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Effect of encapsulation on the viability of probiotics in yoghurt

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Summary: *Background:* It is well established that probiotic bacteria exert myriad of beneficial effects on human health, including antibiotic therapy, improved symptoms of lactose intolerance, resistance against cancer, reduced incidence of diarrhea in humans and production of antimicrobial substances and reducing cholesterol level. The objective of this study was to evaluate the stability of Probiotics in the yoghurt with and without encapsulation. *Methods:* Probiotic yoghurt was compared with control yoghurt in terms of chemical, physical, microbial and sensory properties over a period of 15 days of storage. Yoghurt was prepared with free lactic acid bacteria and with encapsulated bacteria and was stored at 4^0C. Yoghurt was subjected to physiochemical and microbial analysis. *Findings:* The addition of the probiotic bacteria in the yoghurt samples either in encapsulated or without encapsulation significantly affected the results for pH, lactose, acidity, viscosity and syneresis. However, the addition of the culture of probiotic either free or encapsulated form did not bring any distinct difference in color, flavor and taste over the 15 days of storage period.

Key words: encapsulation, probiotics, yoghurt

Introduction

For the past many years, the most debated topic, not only in medicines but also in biomedical sciences, is the recognition of probiotics that resides in the human gut. Research on gut microbiome is progressing rapidly because of the availability of novel and reliable tools for microbial analysis (1). "Probiotic are the live bacteria which when taken in ample quantity give a health advantage on the host" (2). Probiotics are considered as viable microbes, bacteria or yeasts, that when administered in adequate amounts exert beneficial health effects on the host (3) Another definition of probiotics "these are ingredients mostly fermented and selected that permit precise changes, both in the properties and action in the gastrointestinal tract (GIT). Micro biota that gives advantages upon host welfare and health" (4).

Health associated benefits of probiotics include antibiotic therapy, improving symptoms of lactose intolerance, anti cancer activity, reducing incidence of diarrhea in humans, and lowering cholesterol level. They are healthy substitute of good bacteria in the human intestinal tract.

To allow these beneficial microbes to confer the above-mentioned benefits, their survival is of vital importance. The reports regarding the survival and viability of the probiotic indicated that the viability of probiotic bacteria is frequently low in yoghurt (5) that results in less than 10^8 –10^9 cells, which is daily recommended intake. Many brands have been analyzed in Australia (6) and in Europe (7) for the sufficient presence of *Lactobacillus acidophilus* and other *Bifidobacteria* spp.

There are many factors that may affect the viability of these microbes, during the food processing and subsequent storage. It is therefore, important to understand the factors critical to their survival in order to produce a promising food product enriched with bifidobacteria that could confer greater beneficial effects on the consumer.

Due to the harsh processing and storage conditions it is difficult for the probiotic to survive in the products. Conditions like pH, temperature, presence of oxygen (for anaerobic organisms) and storage conditions greatly affect the survival of the probiotic. Metabolites of other bacteria in the food product are also a critical factor for the survival of the probiotic bacteria. Fermenting organisms that present in the yoghurt can have an undesirable effect on the survival of probiotics.

It may be feasible to enhance the survival of probiotic bacteria in dairy foods by different methods. These methods include introducing growth promoters, by adopting dairy product fermentation above pH 5.0, by lower fermentation temperature and enhancing fermentation time and by decreasing storage temperature (6) However, considerable success has not yet been achieved.

To overcome, the problem of the survival of the probiotic encapsulation is the best technology. Encapsulation is a practice that provides a shell of shielding material to organisms, thus protect them and making them able to withstand the unfavorable processing conditions. Encapsulation has been applied to enhance the survival of probiotic in the different conditions (8).

It improves the survival of probiotic organisms during processing in fresh and freeze-dried yoghurt. Encapsulation provides the physical shield to probiotic bacteria to enhance their survival. This physical shield also provides the protection against adverse conditions, like high or low temperature.

It has been demonstrated in many studies that the dairy probiotics have poor ability to survive the processing conditions. It has been come to know that dairy products showed the poor survival due to different factors. These products are also not showing their good performance in the gastro intestinal system regarding the survival of the probiotic bacteria. Encapsulation is providing a protective layer to the probiotics against the unfavorable and adverse environmental conditions to increase the survival. The use of encapsulated ingredients has increased noticeably over the past few years. Encapsulated forms of ingredients achieve longer shelf life in the product (9).

