

## Impact of Banana Puree on Viability of Probiotic Soy Yoghurt During Storage

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The present investigation was made with an attempt to manufacture banana based probiotic soy yoghurt with addition of probiotic culture and banana puree, were examined. Probiotic soy yoghurt samples were produced from soy milk fermented with yoghurt and probiotic culture i.e. *Streptococcus thermophilus* NCDC-074, *Lactobacillus delbrueckii* ssp. *bulgaricus* NCDC-009, *Lactobacillus acidophilus* NCDC-15 and *Bifidobacterium bifidum* NCDC-235. The viability (SPC) of these four strains were analyzed during storage at refrigerated temperature with the selective media of MRS, ST, MRS-sorbitol and MRS-maltose agar. These counts generally decreased during the storage period. Supplementation of banana fortified soy yoghurts with 5, 10 and 15% of banana puree, improved the viability of probiotic bacteria during storage period. However, the yoghurt supplemented with banana puree, at 15% level, showed highest viability ( $10^6$  cfu/g) among all treatment combinations up to four weeks of refrigerated storage.

**Key words:** Soy yogurt, Banana, Storage.

This research aims to expand soybean and banana utilization by developing new product banana based probiotic soy yogurt fermented by typical yoghurt bacteria and probiotic bacteria (*Lactobacillus acidophilus* and *Bifidobacterium bifidum*). These products are particularly popular among children, aged people and health conscious consumers (Garcia *et al.*, 1998; Corte, 2008). The therapeutic dietetic properties of fermented dairy foods have been attracting the human population since ages.

Fermented dairy foods have long been considered safe and nutritious. The beneficiary role of yoghurt may be further enhanced by the supplementation of *Lactobacillus* and *Bifidobacterium* sp., resulting in a product termed AB yoghurt (Shah, 2000). *L. acidophilus*, and *Bifidobacterium* sp. are considered probiotic organisms since they are believed to exert beneficial health effects in the host by modulating the intestinal microflora (Schrezenmeir and De

Vrese, 2001). A number of health benefits have been proposed including antimicrobial, antimutagenic, anticarcinogenic and antihypertensive properties, and reduction in serum cholesterol, alleviation of lactose intolerance, and reduction of allergic symptoms (Schrezenmeir and De Vrese, 2001; Lourens-Hattingh and Viljoen, 2001).

Probiotic organisms are 'live microorganisms which, when consumed in adequate amounts, confer a health benefit on the host' (FAO/WHO, 2002). To observe a positive health effect of their consumption, a minimum level of live microorganisms is required; this level, depending on the strains used and the required health effect, is usually between  $10^8$  and  $10^{11}$  cfu/d (Vanderhoof and Young, 1998). Therefore, assuming a daily consumption of fermented dairy products of 100 g, they should contain between  $10^6$  cfu/g to  $10^9$  cfu/g of these live bacteria at the time of consumption. While this is the case for a limited number of strains, the precise mechanism by which probiotic organisms exert a health effect in vivo is not clearly understood. One aspect that is clear, however, is that some strains produce certain

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health-promoting metabolites (including proteins and fatty acids) which are desirable from a nutritional and/or physiological perspective. However, it should be emphasized that the ingestion of probiotic fermented foods opens up the possibility that these health-promoting metabolites may also be produced in vivo (Tamime, 2005).

Soybeans are less expensive and more plentiful than bovine milk. Moreover, soymilk has no cholesterol. Soy based products have already been developed which compete favorably with dairy products. Soy yogurt can command a market on the strength of being cholesterol free. Soy products contain the isoflavone phytoestrogens with potential anticarcinogenic activity (Wolfe *et al.*, 2003).

