In vitro studies on survival of encapsulated *Bifidobacterium* animalis sub sp lactis in yogurt and simulated gastric acidic condition

Sivakaran, M.[1]*, Ghoddusi, H.[2]

[1] *Home Economics, Faculty of Arts, University of Jaffna, Sri Lanka*
[2] *School of Human Sciences, Faculty of Life Science and Computing, London Metropolitan University, United Kingdom*
**jmenakaj@yahoo.com*

Abstract - Probiotics are the cultures of live microorganisms which, when administered in adequate amounts confer a health benefit on the host. Fermented dairy products are considered as a suitable vehicle to carry probiotics where yogurt is the most common vehicle for incorporating probiotics. However the survival of probiotics can be affected by the food processing conditions as well as the low acidic environment of the gut. Encapsulation of probiotic bacteria can be considered as a suitable method to preserve the probiotics until they reach their site of action. The study was conducted in order to evaluate the effect of encapsulation on the survival of probiotic bacteria in acidic condition. *Bifidobacterium* animalis sub sp lactis (BB12) was encapsulated with sodium alginate and resistant starch and their survival was determined in yogurt as well as in simulated gastric condition. BB12 was mixed with 10 ml of 18g/L sodium alginate and 10 ml of 20g/L resistant starch and dropped in to sunflower oil in order to make encapsulated beads. Calcium chloride solution was used to harden the beads. pH change and titratable acidity of the yogurt added with encapsulated beads were measured. The number of encapsulated BB12 in yogurt declined by about 1 log10 CFU/ ml over the twelve days of storage, while the non-encapsulated bacterial viability declined by about 2 log10 CFU/ ml. The encapsulation of BB12 showed 1 log10 CFU/ ml decline over three hours of exposure to simulated gastric condition (pH 2.0, 3.0 and 4.0) where the non-encapsulated bacteria showed 2 log10 CFU/ ml decline for pH 3.0 or 4.0 at the same time 3 log10 CFU/ ml was observed for pH 2 treatment. A successful encapsulation was made using alginate and starch, but in overall the survival rate of encapsulated and free bacteria did not show any statistical difference.

Key words: BB12, Bifidobacterium, encapsulation, probiotics, yogurt

I. INTRODUCTION

Probiotics are live microorganisms which give health benefits and improve properties of indigenous microbiota. The benefits are by means of suppressing the growth of pathogens, prevention of diarrhea constipation and allergies and nutrient synthesis. To accomplish the positive health effects the probiotics should reach their sites of action alive as well as they have to establish themselves in certain numbers [1]. Probiotics are more stable in dairy products compared to non-dairy products. Yogurt is the most common fermented dairy product added with probiotics while kefir, butter milk and cheese are the other fermented dairy products enriched with the probiotics in the market. Lactobacillus and Bifidobacterium are the predominant groups of probiotics contain numerous strains which are added to dairy food products [2].

Viability of probiotics should be maintained throughout the storage period as well as they must survive in the gut environment. Low pH, acidification during storage, storage temperature, production of hydrogen peroxide and permeation of oxygen through the packaging material causing the toxicity can lead to poor survival in foods [3].

Lack of protection can cause reduced survival of probiotics during the gastric passage due to its low pH in the host, thereby reduces their number reaching the colon. Encapsulation technique is used in food industries as the encapsulated materials give protection against moisture, heat and other extreme conditions. Encapsulation is a better method to increase the survival of bacteria and delivery of them into their site of action in higher number. Alginate is a natural polysaccharide extracted from brown seaweed, can form a gel matrices around the bacterial cells. It can be accepted due to low cost, ease of handling, non-toxic nature, high porosity and tolerance to salt and chelating agents. Starch, prebiotic acts as an additional protectant to the bacterial cells and enhances the survival of bacteria in the encapsulation. The alginate starch combination is preferred mostly as they do not interfere with the probiotic homeostasis [4].

