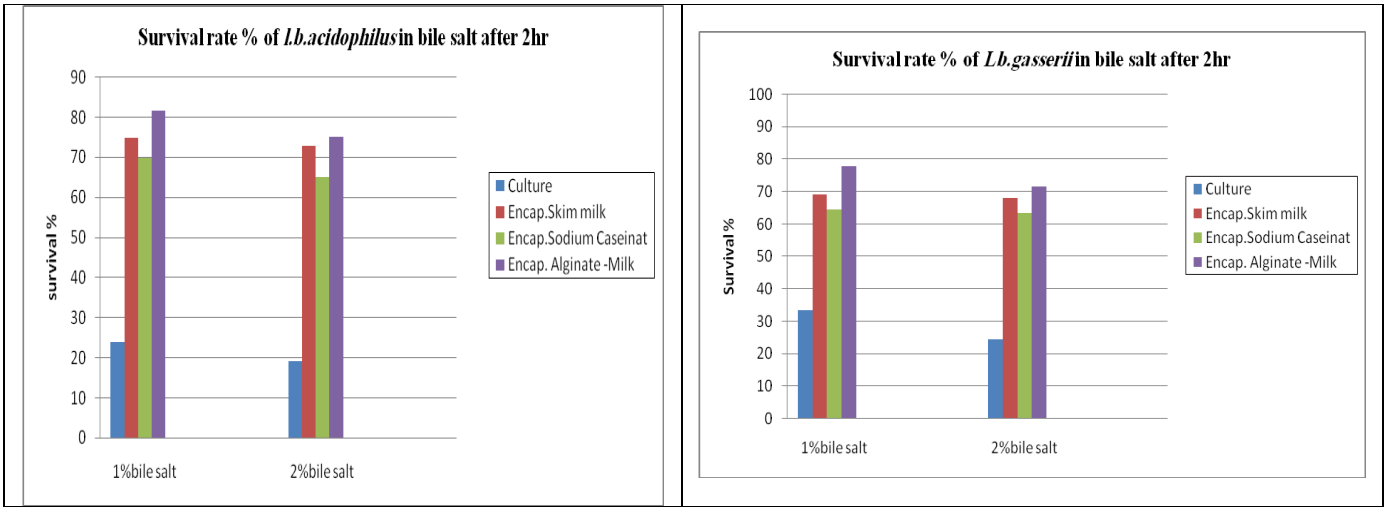
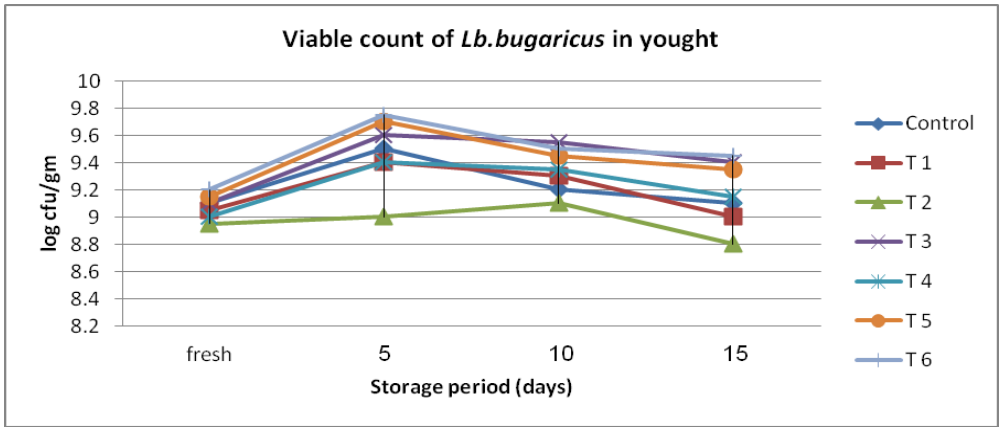


Fig. 3: Viability of free and encapsulated *Lb. acidophilus* and *Lb. gasseri* in bile salt concentrations

Yoghurt production using microencapsulated probiotics with different materials

Data in Fig. (4) Indicated that the counts of both starter cultures were increased in yoghurt samples at the first 5 days of storage period and then slightly decreased at the end of storage period in all samples. The viable counts of *Lb. bulgaricus* ranged between 9.00 to 9.20 log cfu/g in all samples at zero time and reached between8.80 to 9.45 log cfu/gm at the end of storage. The similar results observed for *St. thermophilus* which the viable counts ranged between 8.60 to 8.65 log cfu/g in all samples at zero time and reached between 8.25 to 8.65 log cfu/gm at the end of storage. Also, the results showed there are no differences between control and other samples that contained the encapsulated probiotics with different materials.