On the other hand, soymilk, which serves as a base for a variety of beverages, contains indigestible oligosaccharides such as raffinose and stachyose, which are usually associated with stomach discomfort and flatulence (Rackis *et al.*, 1970). Desai *et al.*, (2002) reported high concentrations of flatulent sugars (raffinose, stachyose) in soy yogurt because the yogurt bacteria cannot utilize these sugars. Some strains of *Bifidobacterium* are able to reduce the concentration of raffinose and stachyose, eliminating the potential cause of flatulence, and also decrease the levels of pentanal and n-hexanal responsible for the beany flavour (Desai *et al.*, 2002; Tsangalis and Shah, 2004).

Prebiotics nourish Probiotic bacteria and yeasts so that they can grow faster. Banana content of insoluble and soluble dietary fibers (DF), pectin and fructooligosaccharides. These prebiotics are in fact able to selectively stimulate the growth and activity of the gut microbiota, particularly lactobacilli and bifidobacteria (Davis and Milner, 2009). FOS are dietary fibers that help keep the stomach and bowels healthy. They do this by nourishing and promoting the naturally present, "friendly" bacteria (*Bifidobacteria* and *Lactobacilli* in particular) capable of warding off infection in the digestive system. Because of these properties FOS is considered a "Prebiotic". The dietary intake of fibers and probiotics exert a positive impact on the development of the intestinal micro-biota and are reported to relieve constipation and reduce the incidence of colon cancer

(Farnworth, 2008).

Some foods naturally contain prebiotics. Examples are wheat, chicory, onions, bananas, garlic, asparagus, Jerusalem artichokes and leeks, although the amount of prebiotics in these foods varies caseament P. A. (1999) and Roberfroid M. B. (2000).

The combination of soy and prebiotics may lower cholesterol and boost heart health. Banana (*Musa sp.*, Musaceae) is a good sources of prebiotics, having 1% prebiotic fiber, 22% sugar, no harm in eating a banana and the soybean is an always typical source of prebiotics. Banana by product contains around 43–49 g of total dietary fiber, 1 g of inulin, 6 g of fructo-oligosaccharide and 10–20 g of pectin per 100 g of dry matter, in addition to significant amounts of  $\pm$ -linolenic acid (ALA), essential amino acids and micronutrients such as Mg, K, P and Ca (Emaga *et al.*, 2007, 2008; Mohapatra *et al.*, 2010).

Throughout the world, probiotics and prebiotics are leaders in sales in the functional foods category. Prebiotics are nonviable food components that exert a benefit on the health of the host, associated with modulation of the intestinal flora (FAO/WHO, 2007). On the other hand, probiotics are live microorganisms, administered in quantities adequate to confer health benefits (FAO/WHO, 2001). Synbiotic may be defined as the combination of probiotics (the live bacteria) and the prebiotics (the food components they live on), being mainly used because a true probiotic, without its prebiotic food source does not survive well in the digestive system (Panesar *et al.*, 2009). Functional foods are products that have been enriched with added nutrients or other substances that are considered to provide health benefits over and above their nutritional value. This term covers a broad range of products: typical examples are probiotic yogurts, cholesterol-lowering spreads, and oligosaccharide-added foods (Williamson, 2009).

This study aims to formulate high protein, synbiotic action and acid production in probiotic soy yoghurt which add health benefits and value addition by incorporation of banana puree and probiotic bacteria. In the addition of probiotic bacteria in to the products provides an extra beneficial aspect to the product and therapeutic value.

## MATERIALS AND METHODS

This study was carried out at “Warner School of Food and Dairy Technology, Sam Higginbottom Institute of Agriculture, Technology and Sciences, Allahabad, U.P., India”, with appropriate methodology.

Fresh mixed milk and fresh cream (50%) was obtained from the “Student’s Training Dairy” WSFDT, SHIATS, Allahabad. Skimmed milk powder (brand name ‘Anikspray’), sugar, soybean and banana were procured from local market in Allahabad, U.P., India. Freeze dried Yoghurt culture (*S. thermophilus* NCDC 074 and *L. bulgaricus* NCDC 009) and Probiotic culture (*L. acidophilus* NCDC 15 and *B. bifidum* NCDC 235) which has proven therapeutic benefits (Reddy *et al.*, 2006), were purchased from the National Dairy Research Institute (NDRI), Karnal, Haryana, India.

