RESEARCH ARTICLE FOOD SCIENCE RESEARCH JOURNAL Volume 3 | Issue 2 | October, 2012| 205-210

Viability of probiotics in flavoured yoghurts made with different starter culture during storage

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Six different yoghurts were prepared with mango pulp and pineapple essence and sugar combination with *Lactobacillus bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus, Lactobacillus sporogens, Bifido bifidum, Bifido longum, and Bifido infantis* as starter culture. Three types of yoghurts were developed under each flavours, with different combination of probiotics and termed as A_1, B_1, C_1, A_2, B_2 and C_2. A_1, B_1, C_1 were mango yoghurts and A_2, B_2 and C_2 were pineapple yoghurts. Statistically, the significant difference were found in viable counts from 7 thday to 14th day (P<0.05) in all yoghurts except in A_2 and C_2. The difference of viable counts from 0 day to 7 thday was also found to be significant (P<0.05) in A_2 and C_2. No significant difference was found from 14th day to 30th day in all yoghurts as 90- 96 per cent viability loss of probiotics was observed by 14th day in all yoghurts. Pineapple yoghurt with probiotic blends of *Lactobacillus bulgaricus, Streptococcus thermophilus* and *Lactobacillus sporogens i.e.*, B_2 had higher viability, among the different types of yoghurts developed.

Key Words : Probiolies, Yoghurts, Starter culture, Storage

How to cite this article : Madhu, Prakash, M. Shiva and Kochhar, Anita (2012). Viability of probiotics in flavoured yoghurts made with different starter culture during storage. *Food Sci. Res. J.,* **3**(2): 205-210.

INTRODUCTION

Yoghurt is considered by nutritionists to be a very nutritious and healthy food compared to milk. Probiotic cultures are live bacteria which help in better absorption of nutrients. They play an important role in reduction of serum cholesterol, alleviation of lactose intolerance, reduction of diarrhea, prevention and suppression of colon cancer, stimulation of the immune system etc. Yoghurt is prepared by fermenting milk with starter cultures containing different types of probiotics, normally *streptococcus thermophillus* and *lactobacillus bulgaricus*. Use of different probiotic blends in combination have several health benefits. Lactic acid bacteria (LAB) and bifidobacteria are the most common types of microbes used as probiotics. Probiotics are commonly consumed as part of fermented foods with specially added active live cultures, such as in yogurt, soy yogurt, or as dietary supplements. The introduction of yoghurt with added probiotics could play a significant role in national health care programs especially in developing countries, where diarrhoea and gastrointestinal problems are common. The increase in the per capita annual consumption of yoghurt in the majority of the countries has been attributed to both the ever-increasing availability of fruit or flavoured yoghurt, and to the diversity of presentations of the product. Although the main choice of any probiotic microbial strain to be used as a starter culture or a blend with a starter culture is based on the health aspects beneficial to humans (Gardiner *et al.,* 2002).The aim of the study was to investigate the changes in microbiological properties in mango and pineapple yoghurts made with different probiotic cultures during storage.

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METHODOLOGY

The raw material *viz.,* Double toned milk, powdered sugar, milk powder, pineapple essence, food colour, pasturised mango pulp, and plastic sterile cups used for preparation of yoghurts were purchased from the local market.

The probiotic stock cultures required for the study *i.e.*, *Lactobacillus bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus, Lactobacillus sporogens, Bifido bifidum, Bifido longum,* and *Bifido infantis* in powder form were obtained from National Institute of Nutrition, Hyderabad. The two different flavoured yoghurt *i.e.*, mango and pineapple was prepared by using standard technique as described below in the form of flow chart:

Double toned milk
↓
Pasteurization (for 15 min.)
↓
Addition of milk powder
↓
Stirring (to prevent lump formation)
↓
Cooled the milk to 40-50 °C and poured into six plastic cups
↓
Inoculation with yoghurt culture in all six cups as starter culture given below:
A_1 and A_2 *L.bulgaricus* + *S.thermophillus*
B_1 and B_2 *L.bulgaricus* + *S.thermophillus* + *L. sporogens*
C_1 and C_2 *L.bulgaricus* + *S.thermophillus* + *L. acidophilus* + *B.bifidum* + *B.longum* + *B. infantis*
↓
Incubation at 37 °C for 12-24 hr.
↓

