Effect of milk supplementation on growth and viability of starter and probiotic bacteria in yogurt during refrigerated storage

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Summary

In the present study, the effects of milk supplementation on growth and viability of yogurt (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) and probiotic bacteria (*Lactobacillus acidophilus* and bifidobacteria) were studied during yogurt production and 33 days of refrigerated storage. The incubation time to reach pH = 4.5 was greatly affected by the addition of milk powder (MP), tryptone (TRY) and sucrose (SUC). Also, the increase in titrable acidity depended on added supplement. Viable counts of *L. delbrueckii* subsp. *bulgaricus* were significantly ($P<0.05$) increased in yogurt supplemented with whey powder (WP), TRY and milk powder plus five fold starter culture (MP-SC). However, milk supplementation did not affect the counts of *S. thermophilus* in probiotic yogurt until the end of storage. Supplementation with TRY and MP-SC promoted the growth and viability of *L. acidophilus*, whereas milk supplementation with whey protein concentrate (WPC), yeast extract (YE), SUC and Cysteine, adversely affected the viability of *L. acidophilus* in probiotic yogurt. Finally, using a high level of inoculums (MP-SC) improved the viability of bifidobacteria during storage for 33 days. In conclusion, tryptone and milk powder plus five fold starter culture were found the most effective supplements to improve growth and viability of starter and probiotic (*L. acidophilus* and bifidobacteria) bacteria in probiotic yogurt during refrigerated storage for a five week period. These findings would be applicable in industrial production of probiotic yogurt.

Key words: Probiotic yogurt, Supplementation, Viability, Starter bacteria

Introduction

Probiotics are defined as viable microorganisms that exhibit beneficial effects on the health of the host upon ingestion by improving properties of indigenous gut microflora (Gomez and Malcata, 1999). Probiotics enhance the population of beneficial bacteria in the human gut, suppress pathogens and build up resistance against preexisting intestinal diseases (Prado *et al*., 2008).

Probiotic microorganisms can not affect the intestinal environment unless their populations reach a certain minimum level (Gilliland *et al*., 2002). There is no general agreement on the minimum concentration for probiotic bacteria, however some researchers suggested a concentration level above 10^5-10^6 viable cell per ml or g of product (Dave and Shah, 1997a), and others stipulate more than 10^7 and 10^8 as satisfactory level (De Vuyst, 2000).

Several members of the lactic acid

bacteria have gained recognition as probiotic bacteria, amongst them; *Lactobacillus acidophilus* and *Bifidobacterium* spp. are more significant (Saarela *et al*., 2000).

Yogurt or yogurt-like products have been used as the most popular vehicle for incorporation of probiotic bacteria. Commercially it is not feasible to ferment milk using only probiotic organisms owing to the longer time required to reduce the pH of milk and also an objectionable taste provided by some of the probiotic bacterial strains (Dave and Shah, 1997a; Lourens-Hattingh and Viljoen, 2001; Tamime *et al*., 2005).

Most of the probiotic yogurts contain live strains of *L. acidophilus* and species of *Bifidobacterium* accompanied by the conventional yogurt organisms, *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *Bulgaricus* (Tamime and Robinson, 1999; Tamime *et al*., 2005). Despite the importance of viability of probiotic bacteria, recent market surveys have revealed poor viability of these microorganisms in commercial yogurt preparations (Shah *et al*., 1995). Several works have been done to improve the growth and viability of probiotic bacteria by adding supplements to the milk base (Dave and Shah, 1997b; Oliveira *et al*., 2001; Sodini *et al*., 2002).

Considering this, most of the efforts have been performed using ABT (*L. acidophilus*, bifidobacteria and *S. thermophilus*) starter cultures that are devoid of *L. delbrueckii* subsp. *bulgaricus* (Dave and Shah, 1998; Oliveira *et al*., 2001).