### Soy milk preparation

100 g of hand washed soybeans were soaked for overnight in 1,000 ml of tap water. After that 0.5% sodium bicarbonate added and stand for 2 hours at 50°C, then blanched with the same carbonated water for 4 minutes. The soybeans were immediately separated and circulated over by tap water. The beans were blended in a high speed blender with 1,000 ml hot water (90°C) for 3 minutes. The mix was filtered with 3 sheets of cheese cloth then boiled for 10-15 minutes. The filtered mixed with 11% nonfat dry milk at the ratio of 80:20 (final concentration of 2.2%) for better fermentation. For soy yoghurt manufacturing, heat treatment of soymilk is usually carried out at 95-100°C for 30 min for soymilk (Liu, 1997).

### Preparation of Soy yoghurt samples

Soy yoghurt mix were standardized to 4.0 % fat, 12% serum solids, 6% sugar and total solids adjusted to 22.06 %. The soy yoghurt mix was prepared by dissolving cream and skim milk powder (SMP) in soy milk at 60°C for adjusting of fat and solid not fat (SNF) respectively. The mix was homogenized and then pasteurized at 85°C for 30 min followed by cooling (37°C) and aseptically inoculating with 0.5% (v/v) of each strains *L. bulgaricus* NCDC 009, *S. thermophilus* NCDC 074, *L. acidophilus* NCDC15, and *B. bifidum* NCDC235, after that incubated at 37°C until the pH of 4.9 is reached. The fermentation time ranged between 8 to 12 hrs for probiotic soy yoghurt. The pH of soy

yoghurt samples were recorded using (Eutech and Oakton instruments, Malaysia) digital pH meter. The banana puree stirred at 0, 5, 10 and 15% with plain yoghurt T<sub>0</sub>, T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> respectively and stored at 4°C for during 28 days.

The microbiological analysis i.e. Std. Plate Count (SPC) of Banana Based Soy Yoghurt were estimated by using standardized Procedure of laid down in I.S(1847) part 2 and manual of dairy bacteriology, ICAR publication (1972).

### Microbiological analyses

The count of *S. thermophiles*, *L. bulgaricus*, *L. acidophilus* and *B. bifidum* in to the formulated soy yoghurt was evaluated on 1<sup>st</sup>, 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup> and 28<sup>th</sup> day. One ml sample was taken from each yogurt sample for serial dilution. Serial tenfold dilutions were prepared in a solution of 0.9% NaCl (w/v) and 0.1% (w/v) bactopectone and suitable dilutions were plated on appropriate media. *L. bulgaricus* were enumerated on Lactobacilli MRS agar, when the incubation is carried out at 45°C for 72 h. *S. thermophiles* were enumerated on ST agar under aerobic incubation at 37°C for 24 h. For determining *B. bifidum* by differential counts between *L. acidophilus* enumerated on MRS-sorbitol agar and the total counts of *L. acidophilus* and *B. bifidum* obtained from MRS-maltose agar were done by Dev and Shah (1960); Shah (2000).

### Statistical analysis

A data of the samples were investigated for microbiological on 1<sup>st</sup>, 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup> and 28<sup>th</sup> day. All analyses were conducted twice. Data obtained from analysis of the samples were evaluated by variance analysis, and the differences among means were detected by Duncan’s multiple range tests (SPSS 1999).

## RESULTS AND DISCUSSION

### Growth of bacterial culture

Fig. 1 shows the growth pattern that occurred when the yogurt producing bacteria *S. thermophilus* and *L. bulgaricus* were together with probiotic bacteria *L. acidophilus* and *B. bifidum* inoculated into the soy yoghurt. Twelve hrs were required to reach a pH of 4.3. These four strains used in this study grew well in the banana based soy yoghurt and their population stabilized 10 hrs incubation period.