Mango pulp addition (15 g) and powdered sugar in three cups *i.e.,* A_1, B_1 and C_1	Pineapple essence addition (5-6 drops) and powdered sugar in three cups *i.e.,* A_2, B_2 and C_2

↓
Stirring
↓
Storage(4 °C)

The prepared products were tested for the viability of probiotics within three hours of preparation as well as in stored samples after 7, 14, 21 and 30 days. The microbiological method *i.e.*, spread plate method (Clark, 1993) used for bacterial count on MRS plate is described below.

One gram of sample was transferred into a test tube containing 9 ml of saline. It was mixed well by shaking for 2 min. and 1 ml from this was taken and diluted further through a series of test tubes, containing 9 ml of sterile normal saline blank solution by an appropriate decimal dilution method. Recommended final dilution was upto 10^8-10^9. Then, from the final diluted tube take 100µl and pour into the MRS agar medium Petridishes and spread into medium by clockwise and anticlockwise via spread plate technique.The plates were incubated at 40°C for 24- 48 hours and the number of colonies in each plates was counted in colony counter machine. These isolated colonies were sub cultured and subjected for gram staining for identification of species.

Lactobacilli and bifidobacteria appeared as large, white colonies in or on the surface of the medium. The average number of colonies, multiplied by the dilution number (dilution factor) and divided by quantity of sample on weight basis and expressed as colony forming units/gram.

$$\text{Number of colony forming units (CFu's) per g of the sample} = \frac{\text{Mean number of Cfu's x Dilution factor}}{\text{Quantity of sample on weight basis}}$$

The results obtained from 10 replications of all organoleptic qualities scores and 2 replications on viable counts of log cfu/g values were analysed by analysis of variance (ANOVA), using complete randomized design (CRD) and Tukey HSD Test for Post-ANOVA Pair-Wise Comparisons. Analysis of variance were applied for testing the significance between the flavours, period of storage, different products and viability of products.

OBSERVATIONS AND ASSESSMENT

The viability of probiotic bacteria in yoghurt depends on the strains used, the interaction between species present, the production of hydrogen peroxide due to bacterial metabolism, and the final acidity of the product.

The percentage loss of probiotic bacteria at different periods of storage at 4°C are presented in Table 1. On the day of preparation and after storing the sample for a period 7th, 14th, 21st and 30thdays, the average percentage of viability loss in mango yoghurts A_1, B_1 and C_1 was 29.9 per cent, 26.53 per cent and 24 per cent at 7th day and 99.99 per cent, 99.7 per cent and 99.9 per cent at 30th day, respectively. In the case of pineapple flavour yoghurts A_2, B_2 and C_2 it was 26 per cent, 27 per cent and 29 per cent on 7th day and 98.4 per cent, 99.4 per cent and 99.95 per cent on 30th day, respectively. $C_1$and C_2 had high viability loss *i.e.*, 93.56 and 92.5 per cent on 14thdays compared to others. The loss of probiotic in all yoghurt could be due to competition for survival of probiotics as, *L. bulgaricus* affect the survival of *L. acidophilus* and bifidobacteria due to the acid and hydrogen peroxide produced during fermentation stage. Due to its proteolytic nature, *L.bulgaricus* grows rapidly and produces acid quickly; whilst appearing to liberate the essential amino acids such as valine, glycine and histidine, that are required to support the growth of bifidobacteria. *S. thermophillus* does not inhibit the growth of probiotic organisms. These observations are in conformity with that of Mortazavian *et al.* (2007) who reported that the decrease in viability of *L.acidophillus* and *B.lactis* at 2°C, 5°C and 8°C of refrigerated storage of 20 days.