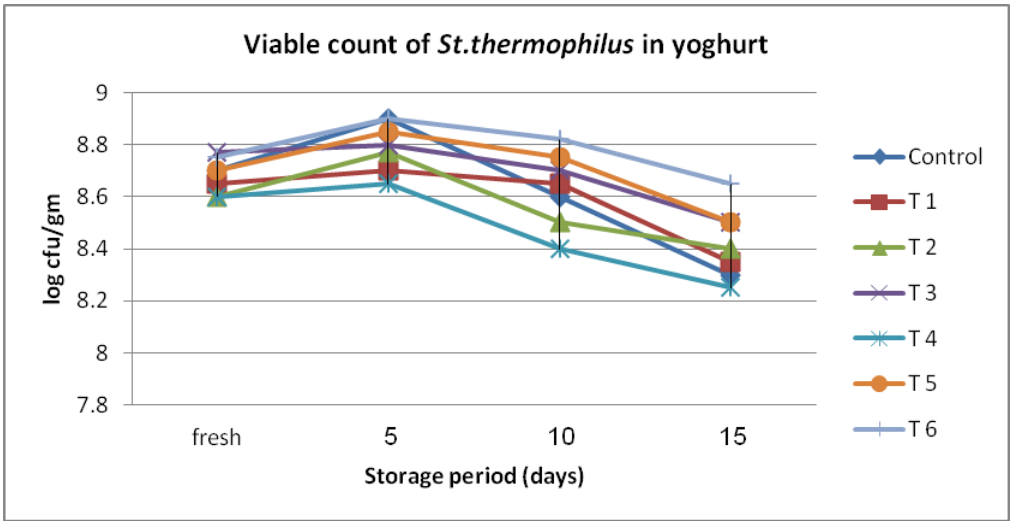


Fig. 4: Viable counts of starter cultures in yoghurt samples during storage for 15 days.

Differences in viable counts of different encapsulated probiotics in yoghurt samples during storage are presented in Fig. (5).Viable counts of all lactobacilli in yoghurt samples are increased gradually during storage periods till 10 days of storage and slight decreased at the end of storage. Moreover, the viable counts in samples that contained microencapsulated lactobacilli more than free cultures especially when used alginate-milk as coating material for encapsulation. Also, the viable counts in yoghurt samples that contained encapsulated probiotics more than 9 log cfu/gm samples at the end of storage compared with free culture which the viable count reached around to 6.5 log cfu/gm samples at the end of storage. The obtained results confirmed the protective effect of microencapsulation on the viability of strains and this in agreement with Hyndman *et al*., **[30]**, Jayalalitha *et al*. **[31]**, Kailasapathy **[28].**