### Microbial counts

The differentiation of *S. thermophiles*, *L. bulgaricus*, *L. acidophilus* and *B. bifidum* has been a problem in cultured dairy foods. The difficulty of cultivating bifidobacteria in milk, because of lack of acid tolerance or oxygen sensitivity, was not encountered in these experiments. In this study, yoghurt and probiotic bacteria grew to high numbers in banana based soy yoghurt during storage period (4°C). Even the high level of growth of all those bacteria when a high percentage (15%) of banana was used. Insufficient growth occurred with 5 and 10% banana. The total colony counts after fermentation of the soy yoghurt to pH 4.9 were  $4 \times 10^8$  cfu/g for all types of bacteria. Initial freezing of the soy yoghurt in the freezer at 4°C for 4 weeks caused a reduction of less than three log cycle in total colony counts.

The *L. bulgaricus*, *S. thermophiles*, *B. bifidum* and *L. acidophilus* count (log<sub>10</sub> cfu/g) of

formulated soy yoghurt during storage period (Table. 1) showed a significant difference. The growth of all four bacteria was better in soy yoghurt supplemented with 15% banana. This might be due to the growth promoting effect of prebiotics. There was a significant difference in the count of *L. bulgaricus*, *S. thermophiles*, *B. bifidum* and *L. acidophilus* between different treatments during storage period at 4°C.

On 7<sup>th</sup> day of storage at 4°C, *S. thermophilus* was at a level of  $1 \times 10^8$  cfu/g, *L. bulgaricus* was at  $5 \times 10^7$  cfu/g, *B. bifidum* was at  $1 \times 10^7$  cfu/g and *L. acidophilus* was at  $8 \times 10^7$  cfu/g. Whereas, on 28<sup>th</sup> day of storage at 4°C, the *S. thermophilus* decreased by two log cycles to  $3 \times 10^6$  cfu/g, *L. bulgaricus* decreased by two log cycle to  $1 \times 10^6$  cfu ml, *B. bifidum* decreased by three log cycles to  $3 \times 10^5$  cfu/g, *L. acidophilus* decreased by two log cycle to  $1 \times 10^6$  cfu/g viability in the soy yoghurt presented in Table 1 and Figure 1.

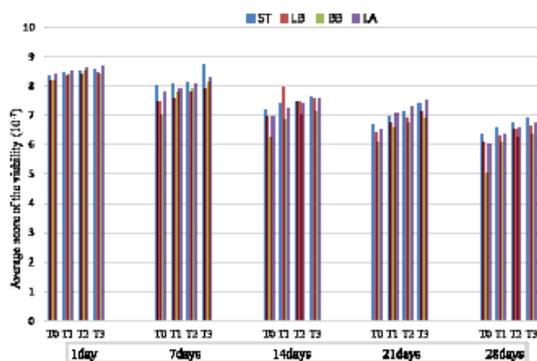
With regard to the *S. thermophiles* counts,