The log cfu/g values at 10^9 of the probiotic strains in developed yoghurt at different periods of storage are presented in Table 2 which shows the variation in the microbial cell count during the refrigerated storage period at 7- day intervals. During

Table 1. Viability loss (%) of probiotics in different period of storage

Flavour	Storage period				
	Yoghurts	7 day	14 day	21 day	30 day
Mango	A_1	29.9 %	96.06%	99.05%	99.99%
	B_1	26.53%	95%	99.02%	99.7%
	C_1	24%	93.56%	97.75%	99.9%
Pineapple	A_2	26%	95%	98.6%	98.4%
	B_2	27%	90.8%	99.16%	99.4%
	C_2	29%	92.5%	94.5%	99.95%

storage, the viable probiotic microorganism in A_1 was 1.733 ± 0.347 x 10^9 on 0 day reduced to 1.466 ± 0.297 x 10^9 on 7th day of storage. From 0 day to 7th day no significant difference was found. But it reduced to 0.277 ±0.038 x 10^9 on 14th day of storage.The analysis of variance in difference of viable counts from 7th day to 14th day were found to be significant ($P<0.05$). No significant difference was found from 14th day to 30th days as viability loss was 90-96 per cent by 14th day itself only in different yoghurts. Luther *et al.* (1931) stated that high counts of viable organisms were obtained at lower or moderate acidities and greater destruction occurred in more acid cultures. According to Dave and Shah (1997) hydrogen peroxide produced by *L. delbruecki* ssp. *bulgaricus* bacteria is the most important viability-reducing factor during refrigerated storage. Similar trends were observed in B_1 and C_1.

Likewise, during storage of pineapple yoghurts, the probiotic counts declined as shown in Table 2. During storage, the viable probiotic microorganism in A_2 which was 1.733 ± 0.347 x 10^9 on 0 day, reduced to 1.210±0.294 x 10^9 on 7th day. In C_2 reduction was from 2.362 ±0.187 x 10^9 to 1.354±0.255 x 10^9 on 7th day of storage. It further reduced to 0.266± 0.084 x 10^9 in A_2, 0.539±0.087 x 10^9 in C_2 on 14th day of storage. The analysis of variance in difference of viable counts from 0 day to 7th day and 7th day to 14th day were found to significant ($P<0.05$). Whereas, in B_2 containing *Lactobacillus bulgaricus, Streptococcus thermophilus* and *Lactobacillus sporogenes* there was no significant difference from 0 day to 7th day ,but significant decrease were found from 7th day to 14th day. No significant difference was found from 14th day to 30th days as viability loss was 90-96 per cent by 14th day itself only in different yoghurts. These observations indicated continuous declining of probiotics in all the developed yoghurt samples during storage. Tamime *et al.* (2005) suggested that probiotic organisms should be present in a food to a minimum concentration of 10^6 cfu g^{-1}, or the daily intake should be about 10^9 cfu g^{-1}. Such high numbers have been suggested to compensate for possible losses in the numbers of the probiotic organisms during passage through the stomach and intestine.In the present study, the level of 10^9 cfu g^{-1} was observed at the time of production *i.e.*, on 0 day in all types of yoghurts.

The viability of probiotic organism in C_1 (7.2x10^9) was more in comparison to other types on 7th day of storage. However, the flavour of pineapple with B_2 blends of probiotics

Table 2. Mean value of viability of probiotics count in the mango and pineapple yoghurt from 0 days to 4 weeks at 7 day intervals (log cfu/g)

