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# The survival of probiotic bacteria and sensory properties of yogurt affected by microencapsulation with resistant starch

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ABSTRACT

Probiotics are live microorganisms, which transit the gastrointestinal tract and their benefits to the health can be achieved through consumption of dairy products. In this study, the survival and effect of the probiotic bacteria (*Lactobacillus casei*) in the free and capsulated form in yogurt were aimed to investigate. Two types of yogurt were prepared by free and induced sodium alginate with 2% starch encapsulated *Lactobacillus casei*. The bacterial survival, acidity and sensory attributes were analyzed during storage for 20 days at the refrigerated condition (4˚C). Titration of acidity in yogurt with free *L.casei* cells was higher compared to yoghurt containing encapsulated *L.casei* cells. The viable cell count of *L.casei* in free form for yoghurt production was $2.3\times10^8$ CFU/ml at inoculation time and $10^7$CFU/g after incubation at 42˚C to reach pH 4.5. When *L.casei* was encapsulated in sodium alginate beads, the probiotic survival raised at rate of $1.0^5$ log CFU/g during the same period of storage due to protection by microencapsulation. The results of sensory indices and eventually total score for each sample confirmed the lack of tangible impact by adding encapsulated bacteria in taste, appearance, oral and non-oral tissues. Final scores of probiotic yogurt samples containing free and encapsulated *L.casei* were not statistically different ($p>0.05$). The results indicated that encapsulation with sodium alginate can significantly increase the survival rate of probiotic bacteria in yogurt compared to probiotic yogurt over an extended shelf life.

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## 1. Introduction

Diet is considered one of the major factors contributing to human health. Due to the increasing awareness of the link between diet and health, the demand for healthy foods has increased over the recent decades. On the other hand, the provision and promotion of such products including probiotic foods are one of the key research priorities of the food industry (1,2). Probiotics are live microorganisms and when ingested in sufficiently high levels, exert health benefits on the host. Lactic acid bacteria (LAB) are the most important probiotic known to have beneficial effects on gastro-intestinal (GI) tract and mainly belong to the genera Lactobacillus and/or Bifidobacterium (3,4,5). Several reports have shown that the survival and viability of probiotic bacteria especially bifidobacteria are often low in yogurt and resulted in less than $10^7$–$10^8$ CFU/g daily which is the recommended intake for the health benefits (5,6). This might be improved to some extent, by applying probiotic growth promoting factors, like using micronutrients such as peptides, amino acids, prebiotics and/or encapsulation technique (6). Encapsulation is the technique by which one material or a mixture of materials are coated with or entrapped

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within another material or system (4,7). Microencapsulation protective biopolymers used as coating agents during encapsulation reduce the injury and cell loss by decreasing the influence of the numerous factors such as acid, high temperature, attack by bacteriophages and enable targeted delivery of probiotics to the Colon (1,8). The most common biomaterial used for probiotics encapsulation is alginate. Other supporting biomaterials include carrageenan, gelatin, chitosan, whey proteins, cellulose acetate phthalate, locust bean gum, the mixture of xanthan-gelan, starches and a mixture of xanthan-gelan gum miscellaneous compounds (9,11,12,13). Homayouni et al., and Fahimdanesh et al., in their studies used two types of probiotics (Lactobacillus casei and Bifidobacterium bifidum/lactis) encapsulated with calcium alginate beads and resistant starch as a prebiotic compound. The results showed that encapsulation can significantly enhance the survival of probiotic bacteria during storage. The sensory qualities were improved by the addition of encapsulated probiotic bacteria (14,15). This study aimed to investigate the effect of incorporating microencapsulated probiotic with alginate and starch on the survival of the probiotic cultures in yogurt and the influence on the sensory properties of yogurt was also studied.

## 2. Materials and methods

### 2.1. Bacterial strain, growth conditions, and preparation of cell suspension

The lyophilized culture of *Lactobacillus casei* (Diprox. 543087 lot. 307) and the direct vat starter (DVS) which was composed by *Lactobacillus delbrueckii ssp. bulgaricus* and *Streptococcus thermophilus* were used. *L.casei* was grown in de Man, Rogosa and Sharpe (MRS; Merck, Darmstadt, Germany) broth for approximately 18 h at 37°C. Bacterial cells were obtained by centrifugation (Heraus) at 400 rpm for 20 min and rinsed twice with saline solution (0.9 g of NaCl 100 ml-1) under the same centrifugation conditions. Washed cells were then re-suspended in saline and used either directly (free cells) or in the encapsulation process.