Lactobacillus delbrueckii subsp. *bulgaricus* may produce lactic acid during storage. This process is known as "post-acidification" and, if it occurs, causes a loss in viability of the probiotic bacteria (Tamime *et al*., 2005).

However, *L. delbrueckii* subsp. *bulgaricus* has a critical role in producing lactic acid and flavor in yogurt. When it is excluded from starter culture, the texture, acidity and aroma of the final product could be profoundly affected. Therefore, in recent years some yogurt products have been made using AB-yogurt cultures (including live strains of *L. acidophilus* and species of *Bifidobacterium* in addition to the conventional yogurt organisms, *S. thermophilus* and *L. delbrueckii* subsp. *Bulgaricus* (Lourens-Hattingh and Viljoen, 2001). Sodini *et al.* (2002) compared four dairy ingredients in combination with two starter cultures. One starter culture contained only *S. thermophilus* with probiotic bacteria and the other was a combination of *S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus* and probiotic bacteria. They showed that the use of single starter culture and the addition of casein hydrolysate gave the best probiotic cell counts.

The effect of milk supplementation on growth and viability of probiotic and yogurt bacteria in such fermented products have not been studied precisely and information is presently lacking on the effects of micronutrients on AB-yogurt culture performance.

The aim of this study was to examine the effects of milk supplementation on the growth and viability of yogurt and probiotic bacteria using AB-yogurt commercial starter culture. In addition, the influence of milk supplementation on pH and titrable acidity were assessed.

Materials and Methods

Starter culture and milk ingredients

A commercial AB-yogurt starter culture was used in this study. The actual name of the commercial starter culture and the name of the supplier are not given for the sake of confidentiality. The starter culture was delivered in freeze dried DVS (Direct Vat System) form.

The storage and maintenance of the culture was carried out as per the recommendation of the manufacturer.

Different experimental groups which were supplemented with various dairy and non-dairy ingredients are summarized in Table 1. Briefly, the studied supplements included tryptone, whey and milk powder, yeast extract, sucrose and some of their combinations. A higher inoculum of starter culture was applied, too.

Yogurt production and storage

Pasteurized (<30,000 cfu/ml for total count) and homogenized milk containing

Table 1: Milk supplemented with various dairy and non-dairy products in different treatment groups

Treatment groups	Supplements
Control	None
TRY	Tryptone, 0.2% W/V
WP	Whey powder, 0.2% W/V
WPC	Whey powder concentrate, 0.2% W/V
MP	Milk powder, 2% W/V
YE	Yeast extract ,0.2% W/V
Suc	Sucrose, 0.2% W/V
TC	Tryptone, 0.2% W/V + Cysteine, 500 mg /L
TCS	Tryptone, 0.2% W/V + Cysteine, 500 mg /L + Sucrose, 0.2% W/V
MP-SC	Milk powder 2% W/V + 5 fold starter culture

2.5% fat, 4% protein, 5.5% lactose and 0.33% ash was obtained from a dairy plant, heated to 50°C and then fortified with supplements as shown in Table 1. The mixtures were subsequently heated to 85°C for 30 min, cooled to 43°C, and the starter culture was added (as recommended by the supplier). The inoculated fortified milk samples was dispensed into 100-ml polystyrene cups. The cups were heat-sealed with aluminum foil.

Incubation was carried out at 43 ± 0.5°C and fermentation was stopped at pH = 4.5.

The time taken to reach pH = 4.5 was recorded for each group in order to study the effects of the supplements. When the fermentation was terminated, yogurt cups were stored at 4°C for 33 days.

Chemical analysis (pH and titrable acidity determination)

The pH values of the inoculated mixtures and yogurts were measured using a pH meter (Hach pH meter, Hach Co., USA). The titrable acidity (TA) was determined by the AOAC method and expressed as percent of lactic acid (AOAC, 1984).

The pH and TA values of samples were measured from 45 min after the beginning of the fermentation at intervals of 30 min, until the pH = 4.5 was reached.