Flavours	yoghurts	0 day	7 day	14 day	21 day	30 day
Mango	A_1	1.733 ± 0.347_a	1.4661 ± 0.297_a	$0.277{\pm}0.038_b$	0.082 $\pm 0.009_b$	0.046± 0.021_b
		(5×10^9)	(3.5×10^9)	(3.2×10^8)	(8.5×10^7)	(4.8×10^7)
	B_1	$2.291{\pm}0.151_a$	$2.035{\pm}0.195_a$	$0.366{\pm}0.104_b$	0.084± 0.007_b	0.026± 0.005_b
		(9×10^9)	(6.8×10^9)	(4.5×10^8)	(8.9×10^7)	(2.0×10^7)
	C_1	2.362 $\pm 0.187_a$	$2.067{\pm}0.275_a$	$0.509{\pm}0.138_b$	$0.195{\pm}0.082_b$	0.009 ± 0.002_b
		(9.8×10^9)	(7.2×10^9)	(6.8×10^8)	(2.2×10^7)	(9.0×10^6)
Pineapple	A_2	1.733 ± 0.347_a	$1.210{\pm}0.294_{ab}$	0.266± 0.084_b	$0.067{\pm}0.019_b$	0.063± 0.005_b
		(5×10^9)	(2.5×10^9)	(3.0×10^8)	(7.0×10^7)	(6.5×10^7)
	B_2	$2.291{\pm}0.151_a$	$1.781{\pm}0.394_a$	0.599± 0.011_b	0.072± 0.009_b	$0.047{\pm}0.024_b$
		(9×10^9)	(5.4×10^9)	(8.2×10^8)	(7.5×10^7)	(4.2×10^7)
	C_2	2.362 $\pm 0.187_a$	$1.354{\pm}0.255_b$	$0.539{\pm}0.087_{bc}$	$0.042{\pm}0.012_c$	$0.005{\pm}0.000_c$
		(9.8×10^9)	(3×10^9)	(7.2×10^8)	(4.3×10^7)	(5.0×10^6)

Means with different subscripts in rows were significantly different $P<0.05$
The values in tables are calculated by logarthimic method ,all the values are expressed in 10^9
Values in parentheses are original value

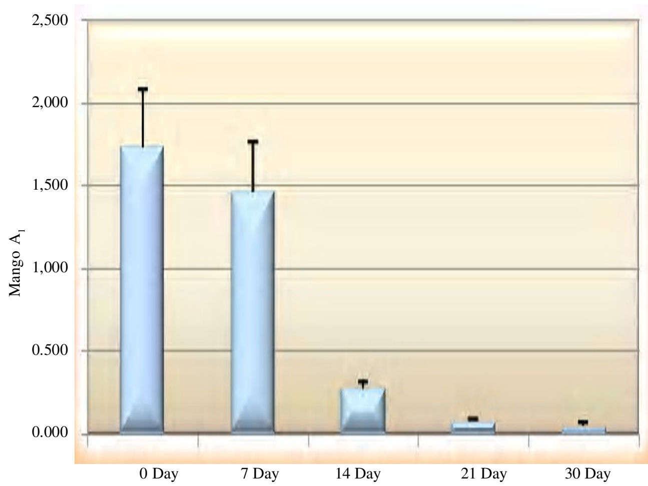

Fig. 1: Viability of probiotics in Mango A_1 yoghurt during different periods of storage

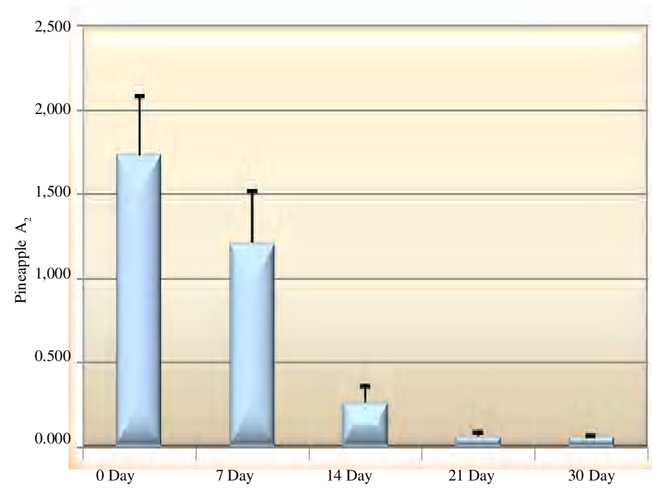

Fig. 4: Viability of probiotics in Pineapple A_2 yoghurt during different periods of storage