Encapsulation has many applications in the food industry that include enhancing shelf life, controlling oxidative reaction, pretense flavors, odors and colors, giving a sustained release, etc. There are several studies that demonstrated the use of encapsulation technique to protect probiotics against adverse environmental and processing (10, 11)

Encapsulation technology usually consist of 3 stages. The first stage includes the addition of the bioactive component like cell in a medium which can be liquid or solid. In the second stage, the liquid matrix is dispersed while a solution is pulverized on the solid matrix. The final stage consists of stabilization by a chemical (12).

In addition to increase the viability of probiotic (cell), encapsulated materials also improve the textural properties of the yoghurt. All encapsulated materials help in increasing the viability of the cell. These materials are very useful and very effective in increasing the different textural properties of different dairy products particularly yoghurt. These materials act as protective cover for the probiotic (13). Therefore, these materials were taken in this manuscript after addition in the yoghurt at different ratio.

Due to all these above mentioned benefits of the probiotics there is a dire need to enhance the survival of the probiotic in foods that contain these microorganisms. The present study was formulated to find out the maximum stability of the lactic acid bacteria and explore the effect of incorporating encapsulated probiotic culture that commonly used as food stabilizer.

Materials and methods

The present study was conducted in Food Microbiology and Dairy technology laboratories, National Institute of Food Science and Technology, University of Agriculture Faisalabad, Pakistan

Encapsulation

Encapsulation of probiotic bacteria was carried out as described by the Sultana et al. 2000 with some modification. A hydrocolloid solution was prepared by adding 2 % sodium alginate in distilled water. In this solution 0.1 % probiotic bacteria were added. This suspension was extruded through a syringe. Syringe was used to form droplets. These droplets were dropped into hardening solution that was prepared by adding the calcium chloride. 0.5 M calcium chloride solution was made. Droplets could stand for thirty minutes in the hardening solution. Beads were collected by centrifugation and were stored at 4 °C for future use.

Physico chemical analysis of milk

Physicochemical analysis such as acidity, pH, total solids, fat, protein and lactose of milk were done according to (AOAC 2000).

Yogurt manufacturing

Yoghurt was manufactured in the Microbiology and Dairy Laboratory, National Institute of Food Science and Technology, University of Agriculture Faisalabad according to the process of (15).

Physicochemical analysis of yoghurt

Chemical analysis of yoghurt

The prepared was analyzed for following parameters.

Lactose

It was determined by the Lane–Eynon method based on the reduction of copper (16).

Acidity

Acidity was determined by direct titration method of (16).

pH

Electronic digital type of pH meter-Hanna 8416 was used for pH determination.

Physical analysis of yogurt

Viscosity

Viscosity of the yogurt was determined by means of a Brookfield DV-I viscometer (LVDVE 230) as described by Gassem and his coworkers (1991) (17).

Syneresis

Syneresis was measured by the method described by Peri et al. (1985) (18).

Treatment plan

Following treatment plan (Table 1) was adopted during this study with three replications.

Microbiological analysis

Total plate count

Total plate count of the microorganism in the yoghurt was determined by adopting the method of (19). The data obtained was analyzed by using Completely Randomized Design (CRD) and the means were compared by Duncan's Multiple Range test (DMR) as described by (20).

Results

The present study was conducted to investigate the affect of the encapsulation on the stability of the probiotic bacteria in the yoghurt. The purpose was also to evaluate the physiochemical and sensory attributes of the yoghurt prepared by encapsulated lactic acid bacteria (LAB).

Table 1. Treatment plan

T0	Traditional yoghurt (Control)
T1	Yoghurt with free LAB (probiotic bacteria)
T2	Yoghurt with encapsulated LAB (probiotic bacteria)

The yoghurt samples were prepared with encapsulated bacteria and without encapsulated bacteria and with traditional yoghurt. The yoghurt was stored at 4 °C and subjected to physiochemical and microbiological analysis. The sensory evaluation was also carried out. The results obtained have been presented and discussed under different heading and subheadings.

Analysis of the milk for preparation of the yoghurt

Milk procured from the commercial market and subjected for analyzing the fat, acidity, pH and total solids.

Physiochemical analysis of the yoghurt

The data regarding the physical analysis (viscosity and syneresis), chemical analysis (pH, acidity, and lactose), microbiological analysis (total plate count) The results that were obtained regarding the different parameters are described individually.