### 2.2. Encapsulation procedure

All glassware and solutions were sterilized at 121°C for 15 min. A 2% sodium alginate mixture was prepared containing 2% Hi-maize resistant starch (Sigma-Aldrich. Lot No. 123K56178) as a filling material and 1% culture of *L.casei* which then was dropped into 300 ml canola oil (sergeant-weltch, Lot No.120962-030-0). Afterward, the mixture was continuously stirred (400 rpm, 20 min) until it was mixed. Then the calcium chloride solution (0.1 M) was added and the generated beads fell into the hardening solution at the bottom of the encapsulation vessel. The mixture was allowed to stand 30 min for further stabilization of the beads. After draining of the oil layer, the collected beads were washed with the saline solution containing 5% glycerol and then stored at 4 °C.

### 2.3. Kinetics of acidification

To evaluate the metabolic activity of encapsulated cells and also the permeability of bead wall, 1 mL of free and encapsulated *L.casei* suspension which corresponded to approximately 2.1-2.3×$10^8$ CFU / mL-1 was inoculated into 100 mL of MRS broth medium. The cultures were then incubated at 37°C until the pH of 4.00 was reached. The pH changes in each culture were monitored at intervals over the test period.

### 2.4. Yogurt preparation

For set-type yogurt production, bovine milk was enriched with 0.5-2.5% (w/v) skim milk powder (fat 2.5%, total solids 12.24%) was heated to 45°C, homogenized under pressure of 200 bar, pasteurized at 90-95°C for 5 min and then cooled to 45°C. After inoculation with the yogurt starter culture (1% v/v), the probiotic cultures were added as free and encapsulated cultures at an initial population of 2.3×$10^8$ CFU $g^{-1}$. The yogurt mix was distributed to 100 mL polyethylene cups, sealed with an aluminum cover and incubated at 42°C until the pH was reached 4.5. Fermentation was stopped by quick cooling of the yogurt containers which were then stored at 4°C for up to 20 days. Microbiological and physicochemical analysis of the final product was conducted after 1, 5, 10, 15 and 20 days of refrigerated storage. At each sampling date, three cups of every treatment were randomly selected to be tested in duplicate. The experiment was carried out in three replicates.

### 2.5. Enumeration of free and encapsulated probiotic bacteria

To release the entrapped bacteria from the beads, 10 g of yogurt sample was mixed with 100 mL of phosphate buffer (0.1 M, pH 7.0) and then homogenized for 10 min in a laboratory stomacher. The samples containing free probiotic cells were treated

similarly to maintain the same treatment conditions. Serial dilutions of the yogurt slurry were then conducted in sterile saline solution and appropriate dilutions were pour-plated into MRS Agar-OX-bile (Merck, Sigma) in duplicate, dishes were incubated aerobically at 37°C for 72 h. the microbiological count data were expressed as log10 of colony forming units per gram of yogurt (CFU $g^{-1}$).

2.6. Physicochemical analysis

The pH values of yogurt samples were measured using a pH meter (Knick- Germany) calibrated with commercial pH 4.00 and 7.00 buffer solutions. For total titratable acidity (TTA), approximately 10g of each sample was diluted with equal volume of water and titrated with a 0.1 N NaOH solution using 3- 4 drops of indicator phenolphthalein to faint pink endpoint persisting 30s. The results were expressed in Dornic degrees (°D). To determine the dry matters, samples (5g) were dried to constant weight in an air oven (Electronic Fater Feb 50 liters) regulated to 102±2°C. Specific gravity was determined using Thermo-lactodensimeter (16).

2.7. Sensory evaluation

Sensory analysis of the yogurt samples containing free or encapsulated *L.casei* was conducted after 2 days of refrigerated storage by a sensory panel composed of ten non-smoker assessors. These panelists were selected based on their ability to recognize four principle tastes and well trained in the sensory evaluation of dairy products. Yogurt attributes including "odor and taste", "mouth feel" and "color and appearance" were scored on an increasing scale ranging from 0 (very poor) to 4 (excellent).

2.8. Statistical analysis

All data collected were analyzed with the Minitab software (version 16). Averages and standard errors were obtained for each stage all three trials (10,17,18).

## 3. Results

3.1. PH and Acidity changes during storage

The pH and acidity changes in the free and encapsulated probiotic yogurts stored at 4 ℃ for a period of twenty days are shown in Fig1 and Fig 2.