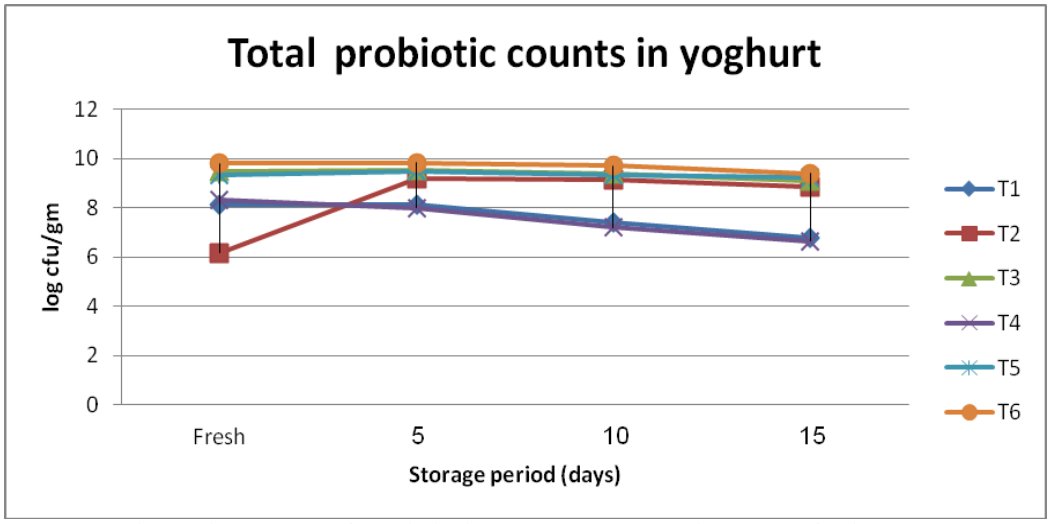


Fig. 5: Viable counts of probiotics in yoghurt samples during storage for 15 days.

As shown in Fig.(6) the survival of free culture in yoghurt was reduced 1.8 and 1 log cycle after 90 min for *Lb. acidophilus* and *Lb. gasseri,* respectively at the end of storage. On the other hand, microencapsulation using alginate- milk and skim milk offered protection throughout the storage time when exposed to SGJ which the survival of encapsulated *Lb. acidophilus* with alginate- milk and skim milk decreased to 7.50 and 7.25 log cfu/gm, respectively at the end of storage. Also, counts of encapsulated *Lb.gasseri* using alginate-milk and skim milk decreased to reach 7.65 and 7.30 log cfu/gm, respectively at

the end of storage. These results could be attributed to the encapsulation materials that used in capsulation technique, also, founding encapsulated cells in products as yoghurt that contained high protein which offered extra protection against SGJ. Our results confirmed by Shi *et al*.**[16]** , Lee and Heo**[32]**.

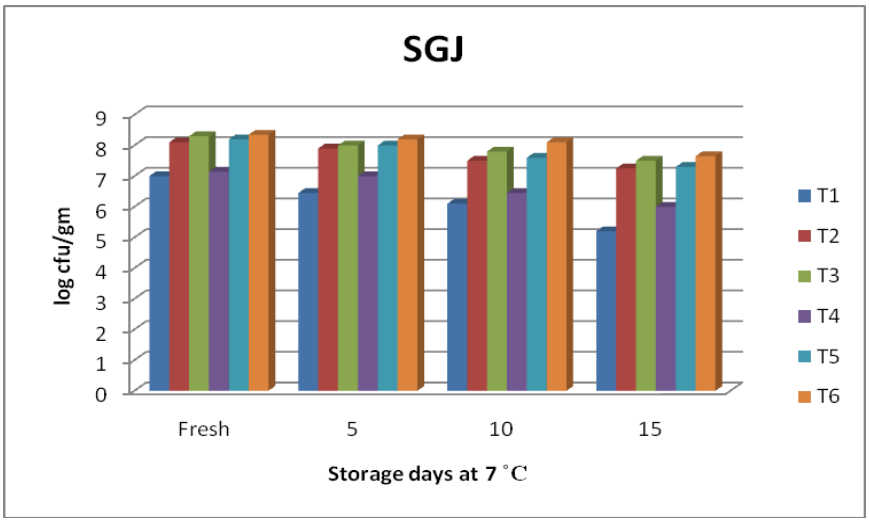


Fig. 6: Survival of probiotics in yoghurt when exposed to SGJ for 90 min during storage periods

The survival of free and encapsulated probiotics with different materials when exposed to SIJ were shown in Fig.(7). The data indicated that survival of free culture in yoghurt was reduced to 2.40 and 2.60 log cycles after 90 min for *Lb. acidophilus* and *Lb. gasseri,* respectively at the end of storage. Also, the viable counts of free culture in yoghurt gradually decreased during the storage when exposed to SIJ for 90 min. On the contrary, the encapsulated probiotics in yoghurt more stable than free cultures during exposure to SIJ for 90 min, the survival of encapsulated *Lb. acidophilus* decreased only0.85 and0.80 log cycle for encapsulated by skim milk and alginate-milk respectively as compared to the initial counts. Moreover encapsulated *Lb. gasseri* by skim milk and alginate-milk reduced only 1.25 and 1.00 log cycle during exposure to SIJ for 90 min as compared to the initial counts at the end of storage .Our results are similar to those obtained by Sultana *et al*. **[33].**