Chemical analysis of the yoghurt

pH

pH is the fundamental criterion for the classification of substances. It is the negative logarithm of the hydrogen ion. The data on the pH of different yoghurt samples during storage period is shown in the Graph 1. The statistical data showed that there are highly significant effects for treatment, storage and the interaction of storage days and treatments. The pH of different yoghurts significantly decreased during storage.

The pH value after 15 days of storage decreased from 4.66 to 4.22. Maximum pH value was observed on the first day that was 4.66. There was a gradual decrease in pH with respect to storage. The pH decreased because of acid production in the yoghurt as storage period increased.

Highly significant value of pH was observed in the yoghurt samples prepared with the addition of probiotics bacteria that were without encapsulation. This value (5.21) obtained was significantly higher than other two treatments (T_0 and T_2). pH of the control (T_0) was significantly higher than the pH value of the yoghurt with encapsulated bacteria. The minimum value of pH 4.40 was observed in the yoghurt with encapsulated bacteria. The reason could be bacteria when encapsulated produced acids with fewer paces, hence less decrease in the pH (Figure 1).

Lactose

The data regarding lactose of yoghurt under different treatments and storage is shown in the Graph 2. The lactose decreased with the passage of time when stored at 4 °C. The results for the treatment, storage and interaction of treatment and storage were found to be highly significant. The value for the lactose during the 15 days of storage decreased from 7.39% to 6.52%. A decreasing trend was observed during the storage of the yoghurt.

The maximum value for the lactose was on the first day and it was significantly higher than all other days of storage. It decreased to 7.27 % on the 5th day of storage. Similarly it decreased 7.10% and 6.50% on the 10th and 15th days respectively during the storage period. The minimum value was observed on the 15th day of storage.

Decreasing trend for lactose was observed in all treatments. Significantly maximum value (7.32%) lactose was observed in the yoghurt produced with encapsulated bacteria. This value was greater as compared to the other two treatments (T_0, T_1). The reason could be the bacteria when encapsulated use less quantity of lactose, hence slow changes in the pH and acidity. The bacteria when encapsulated become slow acid producer. The lactose value for the yoghurt with free probiotics and control was 6.91 % and 6.98 % respectively. The minimum value (6.91%) was observed in the yoghurt sample prepared with free probiotics which was sig-

Table 2. Physicochemical analysis of milk

pH	Acidity	Fat (%)	Protein (%)	Lactose (%)	Total solids (%)
6.68±0.45	0.11±0.45	4.15±0.45	3.80±0.45	4.76±0.45	14.63±0.45

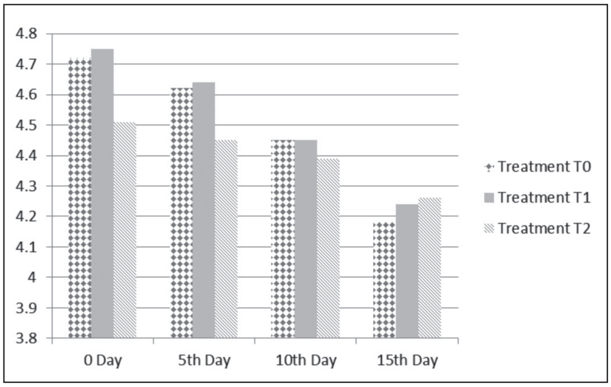

Figure 1. Effect of encapsulated lactic acid bacteria (LAB) on pH of the yoghurt

nificantly lower than all others. The reason could be due to the maximum lactose utilization showing the changes in the lactose content in yoghurt.

The interactive effect of storage days and treatments was also found to be significant. Significantly maximum value (7.66%) was observed in the interaction T_2S_1 (yoghurt with encapsulated bacteria and 1st day). In case of other interaction significantly less value of lactose (6.26 %) was observed. A decreasing trend was observed in the interaction of the treatment and the storage days. Minimum interaction value was observed on the 15th day of storage in the interaction of T_1 and S_4 (Figure 2).

Acidity

The data regarding the acidity of the yoghurt under various treatments is shown in the Graph 3. Yoghurt is an acidic product with natural keeping quality. The quality deteriorates quickly as the acidity increases with the passage of time and the yoghurt becomes bitter. The acidity of the yoghurt during the storage increases this could be due to the conversion of lactose into lactic acid.

These conversions of lactose into lactic acid causes decrease in the pH and hence increase in acidity. The acidity value significantly increased from 0.44% to 0.58% during the 15 days of storage. The minimum value 0.44% was observed on the 1st day.