Microbiological analysis

Viable counts of *S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus*, *L. acidophilus* and bifidobacteria were monitored during storage for a period of 33 days at 4°C. Yogurt samples were taken at 0, 7, 14, 23 and 33 days for microbial analysis.

One g of each yogurt sample was diluted with 9 ml of 0.9% sterile normal saline solution and was mixed uniformly with a vortex mixer. Appropriate dilutions were made prior to pour-plating in duplicate onto selective media. *S. thermophilus* were enumerated on M17 agar (Merck, Darmstadt, Germany) after being incubated aerobically at 37°C for 24 h (Dave and Shah, 1998). *L. delbrueckii* subsp. *bulgaricus* was enumerated by culture on MRS agar (Merck, Darmstadt, Germany) adjusted to pH = 5.2 and anaerobic incubation at 43°C for 72 h (Dave and Shah, 1998). MRS-clindomycin-ciprofloxacin agar (MRS-CL/CIP Agar) was used for selective enumeration of *L. acidophilus* by incubating the plates anaerobically at 37°C for 72 h (ISO/DIS 20128 IDF 192, 2005). Selective enumeration of bifidobacteria was performed on MRS agar supplemented with 0.5 mg L^{-1} dichloxallin (Sigma Chemical Co., St Louis, USA), 1 g/l Lithium chloride (Merck, Darmstadt, Germany) and 0.5 g/l cysteine hydrochloride (Merck, Darmstadt, Germany) and anaerobic incubation at 37°C for 72 h.

Statistical analysis

Three independent replicates of each experimental treatment were carried out at two-month intervals. Before statistical analysis, the populations of bacteria were converted to $\log_{10}$ cfu/g. All data were analysed by one way ANOVA using SPSS, version 11.5 software (SPSS, Chicago, Ill.) followed by Duncan's multiple range test. A $P<0.05$ was considered statistically significant.

Results

Total time to reach pH = 4.5 during the fermentation of yogurt samples is shown in

Table 2. Also, the titrable acidity values of yogurt samples when the pH reached to pH = 4.5 are given in Table 3. The times taken to reach pH = 4.5 and the titrable acidity values ranged from 225 to 405 min and 0.62 to 0.72 percent lactic acid, respectively.

Changes in the viable counts of starter and probiotic bacteria in yogurt supplemented with various ingredients during 33 days of refrigerated storage are shown in Tables 4-7.

Statistical analysis showed that, when the pH reached to 4.5, the total number of *L. delbrueckii* subsp. *bulgaricus* was higher in samples supplemented with tryptone, WP, and MP-SC, than that of the other experimental groups. During storage for 33 days, the counts of *L. delbrueckii* subsp.

Table 2: Total time (mean values) to reach pH = 4.5 during the fermentation of yogurts

Treatment groups	Time (min)
Control	405
WP, WPC	315
YE, TC	285
TRY, SUC, TCS, MP-SC	255
MP	225

Table 3: Titrable acidity values (mean values) of yogurt samples when the pH reached to 4.5

Treatment groups	Titrable acidity (% lactic acid)
MP, MP-SC	0.72
TRY, WP	0.68
WPC, TC, YE	0.64
TCS, SUC	0.62

Table 4: Changes in the number of *Lactobacillus delbrueckii* subsp. *Bulgaricus* during storage of yogurt supplemented with various ingredients[1]