**Table 1.** Average score of the viability ( $10^{-7}$ cfu/g) of bacterial culture in banana based soy yoghurt

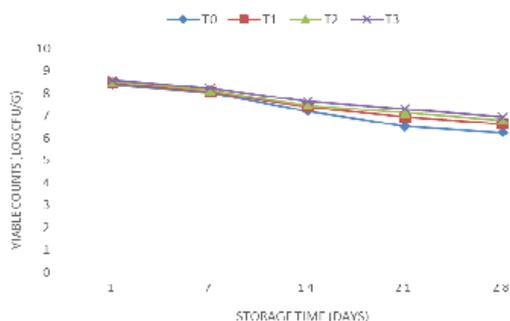
Days	T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>
<i>S. thermophilus</i> ( $10^{-7}$ cfu/g)				
1 <sup>st</sup>	8.38±0.08 <sup>a</sup>	8.44±0.05 <sup>ab</sup>	8.52±0.04 <sup>bc</sup>	8.58±0.08 <sup>c</sup>
7 <sup>th</sup>	8.02±0.08 <sup>a</sup>	8.06±0.05 <sup>a</sup>	8.12±0.08 <sup>ab</sup>	8.22±0.08 <sup>c</sup>
14 <sup>th</sup>	7.20±0.07 <sup>a</sup>	7.40±0.07 <sup>b</sup>	7.46±0.05 <sup>b</sup>	7.64±0.11 <sup>c</sup>
21 <sup>th</sup>	6.52±0.21 <sup>a</sup>	6.94±0.11 <sup>b</sup>	7.12±0.13 <sup>bc</sup>	7.30±0.12 <sup>c</sup>
28 <sup>th</sup>	6.26±0.28 <sup>a</sup>	6.64±0.11 <sup>b</sup>	6.80±0.12 <sup>bc</sup>	6.94±0.16 <sup>c</sup>
<i>L. bulgaricus</i> ( $10^{-7}$ cfu/g)				
1 <sup>st</sup>	8.18±0.13 <sup>a</sup>	8.34±0.08 <sup>b</sup>	8.42±0.08 <sup>bc</sup>	8.48±0.08 <sup>c</sup>
7 <sup>th</sup>	7.48±0.08 <sup>a</sup>	7.56±0.11 <sup>a</sup>	7.84±0.05 <sup>b</sup>	7.90±0.07 <sup>b</sup>
14 <sup>th</sup>	6.98±0.08 <sup>a</sup>	7.28±0.08 <sup>b</sup>	7.42±0.14 <sup>b</sup>	7.64±0.16 <sup>c</sup>
21 <sup>th</sup>	6.32±0.14 <sup>a</sup>	6.72±0.21 <sup>b</sup>	6.86±0.18 <sup>bc</sup>	7.04 ±0.21 <sup>c</sup>
28 <sup>th</sup>	6.02±0.17 <sup>a</sup>	6.24±0.15 <sup>b</sup>	6.44±0.15 <sup>bc</sup>	6.60±0.12 <sup>c</sup>
<i>B. bifidum</i> ( $10^{-7}$ cfu/g)				
1 <sup>st</sup>	8.18±0.13 <sup>a</sup>	8.44±0.05 <sup>b</sup>	8.50±0.07 <sup>bc</sup>	8.60±0.07 <sup>c</sup>
7 <sup>th</sup>	7.10±0.15 <sup>a</sup>	7.78±0.08 <sup>b</sup>	8.04±0.16 <sup>c</sup>	8.10±0.19 <sup>c</sup>
14 <sup>th</sup>	6.38±0.24 <sup>a</sup>	6.92±0.21 <sup>b</sup>	7.18±0.21 <sup>b</sup>	7.24±0.20 <sup>b</sup>
21 <sup>th</sup>	6.08±0.08 <sup>a</sup>	6.60±0.07 <sup>b</sup>	6.90±0.25 <sup>c</sup>	6.90±0.12 <sup>c</sup>
28 <sup>th</sup>	5.34±0.33 <sup>a</sup>	6.08±0.08 <sup>b</sup>	6.42±0.17 <sup>c</sup>	6.26±0.16 <sup>b</sup>
<i>L. acidophilus</i> ( $10^{-7}$ cfu/g)				
1 <sup>st</sup>	8.40±0.10 <sup>a</sup>	8.56±0.08 <sup>a</sup>	8.60±0.07 <sup>a</sup>	8.68±0.26 <sup>a</sup>
7 <sup>th</sup>	7.80±0.12 <sup>a</sup>	7.92±0.08 <sup>a</sup>	8.06±0.11 <sup>b</sup>	8.28±0.08 <sup>c</sup>
14 <sup>th</sup>	6.94±0.11 <sup>a</sup>	7.24±0.08 <sup>b</sup>	7.40±0.07 <sup>c</sup>	7.58 ±0.08 <sup>d</sup>
21 <sup>th</sup>	6.0±0.50 <sup>a</sup>	7.06±0.11 <sup>ab</sup>	7.32±0.08 <sup>bc</sup>	7.50±0.07 <sup>c</sup>
28 <sup>th</sup>	6.02±0.08 <sup>a</sup>	6.34±0.05 <sup>b</sup>	6.56±0.08 <sup>c</sup>	6.74±0.11 <sup>d</sup>