3.2. Physiochemical analysis

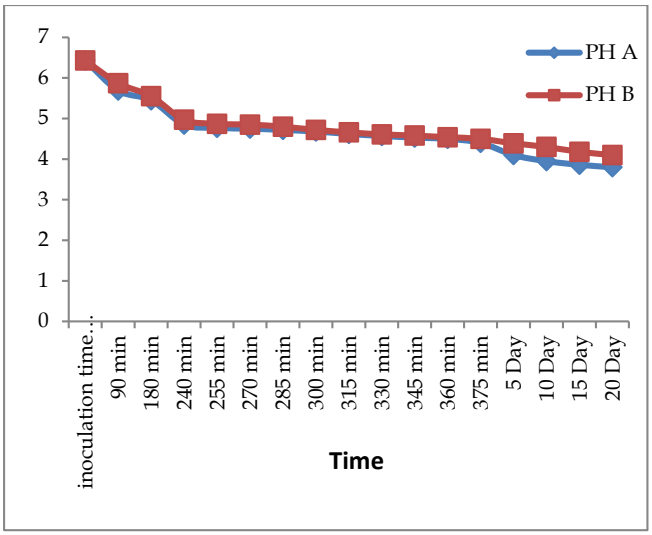


**Fig1.** Changes in pH of probiotic yogurt (A) and encapsulated probiotic yogurt (B)

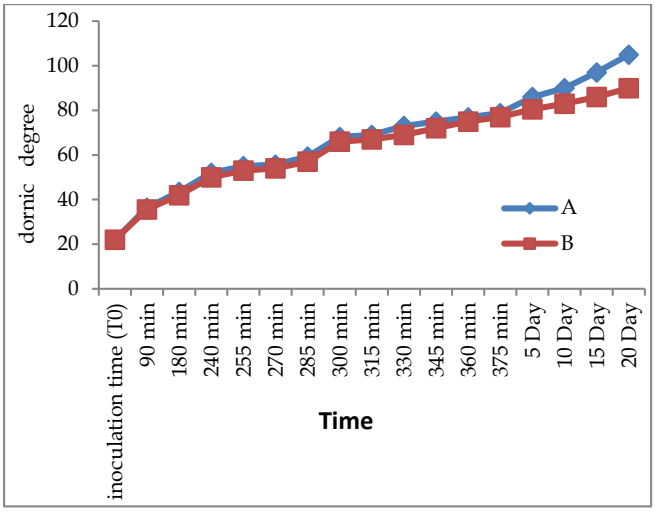


**Fig2.** Changes in acidity of probiotic yogurt (A) and encapsulated probiotic yogurt (B)

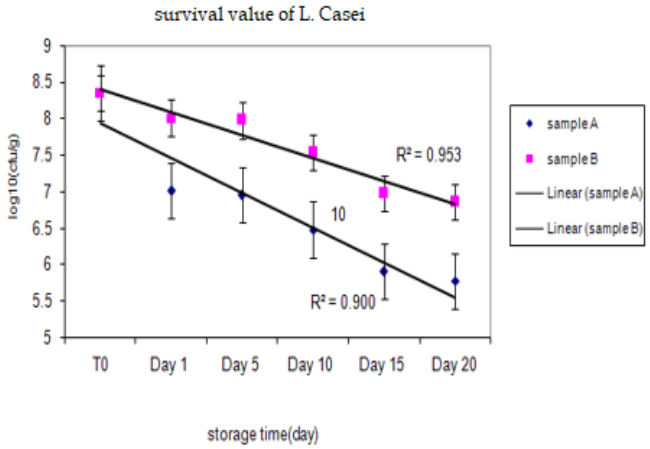


**Fig 3.** Survival of free (sample A) and encapsulated (sample B) *Lactobacillus casei* in yogurt during storage

**Table 1**. Number of free (A) and capsulated (B) *L. casei* in yogurt during 20 days storage (Mean±SE*)

| Storage time | CFU/ml(g) | | S -value (%)** | |
|---|---|---|---|---|
| | Sample A | Sample B | Sample A | Sample B |
| **T0** | 8.36 ± 0.12 | 8 ± 0.12 | | |
| **Day1** (PH 4.5) | 6 ± 0.11 | 8 ± 0.07 | 4.55 ± 0.51 | 44.7 ± 1.7 |
| **Day 5** | 6.96 ± 0.44 | 7.97 ± 0.52 | 4 ± 0.3 | 41.4 ± 3.2 |
| **Day 10** | 6.47 ± 0.35 | 7.54 ± 0.36 | 1.31 ± 0.14 | 15.0 ± 1.9 |
| **Day 15** | 5.90 ± 0.82 | 6.99 ± 0.79 | 0.35 ± 0.024 | 4.24 ± 0.24 |
| **Day 20** | 5.77 ± 0.82 | 6.85 ± 0.37 | 0.26 ± 0.05 | 3.1 ± 0.6 |
| **Determination coefficient ($R^2$)** | 0.9 | 0.95 | | |

*Mean of three replications ± standard error