Treatment groups	Storage time (days)				
	0	7	14	23	33
Control	6.45 ± 0.00^{aC}	6.40 ± 0.40^{a}	6.38 ± 0.17^{aB}	5.79 ± 0.24^{a}	5.98 ± 0.16^{a}
TRY	7.89 ± 0.41^{aB}	6.28 ± 0.28^{b}	6.73 ± 0.22^{bA}	5.98 ± 0.41^{b}	5.25 ± 0.95^{c}
WP	7.43 ± 1.16^{aB}	6.50 ± 0.00^{a}	6.49 ± 0.02^{aB}	5.73 ± 0.58^{a}	6.19 ± 0.11^{a}
WPC	6.77 ± 0.10^{aC}	6.39 ± 0.52^{a}	6.30 ± 0.24^{aB}	6.39 ± 0.60^{a}	5.95 ± 0.10^{a}
MP	6.37 ± 0.00^{aC}	6.09 ± 0.12^{a}	6.23 ± 0.00^{aB}	6.25 ± 0.18^{a}	5.82 ± 0.12^{a}
YE	6.36 ± 0.16^{bC}	6.22 ± 0.09^{b}	6.52 ± 0.17^{bB}	6.71 ± 0.03^{a}	5.95 ± 0.01^{b}
SUC	6.56 ± 0.20^{aC}	6.20 ± 0.48^{a}	6.32 ± 0.32^{aB}	5.95 ± 0.04^{a}	5.37 ± 0.13^{a}
TC	6.27 ± 0.00^{aC}	6.50 ± 0.22^{a}	6.65 ± 0.12^{aB}	6.71 ± 0.02^{b}	5.93 ± 0.04^{a}
TCS	6.34 ± 0.30^{aC}	6.09 ± 0.30^{a}	6.23 ± 0.06^{aB}	6.26 ± 0.06^{a}	5.82 ± 0.37^{b}
MP-SC	8.95 ± 0.21^{aA}	6.00 ± 0.02^{c}	6.86 ± 0.15^{bA}	6.70 ± 0.08^{b}	5.06 ± 0.31^{d}

All values are mean ± standard deviation. [1] The abbreviations are the same as in Table 1. [A-D] Values within each column with different superscripts are significantly different (P<0.05). [a-d] Values within each row with different superscripts are significantly different (P<0.05)

Table 5: Changes in the number of *Streptococcus thermophilus* during storage of yogurt supplemented with various ingredients

Treatment groups	Storage time (days)				
	0	7	14	23	33
Control	8.39 ± 0.10^{bB}	9.26 ± 0.31^{aA}	8.56 ± 0.15^{bB}	8.81 ± 0.13^{b}	9.06 ± 0.15^{bA}
TRY	9.63 ± 0.15^{aA}	8.66 ± 0.29^{bB}	8.84 ± 0.06^{bB}	8.80 ± 0.10^{b}	8.45 ± 0.28^{cB}
WP	8.81 ± 0.31^{aB}	8.88 ± 0.24^{aB}	9.20 ± 0.45^{aB}	8.84 ± 0.25^{a}	8.52 ± 0.07^{aB}
WPC	8.70 ± 0.74^{aB}	8.67 ± 0.37^{aB}	9.44 ± 0.55^{aA}	8.65 ± 0.19^{a}	8.45 ± 0.10^{aB}
MP	8.72 ± 0.46^{aB}	8.67 ± 0.45^{aB}	8.18 ± 0.67^{aB}	8.26 ± 0.19^{a}	8.22 ± 0.22^{aB}
YE	8.45 ± 0.08^{aB}	8.70 ± 0.44^{aB}	8.56 ± 0.40^{aB}	8.64 ± 0.34^{a}	8.22 ± 0.05^{aB}
SUC	8.76 ± 0.12^{aB}	8.76 ± 0.52^{aB}	8.80 ± 0.05^{aB}	8.69 ± 0.08^{a}	8.48 ± 0.16^{aB}
TC	8.40 ± 0.45^{aB}	8.89 ± 0.29^{aB}	8.55 ± 0.03^{aB}	8.36 ± 0.11^{a}	8.12 ± 0.16^{aB}
TCS	8.90 ± 0.40^{aB}	8.49 ± 0.54^{aB}	8.18 ± 0.02^{aC}	8.36 ± 0.07^{a}	8.20 ± 0.12^{aB}
MP-SC	8.85 ± 0.40^{aB}	7.71 ± 0.40^{aB}	9.17 ± 0.05^{aB}	8.59 ± 0.20^{a}	8.24 ± 0.12^{aB}