Means bearing different superscripts with in the row differ significantly (P< 0.05)

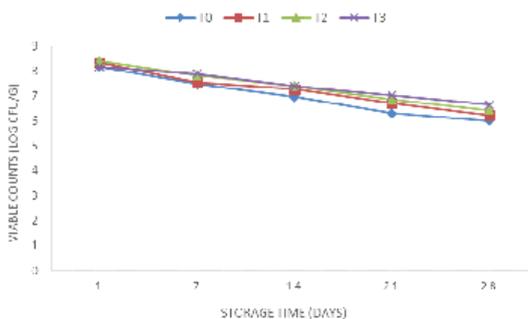
on 1<sup>st</sup> day and 7<sup>th</sup> day T<sub>3</sub> sample was showed significantly ( $P < 0.05$ ) higher than compared to the others T<sub>2</sub>, T<sub>1</sub> and T<sub>0</sub> sample. On 14<sup>th</sup>, 21<sup>st</sup> and especially 28<sup>th</sup> day, T<sub>3</sub> sample showed significantly ( $P < 0.05$ ) higher than followed by T<sub>2</sub>, T<sub>1</sub> and T<sub>0</sub>, respectively and that day T<sub>3</sub> sample are more effectively compare to 1<sup>st</sup> and on 7<sup>th</sup> day (Fig.2).



**Fig. 1.** Average score of the viability of bacterial count on 1<sup>st</sup>, 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup> and 28<sup>th</sup> day



**Fig. 2.** Average growth rate of the viability of *Streptococcus thermophilus* on storage period



**Fig. 3.** Average growth rate of the viability of *Lactobacillus delbrueckii subsp. bulgaricus* on storage period

With regard to the *L. bulgaricus* counts, on 1<sup>st</sup> day T<sub>2</sub> banana based soy yoghurts showed significantly ( $P < 0.05$ ) higher than compared to the others T<sub>1</sub>, T<sub>0</sub> and T<sub>3</sub>. Whenever, on 7<sup>th</sup> day T<sub>0</sub> and T<sub>1</sub> have not significance difference but T<sub>2</sub> and T<sub>3</sub> (higher) have significance difference. On 14<sup>th</sup>, 21<sup>st</sup> and especially 28<sup>th</sup> day, T<sub>3</sub> sample showed significantly higher than compared to the others T<sub>2</sub>, T<sub>1</sub> and T<sub>0</sub> and that day T<sub>3</sub> sample are more effectively compare to 1<sup>st</sup> and on 7<sup>th</sup> day (Fig.3).

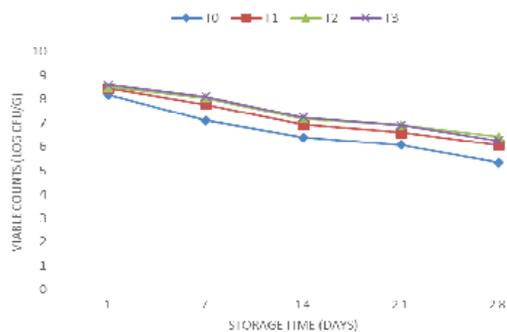
The counts of *B. bifidum*, on 1<sup>st</sup> day T<sub>3</sub> sample was showed significantly higher than compared to the other formulation. Whenever on 7<sup>th</sup> day T<sub>0</sub> between T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> have higher significance difference. On 14<sup>th</sup>, 21<sup>st</sup> and especially 28<sup>th</sup> day, T<sub>3</sub> sample showed significantly ( $P < 0.05$ ) higher than compared to the others T<sub>2</sub>, T<sub>1</sub> and T<sub>0</sub> and in which shows banana are more effectively compare to control (Fig.4).