Survival of encapsulated probiotics in yoghurt samples when exposed to 2% bile salts during storage periods

Both free cultures found in yoghurt samples showed a loss of viability when exposed to 2% bile salt concentration after 90 min (Fig. 8). On the contrary, the

viability of both encapsulated probiotics with different materials slight reduced when exposed to 2% bile salt. The viable count of *Lb. acidophilus* encapsulated with skim milk or alginate- milk reduced only 1.00and 0.85 log cycles during exposure to bile salt for 90 min. Also, encapsulated *Lb. gasseri* by the same materials reduced only0.90 and 0.80 log cycle at 90 min of exposure to 2% bile salt. Our results confirmed results by other authors Kailasapathy **[28]**, Ding and Shah **[34].**

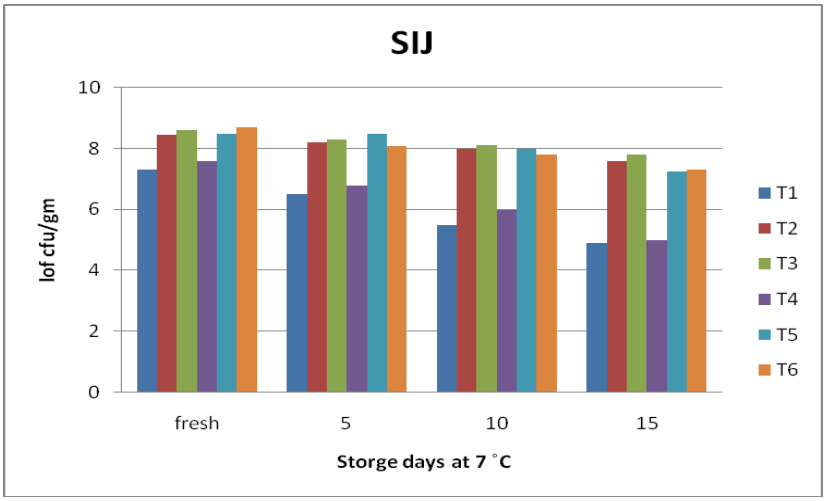


Fig. 7: Survival of probiotics in yoghurt samples when exposed SIJ for 90 min during storage periods.

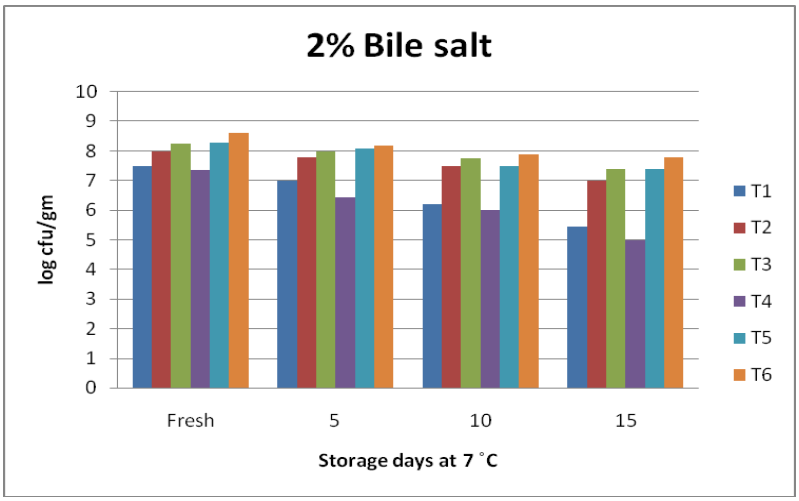


Fig 8: Survival of probiotics in yoghurt samples when exposed to 2% bile salts for 90 min during storage periods.