All values are mean ± standard deviation. [1] The abbreviations are the same as in Table 1. [A-D] Values within each column with different superscripts are significantly different (P<0.05). [a-d] Values within each row with different superscripts are significantly different (P<0.05)

Table 6: Changes in the number of *Lactobacillus acidophilus* during storage of yogurt supplemented with various ingredients

Treatment groups	Storage time (days)				
	0	7	14	23	33
Control	6.36 ± 0.03^{aB}	6.23 ± 0.06^{aB}	6.03 ± 0.01^{aB}	5.81 ± 0.11^{aB}	5.39 ± 0.55^{aB}
TRY	6.07 ± 0.07^{aB}	5.77 ± 0.17^{aB}	6.06 ± 0.04^{aB}	5.74 ± 0.20^{aC}	5.42 ± 0.54^{aB}
WP	6.74 ± 0.05^{aB}	6.19 ± 0.19^{bB}	6.03 ± 0.04^{cB}	5.59 ± 0.24^{cC}	4.56 ± 0.68^{dC}
WPC	6.32 ± 0.32^{aB}	5.70 ± 0.36^{bB}	6.00 ± 0.03^{bB}	5.84 ± 0.13^{bB}	4.93 ± 0.17^{cC}
MP	5.70 ± 0.06^{bB}	6.56 ± 0.13^{aB}	6.15 ± 0.03^{bB}	5.35 ± 0.59^{cD}	4.33 ± 0.28^{dC}
YE	6.23 ± 0.28^{bB}	6.50 ± 0.27^{aB}	6.20 ± 0.03^{bB}	4.81 ± 0.02^{cD}	4.37 ± 0.17^{cC}
SUC	7.52 ± 0.00^{aA}	6.56 ± 0.38^{bB}	5.98 ± 0.20^{cB}	4.95 ± 0.04^{dD}	4.72 ± 0.13^{dC}
TC	6.18 ± 0.35^{bB}	6.80 ± 0.08^{aA}	6.47 ± 0.04^{bB}	4.92 ± 0.02^{cD}	4.36 ± 0.11^{dC}
TCS	6.34 ± 0.14^{bB}	6.68 ± 0.15^{aA}	6.15 ± 0.25^{bB}	4.87 ± 0.02^{cD}	4.48 ± 0.00^{cC}
MP-SC	6.49 ± 0.06^{aB}	6.60 ± 0.15^{aB}	6.80 ± 0.02^{aA}	6.45 ± 0.08^{aA}	5.86 ± 0.17^{bA}

All values are mean ± standard deviation. [1] The abbreviations are the same as in Table 1. [A-D] Values within each column with different superscripts are significantly different (P<0.05). [a-d] Values within each row with different superscripts are significantly different (P<0.05)

Table 7: Changes in the number of bifidobacteria during storage of yogurt supplemented with various ingredients

Treatment groups	Storage time (days)				
	0	7	14	23	33
Control	5.22 ± 0.02^{bB}	5.58 ± 0.01^{aB}	4.85 ± 0.02^{cB}	4.43 ± 0.25^{dB}	4.65 ± 0.09^{dC}
TRY	6.89 ± 0.01^{aA}	5.82 ± 0.34^{bA}	4.93 ± 0.19^{cB}	4.73 ± 0.34^{cB}	4.81 ± 0.13^{cC}
WP	5.26 ± 0.20^{bB}	5.67 ± 0.12^{aC}	5.18 ± 0.07^{bB}	4.44 ± 0.36^{cB}	4.80 ± 0.15^{cC}
WPC	5.44 ± 0.03^{aB}	5.17 ± 0.05^{bB}	4.87 ± 0.17^{cB}	4.80 ± 0.03^{cB}	5.20 ± 0.43^{bB}
MP	5.34 ± 0.39^{aB}	4.69 ± 0.0^{bD}	4.70 ± 0.01^{bB}	4.65 ± 0.04^{bB}	4.15 ± 0.15^{cC}
YE	4.88 ± 0.41^{aB}	4.82 ± 0.15^{aD}	4.74 ± 0.06^{aB}	4.33 ± 0.01^{bB}	4.60 ± 0.18^{aC}
SUC	5.26 ± 0.41^{aB}	4.99 ± 0.25^{bC}	4.90 ± 0.38^{bB}	4.73 ± 0.56^{aB}	4.64 ± 0.39^{bC}
TC	4.73 ± 0.73^{bB}	5.12 ± 0.07^{aC}	5.01 ± 0.24^{bB}	5.37 ± 0.01^{bA}	4.48 ± 0.40^{cC}
TCS	5.25 ± 0.21^{aB}	4.99 ± 0.14^{bC}	4.70 ± 0.20^{bB}	4.73 ± 0.03^{bB}	4.23 ± 0.06^{cC}
MP-SC	5.30 ± 0.17^{aB}	5.40 ± 0.08^{aC}	5.53 ± 0.24^{aA}	5.04 ± 0.02^{aB}	5.40 ± 0.18^{aA}