The *L. acidophilus* counts, on 1<sup>st</sup>, 7<sup>th</sup>, 14<sup>th</sup> and 28<sup>th</sup> day of T<sub>3</sub> banana based soy yoghurts showed significantly higher than compared to the others samles. On 21<sup>st</sup> day showed T<sub>0</sub> between T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> sample significantly higher difference and that day T<sub>3</sub> sample in which shows banana are more effectively compare to control (Fig.5).

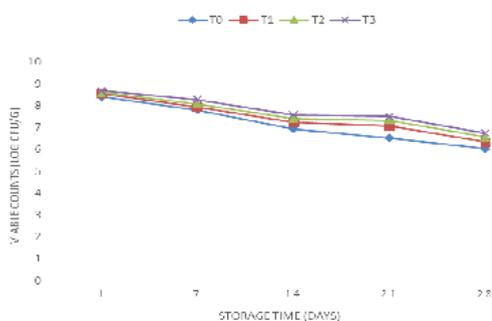
The average scores of the *L. bulgaricus*, *S. thermophilus*, *B. bifidum* and *L. acidophilus* count on 1<sup>st</sup>, 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup> and 28<sup>th</sup> day of during storage also showed (Fig. 2, 3, 4 and 5) a significant difference between T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> respectively and more effectively that control. Donkor *et al.* (2006) observed that a banana fibers increased the counts of all probiotics by no less than 1 log cfu/g compared to controls yoghurts, especially at 28 days (Table No. 1).

The number of probiotic organisms in a probiotic product should meet the suggested minimum value of  $>6$  log cfu/g to achieve optimal potential therapeutic effects (Rasic *et al.*, 1978). Vinderola *et al.* (2003) also stated that probiotic microflora counts decrease during storage. The rate of this loss in cell viability depended on the yogurt type and the use of lactic starter.

Finally, in this study was found the banana was a good medium for the enhancing the growth of yoghurt and probiotic bacteria. Banana together with probiotic bacteria as a synbiotic can stimulate Gut associated Lymphatic Tissue (GALT) system and prevent disruption of the



**Fig. 4.** Average growth rate of the viability of *Bifidobacterium bifidum* on storage period



**Fig. 5.** Average growth rate of the viability of *Lactobacillus acidophilus* on storage period

intestinal microflora which enhances the immunity (Bengmark *et al.*, 2001). The average total viability of selective media per gram of probiotic soy yoghurt seen the total viability of *L. bulgaricus*, *S. thermophiles*, *B. bifidum* and *L. acidophilus* count of soy yoghurt samples  $T_3$  (15%) was given higher stability than  $T_2$ ,  $T_1$  and  $T_0$ . Reid *et al.* (2006) reported that the immature banana along with the probiotic bacteria as a synbiotic are used in the treatment of peptic ulcers as it can form a gel phase in the mucosa which acts as a protective layer and also increases the survival of probiotic bacteria to inhibit the growth of *Helicobacter pylori* by production of lactic acid.

A nature of synbiotic in the gut should be maintained which is “a mixture of a probiotic and a prebiotic, and the rationale for this combination is that the prebiotic is used to stimulate the growth of probiotic in the gut, thereby increasing its effectiveness.” (Macfarlane *et al.*, 2006).

## CONCLUSION

This study find that banana based soy yoghurt may be a suitable medium for delivery of probiotic bacteria and their numbers stability steadily with added banana puree in soy yoghurt during stored at refrigerator temperature. Thus, prebiotic approach through diet increases resident bacteria which are beneficial to human health. In general, all selected probiotic culture showed appreciable viability and above therapeutic value during prolonged storage at 4°C which is an additional of beneficial effect of banana soy yoghurt.

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