pH values and total solids (TS) contents:

pH values of yoghurt samples during storage period were clarified in Fig. (9). It was noticed that as long as increasing storage period, pH values decreased in all samples. Control samples had gained the

highest pH values during storage period. pH values of control samples were (6.02, 5.93, 5.86 and 5.43) at fresh, 5, 10 and 15 days of storage period. The high pH values of control samples might be as a result of absence of probiotic bacteria in all samples. So, acidity was due to the activity of starter culture only. On the other hand, it was obvious that yoghurt treated samples which made with free *Lb. gasseri* had lower pH than *Lb. acidophilus*. From the microbiological data, the decrease in pH values were not due to the activity of starter cultures themselves only, but it was due to the viability of both Lactobacillus species in yoghurt samples. It was lucid that there were no significant differences between yoghurt samples with different capsulated materials (T_3&T_6). As we mentioned before in microbiology results that encapsulated treated samples had more viability of probiotic than control samples. Similar data were observed by Cakmakci *et al*., **[35]**. They used probiotic cultures (*Lactobacillus acidophilus*, *Bifidobacterium bifidum*).

Data in Fig. (10) Represents total solid contents (TS) of yoghurt samples either control or treatment samples. The apparent results displayed that there were no significant differences between control and yoghurt treated samples with both of free probiotic in TS contents. During storage period, total solid contents decreased in all yoghurt samples either control or treated samples. Data demonstrated that yoghurt samples with encapsulated probiotics had obtained lower TS contents than control and yoghurt samples with free probiotics. The lowering in total solid contents may be as a result of using encapsulated materials, for example, alginate is widely used as a gelling worker. Alginate has the capability to form hydro gels with divalent cations such as Ca^{+2} and Ba^{+2} under mild conditions **[36, 37]**. Therefore, encapsulated materials can be holding some water causing decrease in total solid contents.