**S - value: survival value, time required to destroy 1 log of the microorganism.

Physicochemical composition of standardized milk to produce yogurt included fat 2.5 ± 0.1%, pH= 6.55 ± 0.5, total dry matter 12.54 ± 0.1, acidity 16.9 ± 0.7 dornic degree and density 1.035 ± 0.001 g/cm$^3$. Survival of *L.casei* bacteria in free probiotic yogurt (sample A) and encapsulated probiotic yogurt (sample B) during storage until 20 days at refrigerator temperature is shown in Fig 3.

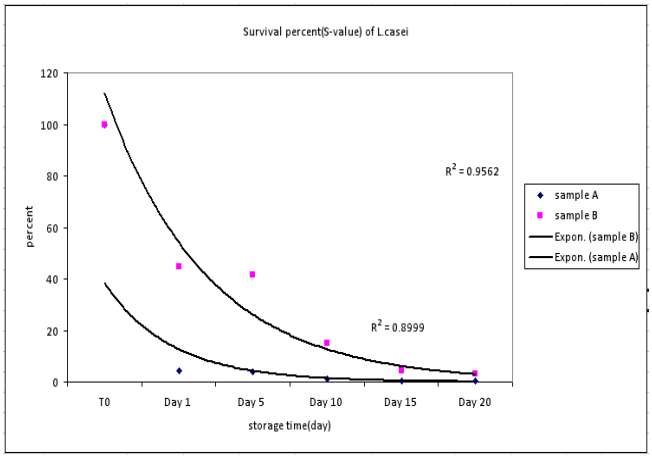


**Fig 4.** Survival of free (sample A) and encapsulated (sample B) *Lactobacillus casei* in yogurt during storage

## 3.3. Sensory evaluation

Sensory evaluation scores of free and encapsulated probiotic yogurt samples for flavor (taste and odor), oral tissue, appearance (color, condition of surface and syneresis) and non-oral tissues (Stirring and spoon vector) are reported in table 2.

## 4. Discussion

The yogurts inoculated with free probiotic bacteria (sample A) and yogurt samples with encapsulated probiotic bacteria reached the pH 4.5 in 360 min and 375 min respectively. It would be due to the lower participation of encapsulated *L.casei* in lactic fermentation as a result of slower intake of nutrients and metabolites release from alginate coated capsules

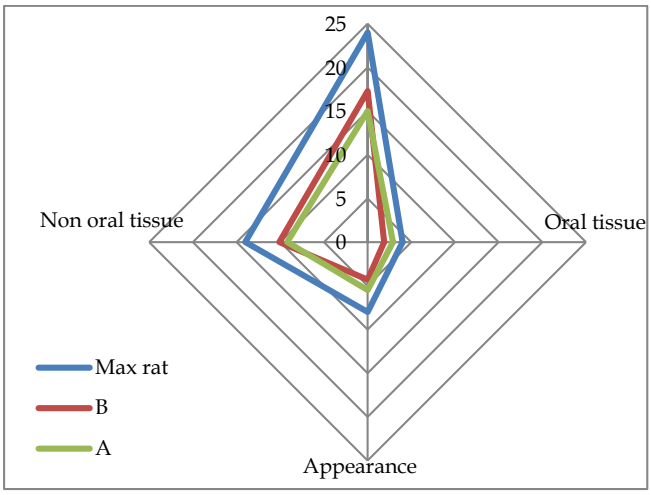


**Fig 5.** Scoring sensory evaluation of probiotic yogurt containing free (A) and encapsulated *L.casei* (B) and resistant starch.