All values are mean ± standard deviation. [1] The abbreviations are the same as in Table 1. [A-D] Values within each column with different superscripts are significantly different (P<0.05). [a-d] Values within each row with different superscripts are significantly different (P<0.05)

bulgaricus gradually declined in all treatments, except those supplemented with TC and yeast extract, which showed the highest counts at 23 and 14 days, respectively.

At 14 days, the highest number of *L. delbrueckii* subsp. *bulgaricus* was recorded in yogurt supplemented with tryptone and MP-SC. At other days, counts of this bacterium had no significant differences between yogurt samples (P<0.05, Table 4).

The number of viable cells of *S. thermophilus* remained high (>10^8 cfu ml^{-1}) until 33 days from the date of production in all yogurt samples. Also, the highest counts of *S. thermophilus* were observed in control group during this period.

During the 33 days storage, the counts of *L. acidophilus* remained more than 10^5 ml^{-1} in yogurts supplemented with TRY, MP-SC and control group. However, the counts of *L. acidophilus* showed a constant decline in other groups during refrigerated storage. The highest counts of *L. acidophilus* were observed in yogurt supplemented with milk powder and inoculated with five fold starter culture.

Bifidobacteria counts remained higher than 10^5 cfu ml^{-1} in yogurt supplemented with MP-SC throughout the 33 days of refrigerated storage.

Discussion

Times taken to reach pH = 4.5 of the yogurt samples indicate a significant effect

of milk supplementation on incubation time, with the shortest value (225 min) observed in the milk supplemented with milk powder and the longest value (405 min) in the non-supplemented milk (control group). In addition, the decrease in pH was faster in yogurts supplemented with milk powder, casein fraction of milk proteins and whey proteins, respectively.

In a study published in (1998), Dave and Shah showed the effects of ingredient supplementation on pH change during a 24-h fermentation of yogurt with ABT starter culture. Results in this study, however, differ from our observation in that the acidification rate in yogurt with AB starter cultures were faster than yogurts fermented with ABT starter cultures. It could be due to the acidification potency of *L. delbrueckii* subsp. *bulgaricus*, and excluding this bacterium from the starter culture causes an increase in the incubation period. Furthermore, in ABT cultures, there is no symbiotic relationship; as a result, the incubation period to reach pH = 4.5 is longer for these cultures (Dave and Shah, 1998).

Therefore, using AB starter cultures might lead to shorter incubation time and increased productivity.

According to Sodini *et al*. (2002), the effect of supplement ingredients on fermentation time depends on the type of starter culture and there is no general rule that explains the effect of supplements on fermentation time, however, these supplements often decrease yogurt incubation time.

Titrable acidity values of yogurt samples supplemented with milk powder (MP and MP-SC) were significantly higher than other groups. It may be due to differences in buffering capacity of supplements.