II. MATERIALS AND METHODS

1. Encapsulation procedure

Harvested BB12 were centrifuged at 6500 rpm for 10 minutes and were washed with saline solution. 10 ml of 20g/L starch solution and 10 ml of 18g/L sodium alginate solution were mixed with the washed bacterial cells. The oil mixture (100 ml of sunflower oil +1 ml of Tween 80) was tempered to 40 oC for 2 minutes on a stirrer hotplate. The alginate, starch, bacterial cell mixture was drawn in a sterile syringe fitted with 21G needle. The mix was dropped in to the oil by keeping a same distance between the oil and the needle all the time in order to get uniform size of beads. 100 ml calcium chloride solution was added along the walls of the beaker to harden the beads. The oil was removed after 20 minutes and the beads were washed with calcium chloride with 5% of glycerol. The beads were preserved in 1M CaCl2 solution at 4 oC [5].

2. Preparation of yogurt
Sterilized skim milk (500 ml) was warmed to 40 oC and semi skimmed milk powder (10g) was added. Heating was carried out at 80 oC for 30 minutes. After cooling the milk to 40 oC, X11 started culture was added and allowed to ferment at 30 oC in Duran bottles. After reaching desired pH, yogurt samples were divided into three groups and the first group was added with encapsulated beads, second with a loop full of free probiotic bacteria and last group was set as control at 4 oC.

3. Study of encapsulated and free probiotic bacteria in acidic conditions
In combination with the selective supplement Lithium-mupirocin the TOS propionate agar allows the direct enumeration of viable BB12 in yogurt [3]. The pH, titratable acidity (Fadelaet al., 2008) and the survival of the bacteria (Kailasapathy, 2006) were tested on day 0, 3, 6, 9 and 12. Hourly survival study was carried out for the encapsulated and free bacteria for 3 hours (pH 2.0, 3.0, 4.0).

4. Data analysis
Data (obtained from three replicates of samples) were analyzed using ANOVA using Minitab16 statistical package at 95% of confidential interval.

III. RESULTS AND DISCUSSION

Spherical shaped, single separated beads were obtained after encapsulation (Figure 1). Average weight of a bead was 5.4+0.3 mg.

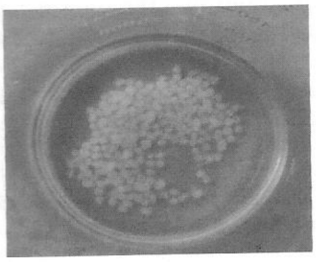

Fig. 1: Encapsulated BB12 beads in 1MCaCl2 solution

Starch plays a major role in the nourishment of the probiotic as a prebiotic. It synergistic with alginate and give more protection to the cells.

1. Survival of encapsulated and free bifidobacteria in yogurt
The survival study could have carried out in a commercial yogurt but many of the commercial plain yogurt also contain some probiotics, including bifidobacteria, Thus,the enumeration results would not be reliable. The yogurt was prepared using sterilized milk inoculated with X-11.

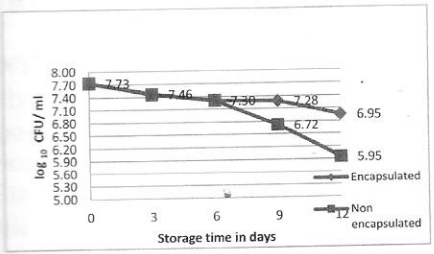

Fig 2: Survival of encapsulated and free BB12 for 12 days of storage

Survival of encapsulated BB12 in the yogurts showed 1 log10 cycle decrease while non-encapsulated BB12 decreased by 2 log10 CFU/ml after 12 days of storage. However, the difference was not significant (p>0.05). Perhaps a longer monitoring period could have revealed the faster decrease in the viability of free bacteria which is showed in several studies such as storage at more than 6 weeks [7].

3. pH changes during the storage of yogurt

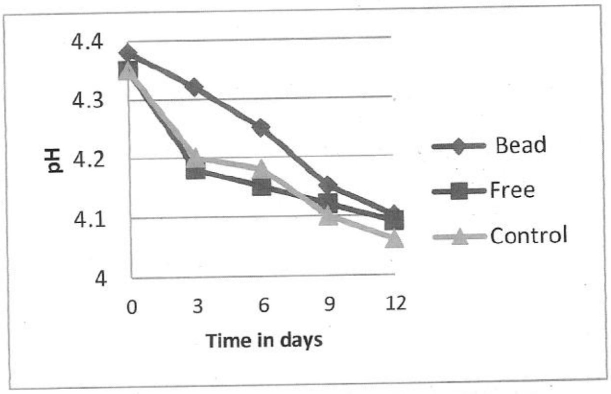

Fig 3: pH change of the yogurt added with BB12 over 12 days of storage

The rate of pH decline of yogurt containing encapsulated BB12 was lower than the rate of pH decline of control or free BB12 added yogurt. The encapsulated cells have a barrier to absorb the nutrition as well as release acid [1].