(14). Furthermore, the increasing trend of titratable acidity in samples containing free *L.casei* cells (sample A) compared to samples containing encapsulated *L.casei* cells (sample B) can be seen in Fig 2. Moreover, the acidic activity of both free and encapsulated *L.casei* bacteria in MRS - Broth medium was evaluated for the mean time to reach the pH 4. In the presence of free cells, it took 21 h to reach the pH 4, however, after 21h the pH of the medium reached 6.1 in the presence of encapsulated bacteria and to reach the pH 4 about 49 h was required. This result demonstrates the viability, metabolic activity and acid production of the encapsulated bacterial in alginate (5).

The results showed that in free *L. casei*, the number of bacteria decreased approximately 2.58 log, while the encapsulated *L.casei* bacteria in the similar storage temperature decreased around 1.5 log. Decreasing number of *L.casei* in probiotic yogurt in free and capsulation condition showed a significant difference ($p<0.05$) compared together, which shows the influence of microencapsulation on the survival of *L. casei*. In several studies, encapsulated probiotic bacteria

**Table 2.** Mean sensory scores of probiotic yogurt containing free (A) and encapsulated (B) *L.casei* and resistant starch.

| Attributes | Average initial scores | | Important factor | The final score (of 50) | | Maximum rating |
|---|---|---|---|---|---|---|
| | A | B | ISIRI 695 | A | B | |
| **Flavor (taste and odor)** | 2.5 | 2.9 | 6 | 15 | 17.3 | 24 |
| **Oral tissue** | 2.6 | 2.9 | 3.5 | 9.2 | 10.1 | 14 |
| **Appearance (color, condition of surface and Syneresis)** | 2.8 | 2.1 | 2 | 5.5 | 4.3 | 8 |
| **Non-oral tissues (blink and spoon vector)** | 2.9 | 1.9 | 1 | 2.9 | 1.9 | 4 |
| **Final score of 50** | | | | 32.6 | 33.4 | 50 |

especially *L.casei* in yogurt promote survival during the storage period, while the number of probiotic bacteria has been reported to decrease after reaching pH 4.5 (2,8,19). However, this study showed that in probiotic yogurt with encapsulated *L.casei* in sodium alginate and resistant starch, the survival of the bacteria was observed approximately up to 40 % more than that of with free *L.casei*. Many of probiotic bacteria are sensitive to PH below 4.6. Alginate covers a protective layer to protect the bacteria in pH below 4.5, therefore it prevents reduction of the number and retards the bacterial death (19). S-value is an important indicator subjected to the viability of bacteria and the equivalent time required for the loss of the initial number of bacteria to one log. S-value for free and encapsulated *L.casei* bacteria during storage time is shown in table 2. S-value comparison of free and encapsulated bacteria in first, fifth, tenth, fifteenth and twentieth days indicates a significant difference ($p<0.05$) related to the impact of pH on the viability of probiotic bacteria. Fermentation by starter bacteria makes the yogurt pH begins to drop during incubation at 42°C. The most significant step in the death of *L.casei* cells was recorded during the fermentation process and pH drop on the first day.

Therefore, the pH reduction is one of the main causes of injury and death for *L.casei* bacteria during fermentation at 42˚C. The injury and death of probiotic cells at this stage, are most likely caused by the starter bacteria activity and their interaction with *L.casei* bacteria, as well as low pH resulted from fermentation. In this regard, the resistance of *L.casei* against these threats in capsulated form is significantly higher than free *L.casei* ($P<0.05$). As seen in Fig 4, the viability rate after reach to pH 4.5 in the first day for free bacteria was 4.5% of the initial number and for encapsulated *L.casei* it was 44.8% which until the end of the storage period (20 days) at 4˚C it decreased to 0.26% and 3.1% respectively. This result confirms that capsulated *L.casei* with sodium alginate accompanied by corn resistant starch promotes the survival of the bacteria from the first day during fermentation and also during storage at 4˚C.

The results of sensory indices and eventually total score for each sample confirmed the lack of tangible impact by adding encapsulated bacteria with sodium alginate blend cornstarch in taste, appearance, oral and non-oral tissues. Final scores of probiotic yogurt samples containing free and encapsulated *L.casei*, were recorded as 32.6 and 33.4 out of 50 respectively that were not statistically different ($P>0.05$).