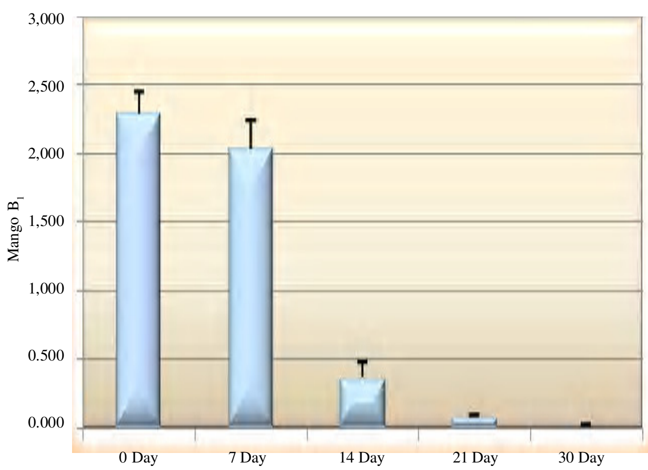

Fig. 2: Viability of probiotics in Mango B_1 yoghurt during different periods of storage

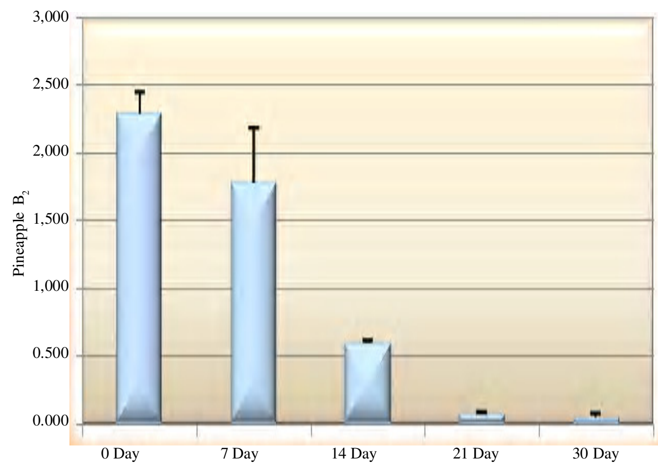

Fig. 5: Viability of probiotics in Pineapple B_2 yoghurt during different periods of storage

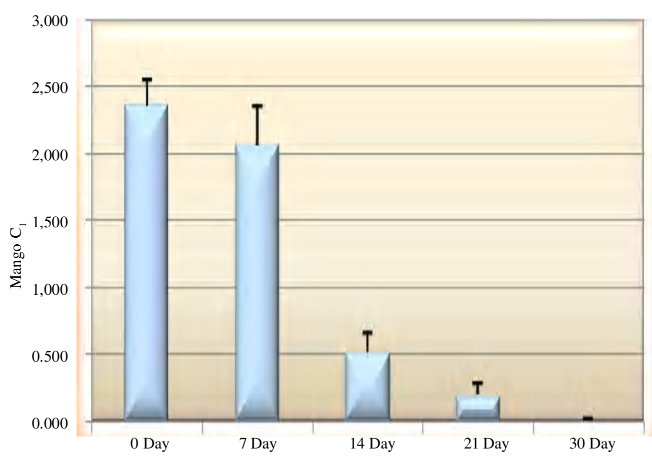

Fig. 3: Viability of probiotics in Mango C_1 yoghurt during different periods of storage

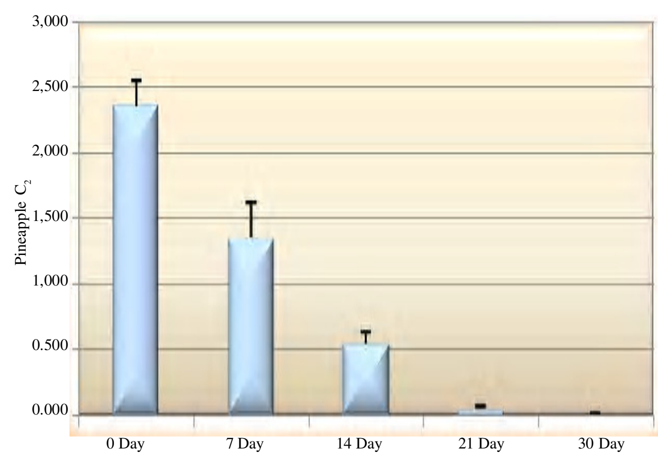

Fig. 6: Viability of probiotics in Pineapple C_2 yoghurt during different periods of storage

i.e., 8.2×10^8 significantly increased the viability when compared to A_1, B_1, C_1, A_2 and C_2 at 14th day. It could be due to *L. sporogenes* which have longer vaibility than others. In support of these finding a stability study, done by Losada and Olleros (2002) reported that *L. sporogenes* were highly viable during storage.