Milk supplementation with various ingredients had no notable effects on *L. delbrueckii* subsp. *bulgaricus* counts during the 33 days of refrigerated storage. Dave and Shah (1997b) studied the effects of milk supplementation with various concentration of cysteine on growth and viability of *L. delbrueckii* subsp. *bulgaricus* during refrigerated storage for 25-30 days. They showed that the growth of this bacterium was enhanced at 50 mg L^{-1} and was suppressed at 250 and 500 mg L^{-1} cysteine. However, at the end of 33 days, the counts of *L. delbrueckii* subsp. *bulgaricus* were higher with increasing concentration of cysteine.

In our investigation, counts of *S. thermophilus* remained higher than counts of lactobacilli during the storage of all samples. In this regard, Kneifel *et al*. (1993) showed that approximately 80% of commercial yogurts had higher counts of cocci than rods. The counts of *S. thermophilus* were highest in yogurt supplemented with tryptone at 0 day, which is in agreement with the report of Dave and Shah (1998) (Table 5). The counts of *S. thermophilus* increased slightly at 7 days in the control, WP, and TC groups. In conclusion, milk supplements did not affect the *S. thermophilus* counts in probiotic yogurt during refrigerated storage at 4°C. Dave and Shah (1998) reported changes in counts of *S. thermophilus* in ABT yogurt supplemented with different ingredients, but it should be noted that they have no applied statistical analysis tools to compare their results. For instance, they reported that the incorporation of cysteine at 250 or 500 mg L^{-1} adversely affected the growth of *S. thermophilus*. But cysteine at 50 mg L^{-1} was found to promote the growth of *S. thermophilus* (Dave and Shah, 1997b; Dave and Shah, 1998). Wang *et al.* (2002) showed that, 3-4 log reduction in the number of *S. thermophilus* of fermented soymilk drinks supplemented with cysteine occurred when it was held at 25°C for 10 days compared to the 4°C, whilst our results did not show any significant effect of yogurt supplemented with cysteine. On average, the survival of *S. thermophilus* was improved compared to the yogurt probiotic bacteria.

Dave and Shah (1998) have also reported that at 0 day, counts of *L. acidophilus* were lower in the ABT probiotic yogurt sample supplemented with WP, WPC or TRY than that observed in control yogurt, but these results are contrary to our findings. The addition of 500 mg L^{-1} of cysteine promoted the growth of *L. acidophilus* until two weeks from the date of production. This confirmed the findings of Dave and Shah (1997b and 1998) who observed an improvement in the viability of *L. acidophilus* in yogurt supplemented with 250 or 500 mg L^{-1} of cysteine.

They concluded that, the adverse effect of cysteine on *S. thermophilus* is more likely caused by a prolonged fermentation time and perhaps favored the multiplication of *L. acidophilus* in yogurt supplemented with cysteine (Dave and Shah, 1997b). In the present study, improved viability of *L. acidophilus* was neither due to the adverse effect of cysteine on *S. thermophilus* nor the longer fermentation time, as there was no significant ($P<0.05$) difference for the counts of *S. thermophilus* between yogurts supplemented with cysteine by other yogurt samples, or the counts of *L. acidophilus* yogurts with shorter fermentation time.

The present study showed that milk supplementation with the mentioned ingredients (except for TRY and MP-SC) has a negative impact on viability of *L. acidophilus* in AB probiotic yogurt during 33 days of refrigerated storage.

During 33 days of refrigerated storage, the counts of *L. acidophilus* were highest in yogurt supplemented with milk powder and inoculated with five fold starter culture.

When the time to reach pH = 4.5 was taken into consideration, the counts of bifidobacteria were significantly ($P<0.05$) higher in the yogurt supplemented with tryptone than that shown in other yogurt samples (Table 7). Using a high level of inoculums will ensure a high cell count at the end of the inoculation and survival of the probiotic bacteria during storage until consumption (Samona and Robinson, 1994). In the present study, fivefold increase in inoculums caused a significant increase ($P<0.05$) in the survival of bifidobacteria during storage period. In contrast, Dave and Shah (1997b) showed that increased inoculums did not improve the viability of bifidobacteria in yogurt made with ABT starter culture.