4. Titratable acidity changes during storage of yogurt
The yogurts added with the encapsulated beads showed the slow increase in the acidity.

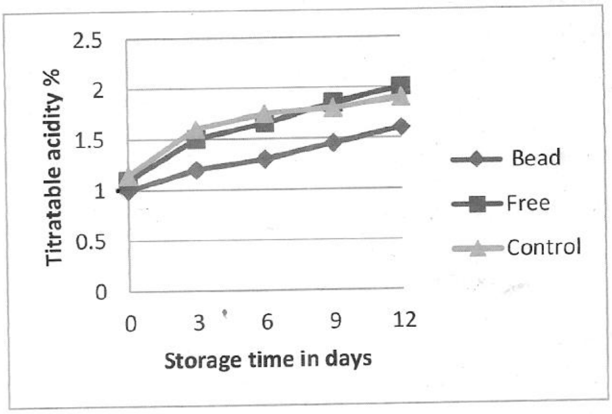

Fig 4 : Titratable acidity change of the yogurt added with BB12 over 12 days of storage

Yogurt samples added with the encapsulated bacteria showed a little higher pH and lower titratable acidity compared with the other treatments on the initial day (day 0). This may be due to the preservation of beads in the CaCl2. There was a change in pH and titratable acidity observed even in the control yogurts which were not added with any form of probiotics. Because they had the yogurt starter culture bacteria which can convert lactose into lactic acid and show a noticeable change in pH [6].

5. Survival of encapsulated bacteria in simulated high acidic gastric conditions

Solid foods remain in the stomach for 2-4 hours and liquid foods spend 20 minutes before emptying the stomach. The foods need to pass an adverse pH condition through the gastro intestinal tract. The probiotic bacteria must survive when they pass through the digestive tract and reach the colon in large number. Effect of pH on the free and encapsulated bacteria was monitored over 3 hour period to evaluate the changes in viability of BB12 in simulated conditions relevant upper gastro intestinal track.

Table I: Survival of encapsulated and free BB12 in simulated gastric acidic condition

pH	State of the bacteria	$\log_{10}$ CFU/ml BB12 at each hour of incubation			
		0	1	2	3
2.0	Beads	6.45	6.28	5.53	5.48
	Free	6.79	4.00	3.48	3.26
3.0	Beads	6. 53	6.26	5.66	5.38
	Free	6.04	5.38	4.52	4.40
4.0	Beads	6.85	6.00	5.81	5.48
	Free	6.64	5.86	5.49	4.36

Three hours exposure of the bacteria to high acidic condition resulted in 1 log10 CFU/ml decline in the survival of encapsulated BB12. Free BB12 showed 2 log10 CFU/ml decrease in their survival when exposed to pH 3.0 and pH 4.0 for 3 hours. But 3 log10 CFU/ml decline was observed for the free BB12 tested at pH 2.0 for 3 hours which showed a statistical difference with the treatment of encapsulated BB12 ($p = 0.03$) The decrease in the viable population of free BB12 is similar to the findings by Annanet al., 2008 [8] for B. adolescentis. The survival of encapsulated and free BB12 in pH 3.0 and pH 4.0 did not show any statistical difference ($p>0.05$).

6. Improvements and suggestion

The survival study can be carried out by increasing the storage time where the number of non -encapsulated bacteria may decline and show a significance difference in the viable count compared with the encapsulated bacteria.

IV. CONCLUSION

Encapsulation is a suitable way to protect the probiotics in acidic foods like yogurt. BB12 strain was successfully encapsulated with sodium alginate and starch. The encapsulation showed retention of survival rate of encapsulated bacteria but sthe survival rate of encapsulated and free bacteria did not show any statistical difference for twelve days of storage. Encapsulation protected the BB12 to survive at pH 3.0 and 4.0.

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