Payot *et al.* (1999) reported that spores of *B. coagulans* / *L. sporogenes* are ellipsoidal bodies located at one of the cellular poles, resistant to heat and adversal environmental conditions, and able to germinate in presence of diluted HCl or NaOH solutions. The longevity of yoghurt was significantly more by fermenting the yoghurt by B_2 blends of probiotics than with other types of probiotic blends. The reduction of probiotics counts observed on 7th day of storage in this study might be due to the incorporation of pineapple essence or mango pulp.

The lowest viability was observed in A_2 containing *L. bulgaricus and S. thermophilus* on 14th day of storage. It could be due to *S. thermophilus and L. bulgaricus* might not be grown properly at 4^0C. Vinderola *et al.* (2000) reported that after 7 days of storage, the counts of *L. bulgaricus* showed a sharp decline (6–10-fold) from the initial counts and finally decreased by 97–99 per cent in all samples at the end of storage. They also reported that *S.thermophilus* counts were higher by at least 1 log order than those for *L. delbrueckii ssp. bulgaricus* in yoghurts containing probiotic bacteria. Kim *et al.* (1993), Medina and Jordano (1994), Lim *et al.* (1995) and Dave and Shah (1997) also reported almost similar results. However, in the present study vaiibility could not be carried out on individual species of probiotics, but viability was tested on a combination of organism. Mortazavian *et al.* (2007) reported that at higher temperatures 5 and 8^0C compared to 2^0C the *L. delbruecki* spp. *bulgaricus* bacteria grow faster, therefore, higher amounts of lactic acid and hydrogen peroxide could be produced. Chandan and Shahani (1993) suggested that yoghurt must have at least 10^8 cfu/g probiotic counts at the time of preperation. In the present study the viability counts were 10^8 upto 14 day which satisfied the above suggestion.

Bakirci and Kavaz (2008) observed that addition of fruit and sugar in yoghurt led to an increase in the viable counts of probiotic organisms. They also reported that the total viable counts of *L. acidophilus* and *Bifidobacterium* spp. decreased slightly for longer period of storage, but remained at sufficient levels (> 6 log cfu/g) up to 14th days. Con *et al*. (1996) reported that the addition of fruit flavours to yogurt did not show significant effect on the viability of total bacteria.

Overall, it was observed that in all the yoghurt developed, the probiotics used were viable incorporatrd upto 7th day and after that declined from 14th day. These levels of live organisms present upto 7th day are sufficient to have beneficial effect *i.e.*, to have probiotic effect. From the finding of the present study, the yoghurt B_2 containing *Lactobacillus bulgaricus, Streptococcus thermophilus* and *Lactobacillus sporogenes* can be recommended as a good combination of probiotic for higher viability, specially with addition of pineapple essence added yoghurt.

Conclusions:

The demand for fruit flavoured yoghurts is increasing in the recent years. Hence, there is a great scope to develop and popularize fruit yoghurts in India. There are very few research studies on the flavoured yoghurt with different combination of probiotic blends.

During storage, the viable probiotic microorganisms in A_1 were 1.733 x 10^9 on 0 day which reduced to 1.466 x 10^9 on 7th day of storage. From 0 day to 7th day no significant difference was found. But it reduced to 0.277x10^9 on 14th day of storage. The difference in viable counts from 7th day to 14th day were found to be significant ($P<0.05$). After that significant difference was not found from 14th day to 30th day. Similar trends were observed in B_1 and C_1. The viability of probiotic organisms in C_1 2.067× 10^9 (7.2×10^9) was more in comparison to other types on 7th day of storage.