## 5. Conclusion

This study showed that the protecting capsule can significantly improve and promote the viability of probiotic bacteria in yogurt. Milk fermented products such as yogurt can be used as good carriers for the delivery of probiotic bacteria in the human gut. In free *L.casei* cells, the bacterial number was lowered by 1 log in the 1st day, 2 log in the 5$^{th}$ day and 3 log in the 15$^{th}$ day of storage respectively. However, in the case of the encapsulated cells in the 5$^{th}$ day only 1 log and until the end of the storage period, only 2 logs of the bacterial count was reduced. The final number of encapsulated *L.casei* bacteria in the last day was $7\times10^6$ CFU/g, while for free bacteria it was recorded $6\times10^5$ CFU/g. The number of encapsulated bacteria during storage was relatively satisfactory. In case of appropriate and effective encapsulation of probiotic bacteria used in

yogurt production, their count can be maintained to the proper levels at the end of product shelf life.

**Conflict of interest**

The authors declare that there was no conflict of interest.

**Acknowledgments**

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**References**

1. Semyonov D, Ramon O, Kaplun Z, et al. Microencapsulation of *Lactobacillus paracasei* by spray freeze drying. Food Res Int 2010; 43:193–202.
2. Sahadeva RPK, Leong SF, Chua K H, et al. Survival of commercial probiotic strains to PH and bile. Int Food Res J 2011; 18:1515-1522.
3. Lone KD, Dhole JA. Formulation and evaluation of probiotic tablets containing anti-inflammatory drug. Int J Pharma Sci & Res 2013; 4: 341-346.
4. Moayednia N. Quality evaluation of new developed symbiotic yoghurt over the storage at refrigerator. J Food Biosci & Technol 2014; 4:57-64.
5. Shafiei Y, Razavilar V, Javadi A, et al. Survivability of free and microencapsulated *Lactobacillus plantarum* with alginate and resistant starch in simulated gastrointestinal conditions. J Food, Agri & Environ 2012; 10:207-212.
6. Subbaiah BV, Krishnamoorthy B, Muthukumaran M. Importance and characterization of microencapsulation techniques in pharmaceutical dosage forms - A review. Int J Pharm & Technol 2012; 4:2332-2346.
7. Vivek KB. Use of encapsulated probiotics in dairy based foods. Int J Food, Agri & Vet Sci 2013; 3:188-199.
8. Sultana K, Godward G, Reynolds N, et al. Encapsulation of probiotic bacteria with alginate-starch and evaluation of survival in simulated gastrointestinal conditions and in yoghurt. Int J Food Microbiol 2000; 62:47-55.
9. Lone KD, Dhole JA, Borhade PS. Bio encapsulation of probiotic bacteria by direct compression method. Int J Pharm & Chem Sci 2013; 2:1400-1402.
10. Iyer C, Kailasapathy K. Effect of co-encapsulation of probiotics with prebiotics on increasing the viability of encapsulated bacteria under in vitro acidic and bile salt conditions and in yoghurt. Food Microbiol & Safe 2005; 70:18-23.
11. Hassan A, Nawaz Ch M, Rasco B. Microencapsulation, survival and adherence studies of indigenous probiotics. African J Microbiol Res 2014; 8:766 -775.
12. Amin T, Thakur M, Jain SC. Microencapsulation-the future of probiotic cultures. J Microbiol Biotechnol & Food Sci 2013; 3:35- 43.
13. Gildas K, Vandamme GT. Probiotic encapsulation technology: from microencapsulation to release into the gut. Pharmaceut 2012; 4:149-163.
14. Homayouni A, Azizi A, Ehsani MR. Effect of microencapsulation and resistant starch on the probiotic survival and sensory properties of symbiotic ice-cream. Food Chem 2008; 111:50-55.
15. Fahimdanesh M, Mohammadi N, Ahari H, et al. Effect of microencapsulation plus resistant starch on survival of *Lactobacillus casei* and *Bifidobacterium bifidum* in mayonnaise sauce. African J Microbiol Res 2012; 6:6853-6858.
16. Arora R, Bhojak N, Joshi R. Comparative aspects of goat and cow milk. Int J Engin Sci Invent 2013; 2:7-10
17. Chan ES, Zhang Z. Bioencapsulation by compression coating of probiotic bacteria for their protection in acidic medium. Process Biochem 2005; 40:3346-3351.
18. Chen KN, Chen MJ. Optimization of incorporated prebiotics as coating material for probiotic microencapsulation. Journal of food science 2005; 70:260-266.
19. 19. Viability of probiotic cultures from yoghurt samples randomly selected from South African retail stores. University of Pretoria, Chapter 2:58-71.