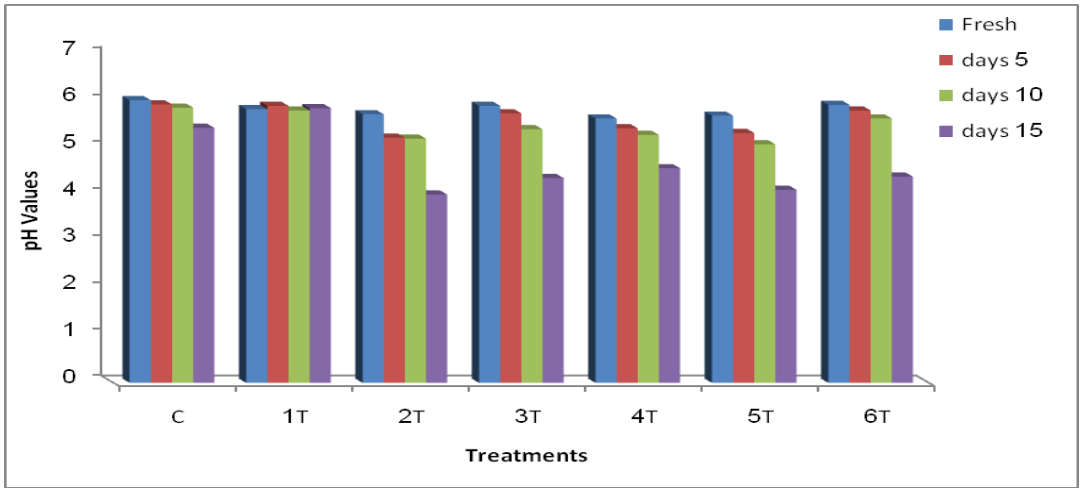


Fig. 9: pH values of yoghurt samples when fresh and after 15 days

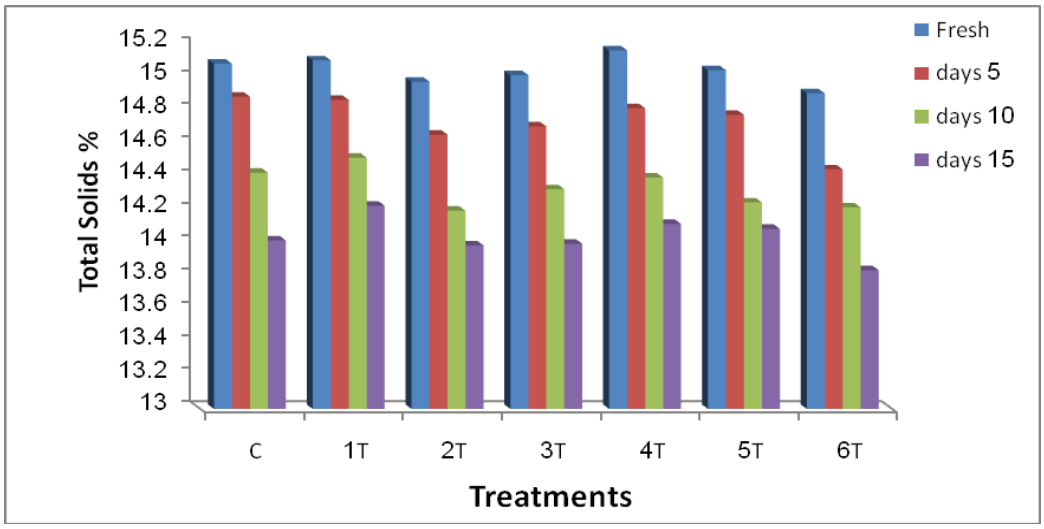


Fig. 10: TS contents of yoghurt product samples when fresh and after 15 days

Texture profile analysis

The results showed in Table (1) demonstrated that there were no significant differences between control and yoghurt treated samples with both of free or capsulated probiotic in hardness in all samples. During storage period, the values of hardness were decreased in all samples. The decrease in the hardness values may be attributed to the higher moisture content in yoghurts with both of free or capsulated probiotic than control. These results were agreement with the TS content of the samples which decreased during storage period. Positive correlation were found between hardness and total solids, TP, pH and S.N/TN and negative correlation were found with Fat/DM and lactose **[38].** The highest hardness was measured in the sample T 2 (yoghurt

contains encapsulated *L. acidophilus* with skim milk) (2.5 – 2) N and sample T 6(yoghurt contains *L.gasseri* encapsulated with alginate- milk) (2.5 – 2) N.

There were slight differences in cohesiveness between all samples of yoghurts. While the cohesiveness of the samples treated with *Lb. acidophilus* were slightly lower than samples treated with *Lb. gasseri*. With the changed levels of cohesiveness, multiplication of hardness and cohesiveness, namely gumminess **[39] ,** also lower in yoghurts treated with *Lb. acidophilus* than other samples. The springiness of yogurts which treated with *Lb. gasseri* were slightly higher than of the others, indicating that it returned more easily to its original shape after the deforming force was removed. Chewiness is defined as the number of masticates required for a certain amount of sample in order to satisfactorily decrease the consistency for swallowing [*39*]**.** Multiplication of gumminess and springiness, namely chewiness showed a similar trend of gumminess. With the development of storage period, all parameters of rheological properties were decreased in all yoghurt samples which treated with the two strains of Lactobacillus bacteria after 15 days of storage. Finally, the results of rheological properties indicated that all samples of yoghurts which contain free or capsulated *Lb. gasseri* were better than control and yoghurt samples contain free or capsulated *Lb. acidophilus*.

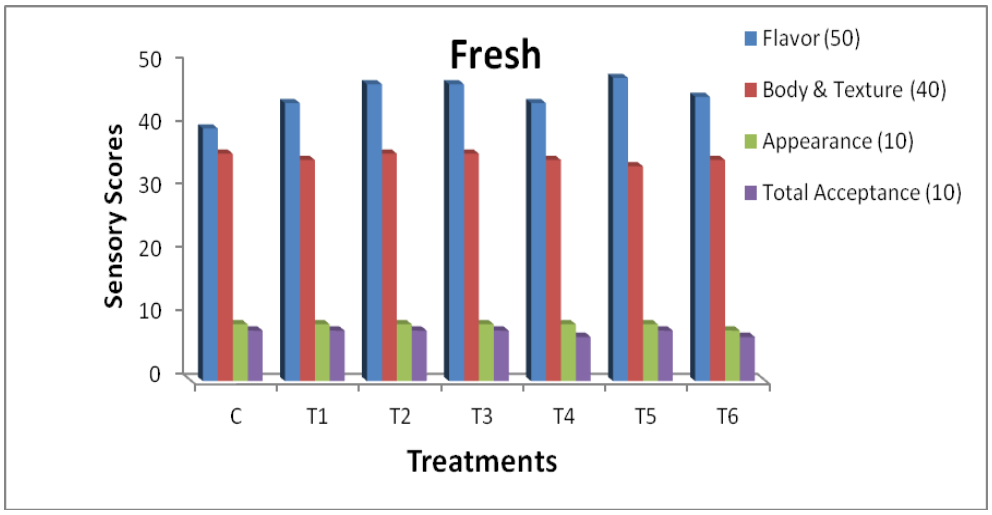
Table 1: Changes in the textural profile parameters of yoghurt samples during storage period