The viability of probiotics in all yoghurts was assessd using spread plate method at 0, 7th day, 14th day, 21st day and 30th day. The viability of probiotic organisms declined from 0 day to 30th day and in C_1 the viability of probiotics was higher *i.e.*, 2.067× 10^9(7.2×10^9) in comparison to other types on 7th day of storage and the viability of B_2 blends of probiotics 0.599× 10^9 (8.2×10^8) was higher on 14th day, compared to other yoghurts. Statistically, the significant difference were found in viable counts from 7th day to 14th day ($P<0.05$) in all yoghurts except in A_2 and C_2. The difference of viable counts from 0 day to 7th day was also found to be significant ($P<0.05$) in A_2 and C_2. No significant difference was found from 14th day to 30th day in all yoghurts as 90-96 per cent viability loss of probiotics was observed by 14th day in all yoghurts. Pineapple yoghurt with probiotic blends of *Lactobacillus bulgaricus, Streptococcus thermophilus,* and *Lactobacillus sporogens i.e.*, B_2 had higher viability, among the different types of yoghurts developed. The probiotic based yoghurts developed can be used as food supplement for human consumption, as these products have commercial value. These can be supplied as neutraceuticals or therapeutical supplements.

LITERATURE CITED

Bakirci, I. and Kavaz, A. (2008). An investigation of some properties of banana yogurts made with commercial ABT-2 starter culture during storage. *Internat J. Dairy Technol.*, **61**(3): 270-276.

Chandan, R.C and Shahani, K.M. (1993). Yoghurt. In: Dairy Science & Technology 2. Product manufacturing, Ed. Y.H. Hui, NEW YORK: PVCH Publishers. pp. 1-56.

Con, A.H.C., Akmakci, S.M.C.¸ Aglar, A. and Gokalp, H.Y. (1996). Effects of different fruits and storage periods on microbiological qualities of fruit-flavoured yoghurt produced in Turkey. *J. Food Protect.*, **59**(4):402-406.

Dave, R.J. and Shah, N.P. (1997). Viability of yoghurt and probiotics bacteria in yoghurt made from commercial starter cultures. *Internat. Dairy J.,* **7**:31-34.

Gardiner, G.E., Bouchier, P., O'Sullivan, E., Kelly, J., Collins, J.K., Fitzgerald, G., Ross, R.P. and Stanton, C. (2002). A spray – dried culture of cheese manufacture. *Internat. Dairy J.,* **12**:749-756.

Kim, E.R., Lee, K.W., Park, Y.H. and Kwak, H.S. (1993). The study of lactic acid bacteria in yoghurt during delivery and storage. *Korean J. Dairy Sci.,* **14**: 260–263.

Lim, K.S., Huh, C.S., Baek, Y.J. and Kim, H.U. (1995). A selective enumeration medium for bifidobacteria in fermented dairy products. *J. Dairy Sci.,* **78**: 2108–2112.

Losada, M.A. and Olleros, T. (2002). Towards a healthier diet for the colon: the influence of FOS's and Lactobacilli on intestinal health. *Nutri. Res.,* **22**: 71-84.

Medina, L.M. and Jordano, R. (1994). Survival of constitutive microflora in commercially fermented milk containing bifidobacteria during refrigerated storage. *J. Food Protect.,* **56**:731–733.

Mortazavian, A.M., Ehsani, M.R., Mousavi, S.M., Rezaei, K., Sohrabvandi, S. and Reinheimer, J.A. (2007). Effect of refrigerated storage temperature on the viability of probiotic micro-organisms in yogurt. *Internat. J. Dairy Technol.*, **60**(2) 123-127.

Payot, T., Chemaly, Z. and Fick, M. (1999). Lactic acid production by *Bacillus coagulans*-kinetic studies and optimization of culture medium for batch and continuous fermentations. *Enzyme & Microbial Technol.,* **24**:191-199.

Tamime, A.Y. (2005). *Probiotic Dairy Products*, Black well Publishing. Oxford. pp. 39-72.

Vinderola, C.G., Bailo, N. and Reinheime, J.A. (2000). Survival of probiotic microflora in Argentinian yoghurts during refrigerated storage. *Food Res. Internat.,* **33**(2):97-102.

Received : *21.06.2012;* **Revised:** *28.07.2012;* **Accepted :** *27.08.2012*