The statistical data revealed that the acidity increased with the increase of the storage of the yoghurt. The results were highly significant for the storage, treatments and interaction. In the treatments mean maximum acidity was observed in yoghurt sample of the treatment with encapsulated bacteria. It was significantly greater than the other two treatments.

The minimum value for the acidity was observed for the control treatment. The value of the acidity for the yoghurt produced with the addition of free lactic acid bacteria was intermediate between the control and the yoghurt with encapsulated bacteria. An increasing trend of the acidity was observed in all the treatments with the passage of time.

With respect to the interaction the storage days and the treatment found to be significant in case of titratable acidity. The statistically significant value (0.63%) was observed in the interactionT_2S_4.The values for the interactions of T_0S_I, T_1S_2 and T_2S_2 were statistically at par with each other. The minimum value (0.38%) was observed in the interaction of T_0S_I (Figure 3).

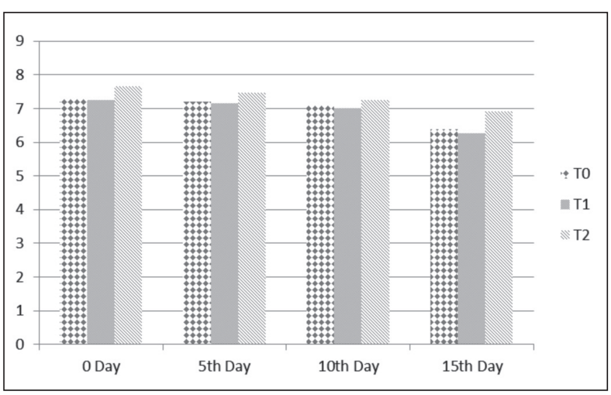

Figure 2. Effect of encapsulated lactic acid bacteria (LAB) on lactose (%) of the yoghurt

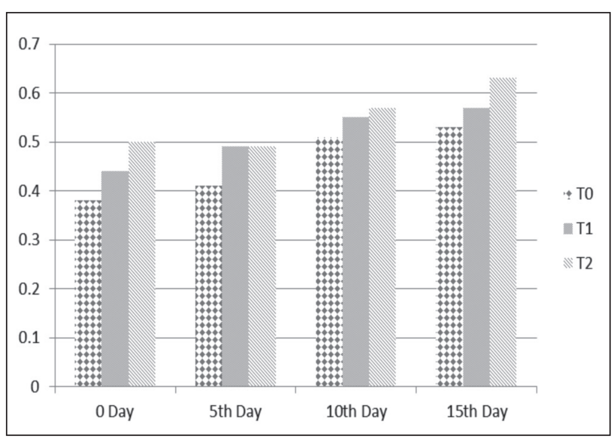

Figure 3. Effect of encapsulated lactic acid bacteria (LAB) on acidity (%) of the yoghurt

Physical analysis of the yoghurt

Viscosity

The data regarding the viscosity of yoghurt under various treatments and storage is shown in Graph 4. The data has shown a decreasing trend in the viscosity during the storage. The results for the treatments, storage and the interaction of the treatment and the storage were found to be highly significant.

The viscosity decreased from 4736 to 4473 centipoises (cp) during the 15 days of storage. Minimum value for the viscosity was observed on the 15th day of storage which was statistically less than all others. The values for lactose on the 5th and 10th day of storage for viscosity were 4687cp and 4573cp, respectively. There was a distinct decreasing trend in the viscosity of the yoghurt during the storage.

The data has shown that the maximum value for lactose observed in the treatment T_2. Yoghurt prepared with the encapsulated bacteria showed 4955cp value for the viscosity which was comparatively greater than the other two treatments (T_0, T_1). The yoghurt prepared with free probiotic bacteria and control showed the 4480cp and 4480 cp, respectively.

The minimum value for the viscosity was observed in the control yoghurt (T_o). The statistical data regarding the interaction and storage has shown the decreasing trend in the viscosity during the storage period of 15 days. The maximum value on first day was observed in the yoghurt sample prepared with the encapsulated bacteria. The reason could be due the addition of the encapsulated bacteria, which were encapsulated by using Sodium alginate. Sodium alginate has stabilizing ability. The minimum value on first day was observed in the control yoghurt. On the 5th, 10th and 15th day of storage same trend was observed (Figure 4).