Samples	Hardness N	Cohesiveness [A are [A/B	Springiness mm	Gumminess N	Chewiness mm.N
Fresh					
C	1.8^{ED}	0.514^{CD}	0.635^{GF}	0.925^{CB}	0.587^{EF}
T1	2.1^{CB}	**0.497FE**	0.650^{EF}	1.044^{ABC}	0.678^{D}
T2	2.5^{B}	0.511^{BCD}	0.647^{GF}	1.277^{AB}	0.826^{C}
T3	2.1^{ED}	0.487^{F}	0.662^{ED}	1.023^{ABC}	0.677^{D}
T4	2.1^{A}	0.562^{A}	0.721^{BC}	1.180^{C}	0.851^{CB}
T5	2.1^{CB}	0.581^{A}	0.727^{B}	1.220^{ABC}	0.887^{B}
T6	2.5^{CB}	0.531^{BC}	0.751^{A}	1.327^{A}	0.997^{A}
15 Days					
C	2.0^{E}	0.507^{CDE}	0.630^{GF}	1.014^{ABC}	0.639^{ED}
T1	1.8^{B}	0.462^{FE}	0.607^{G}	0.832^{C}	0.505^{F}
T2	2.0^{ED}	0.457^{G}	0.588^{I}	0.914^{C}	0.537^{F}
T3	1.7^{B}	0.414^{H}	0.612^{H}	0.704^{C}	0.431^{G}
T4	1.8^{CD}	0.539^{B}	0.700^{C}	0.970^{ABC}	0.679^{D}
T5	1.8^{A}	0.486^{A}	0.679^{D}	0.875^{ABC}	0.594^{ED}
T6	2.0^{B}	0.527^{BC}	0.737^{AB}	1.054^{ABC}	0.777^{C}

Sensory properties of yoghurt:

The popularity of yogurt depends mainly on its sensory characteristics, of which are characterized as the microbial factors, processing parameters, source of milk and the additives used. The mean scores of the sensorial attributes (flavor, body and texture, appearance and overall acceptability) for samples given by the panelists were presented in Fig(11). There were significant differences in the flavor between the encapsulated yoghurt samples (T_2, T_3, T_5, T_6) and the control and free lactobacillus culture samples. The scores of flavor in (T_2, T_3, T_5, T_6) samples were higher than (C, T_1, T_4) samples. The panel found that no significant differences in all samples for body and texture, appearance and acceptability. After 5 days of storage the acidity of the yoghurts increased, and the sensory scores of all samples began to decrease gradually during storage. The lowest acceptability was found in (T_2, T_3) samples. The highest sensory scores recorded in the encapsulated yoghurt samples (T_2, T_3,T_5, T_6). These results indicated that the yoghurt samples with encapsulated culture were more acceptability than control and free culture yoghurt samples.

The panels found that the yoghurt samples with encapsulated probiotics contained small granules which had been felt in the mouth. However the control and yoghurt samples with free probiotics didn't contain these granules. These results may leads to affect on the texture characteristics and may cause week in the texture of yoghurts. These results were agree with Krasaekoopt and Tandhanskul **[40].** They confirmed that presence of probiotic beads affected the texture characteristics of the plain yogurt.