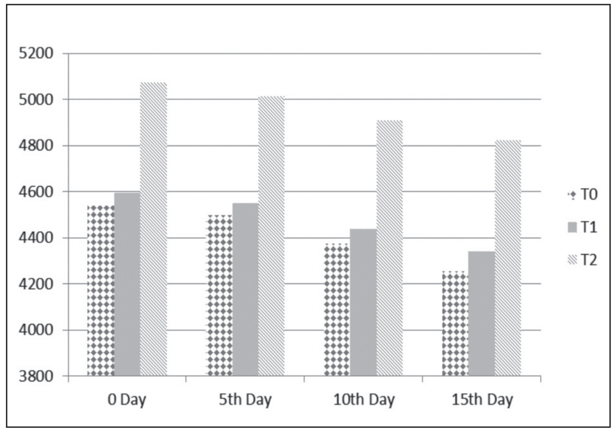

Figure 4. Effect of encapsulated lactic acid bacteria (LAB) on viscosity (cp) of the yoghurt *Syneresis*

The results regarding the syneresis of yoghurt under various treatments during storage are shown in Graph 5. The statistical data has shown the highly significant results for the treatment, storage and the interaction of the treatments and storage. During the storage of the yoghurt syneresis increased. Maximum syneresis was observed at 15th day of storage which was statistically at par with 10th day of storage. The minimum value of 2.02 ml was observed on the first day, on the 5th day of storage the syneresis value was 2.17 ml that was significantly higher than the syneresis value on the first day. There was an increasing trend in the value of syneresis with respect to storage days.

The data for the treatment has shown that the syneresis was affected significantly by the different treatments. Significantly maximum value (2.36ml) for the syneresis was shown by the yoghurt sample produced with the free probiotic bacteria. The reason could be due to the high acid production and proteolysis activity.

Significantly minimum value (2.13ml) was observed in the yoghurt sample produced with the encapsulated bacteria. The reason could be due to the slow acid production by the encapsulated bacteria. The low syneresis could also be due to the Sodium alginate that was used as encapsulating material. Control yoghurt showed 2.20ml value for the syneresis that was less than the yoghurt produced with the free lactic acid bacteria.

Data regarding the interaction of the treatments and storage days found to be significant. Maximum value of syneresis was observed in the interaction of T_1S_4. Which was significantly higher than all other interactions and it was statistically at par with T_1S_3. Significantly minimum value (1.96ml) of interaction of T_2 at first day was observed which was at par with T_0 and first day (Figure 5).

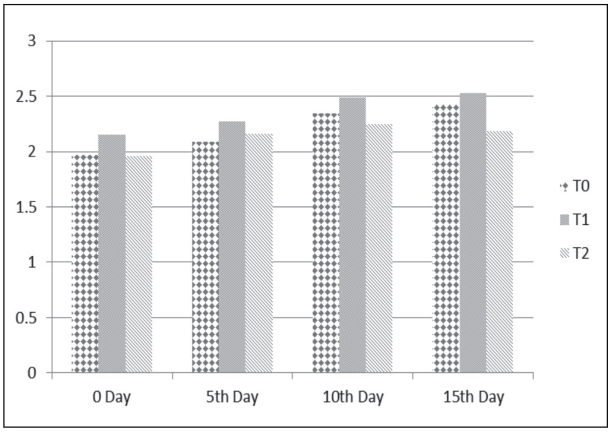

Figure 5. Effect of encapsulated lactic acid bacteria (LAB) on syneresis (ml) of the yoghurt

Microbiological analysis of yoghurt

Total plate count (TPC)

The data regarding the total plate count (TPC) of yoghurt under the various treatments in storage has shown in the Graph 6. The analysis of variance was highly significant for the treatment, storage and interaction. The data showed that the total plate count increased with the passage of time.

Significantly maximum value ($8.9x10^6$) cfu/g was observed on the 15^{th} day of storage and it was statistically at par with the value of 10^{th} day of storage. The minimum value $8.17x10^6$ was observed on the first day of storage and it was significantly less than all other. The result for the total plate count on the 5^{th} day of storage was $8.4x10^6$ that was greater than the value on the first day of storage. With the long storage of the yoghurt it has been investigated that total plate count started to decrease. It could be due to the high acid production. During the storage the acidity increases which becomes undesirable for the lactic acid bacteria.

The data regarding the treatment showed that significantly maximum total plate count was observed in the yoghurt sample prepared with the free probiotic bacteria. The maximum value was $8.9x10^6$ that was for the yoghurt with free (LAB) probiotic bacteria. There was no significant difference in T_0 ($8.43x10^6$) and T_2 ($8.41x10^6$) and both values were significantly lower than control yoghurt (T_1).The data of interaction also showed significant results. Significantly minimum value was observed in interaction of T_0S_1 and it was significantly at par with T_1S_I and T_2S_I at first day and it was also at par with T_0S_4 and T_2S_4 (Figure 6).

Discussions

The reason for the decrease in pH was an increase in the acidity due to conversion of lactose into lactic acid during the storage period. (21) Reported that pH decreased during the storage of yoghurt is due to production of lactic acid. The results are also agreed with (22) that probiotics bacteria with encapsulation are slow acid producer.

The results of the lactose are in accordance with those of work done by (23) who observed a decrease in the lactose content of the yoghurt. The decrease in the lactose content could also be due to the breakdown of sugar metabolized by lactic acid bacteria (24). The result may be compared with the findings of (25) who observed decrease in the lactose content during their studies on the yoghurt.

The results indicated that the acidity increased with the passage of time due to the fermentation and could also be increased due to proteolysis as studied by **(17).** (26) Reported that during the storage period acidity of the yoghurt increased which is due to the excessive sugar fermentation of milk sugar and the presence of lactic acid producing organisms. (27) Found similar results in the titratable acidity when they stud-

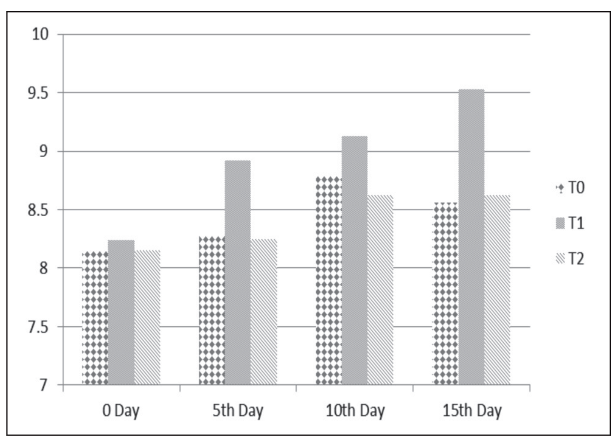

Figure 6. Effect of encapsulated lactic acid bacteria (LAB) on TPC (cfu/ml) of the yoghurt

ied the set type of yoghurt during the storage.

As concerned with viscosity readings are similar to the findings of the (17) who reported a decrease in the viscosity of yoghurt with increase of storage time. The results may be compared with the findings of (28) who concluded decrease in viscosity for plain yoghurt with the passage of storage time. (29) Observed that different type of starter bacteria resulted in change in yoghurt viscosities during storage, implying the role of microorganism in affecting yoghurt viscosity. Starter bacteria have proteases (30), which act on the yoghurt protein matrix over time, resulting in lower viscosities.

The present study increased in the syneresis that could be due to the rearrangement of protein network (31) resulting from change in pH and acidity during the storage of the yoghurt. Similar findings were reported by (32-33) they showed that with the passage of time there is a slight increase in the whey separation of yoghurt during first week of storage but it was increased rapidly during first week of storage. The results of syneresis were in line with the findings of (28-34) who investigated that syneresis in the yoghurt with the passage of time.

Sultana (2000) reported similar results of Total Plate Count (TPC) in her study when she encapsulated probiotics with alginate (35). They found an increase in the total count during the first two week of storage of the yoghurt. They also found that the encapsulation with alginate increase the survival of the bacteria in the acidic conditions. They also found that the addition of the glycerol also helpful for increasing the survival of the probiotics in the yoghurt. During the 8th week storage of the yoghurt they found a decline of 0.5 log (for yoghurt with encapsulated bacteria) and 1 log (with free probiotic bacteria) in the total count of the probiotics. The results are in line with the findings (22**)** who found in his study the survival of free and encapsulated probiotic bacteria in the yoghurt.

Conclusions

As per findings in the studies based on acceptable results in yogurt with encapsulated bacteria, it is concluded that there is a dire need of the application of the encapsulation in the dairy fermented products to prolong the life and health benefits of lactic acid bacteria (probiotics). This would not only be helpful in increasing the survival of the live microorganism but would have good impact on health benefits.

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