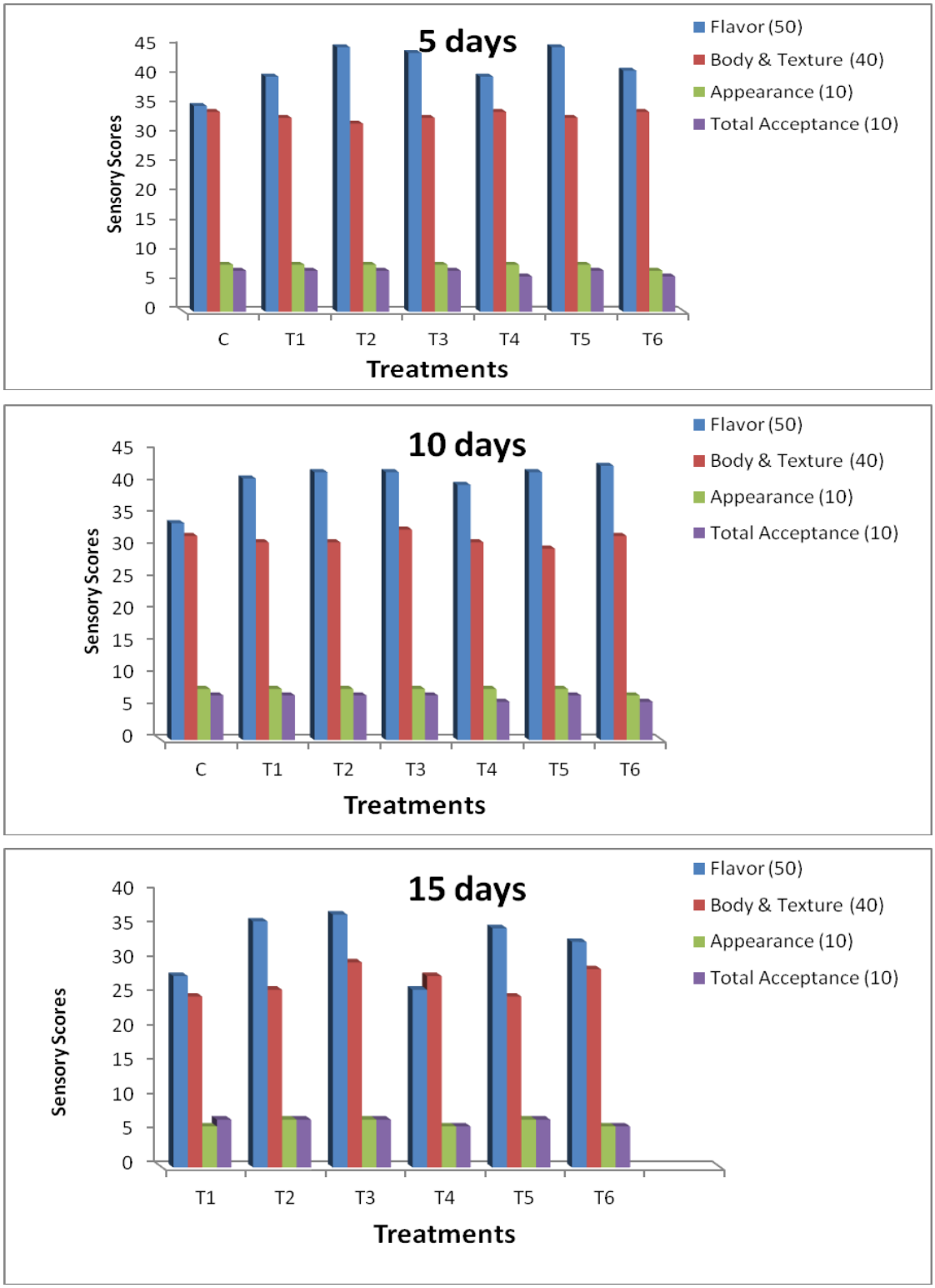


Fig. 11: Sensory scores of yoghurt product samples when fresh and after 15 days

CONCLUSIONS

The microencapsulation methods reported in this paper proved to be very efficient in increasing the viability of probiotic bacteria compared to non-encapsulated free cells. Also, highest efficiency in probiotic protection was observed with alginate-milk microspheres method.

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