At 0 day, counts of bifidobacteria were lowest in yogurt supplemented with YE, but bifidobacteria counts remained constant in this group until the end of storage. The highest counts of bifidobacteria were observed in yogurt with TRY at the first week of refrigerated storage, but the counts decreased to $<10^5$ ml^{-1} from 7 days until the end of storage.

This study presented the behavior of yogurt and probiotic bacteria in the presence of different milk supplements. All batches of yogurt showed different patterns of change in pH, titrable acidity and counts of *Streptococcus thermophiles*, *Lactobacillus delbrueckii* subsp. *Bulgaricus*, *Lactobacillus acidophilus* and bifidobacteria. The time to reach pH = 4.5 decreased considerably in yogurt supplemented with milk powder; also, the increase in titrable acidity was dependent on added supplement. Tryptone and milk powder, in addition to fivefold starter culture were found the most effective supplements to improve growth and viability of starter and probiotic (*L. acidophilus* and bifidobacteria) bacteria in probiotic yogurt during refrigerated storage for five weeks. These results would be applicable to the development of probiotic containing fermented dairy products. Legal and economical aspects should be considered for the industrial application of these supplements in the manufacturing of yogurt.

References

AOAC (1984). *Official methods of analysis*. 14th Edn., Washington D.C., Association of Official Analytical Chemists. P: 1141.

Dave, RI and Shah, NP (1997a). Viability of yoghurt and probiotic bacteria in yoghurts made from commercial starter cultures. Int. Dairy J., 7: 31-41.

Dave, RI and Shah, NP (1997b). Effect of cysteine on the viability of yoghurt and probiotic bacteria in yoghurts made with commercial starter cultures. Int. Dairy J., 7: 537-545.

Dave, RI and Shah, NP (1998). Ingredient supplementation effects on viability of probiotic bacteria in yogurt. J. Dairy Sci., 81: 2804-2816.

De Vuyst, L (2000). Technology aspects related to the application of functional starter culture. Food Technol. Biotech., 38: 105-112.

Gilliland, SE; Reilly, SS; Kim, GB and Kim, HS (2002). Viability during storage of selected probiotic Lactobacilli and bifidobacteria in yogurt-like product. J. Food Sci., 67: 3091-3095.

Gomez, AMP and Malcata, FX (1999). Bifidobacteria spp. and *Lactobacillus acidophilus*: biological, biochemical, technological and therapeutical properties relevant for use as probiotic. Trends Food Sci. Tech., 10: 139-157.

ISO/DIS 20128 IDF 192 (2005). Fermented

milk-enumeration of *Lactobacillus acidophilus*-Colony-count technique at 37°C. International Organization for Standardization and International Dairy Federation.

Kneifel, W; Jaros, D and Erhard, F (1993). Microflora and acidification properties of yogurt and yogurt related products fermented with commercially available starter cultures. Int. J. Food Microbiol., 18: 179-189.

Lourens-Hattingh, A and Viljoen, BC (2001). Yogurt as probiotic carrier food. Int. Dairy J., 11: 1-17.

Oliveira, MN; Sodini, I; Remeuf, F and Corrieu, G (2001). Effect of milk supplementation and culture composition on acidification, textural properties and microbiological stability of fermented milk containing probiotic bacteria. Int. Dairy J., 11: 935-942.

Prado, FC; Parada, JL; Pandey, A and Soccol, CR (2008). Trends in non-dairy probiotic beverages. Food Res. Int., 41: 111-123.

Saarela, M; Mogensen, G; Fonden, R; Mättö, J and Mattila-Sandholm, T (2000). Probiotic bacteria: safety, functional and technological properties. J. Biotechnol., 84: 197-215.

Samona, A and Robinson, RK (1994). Effect of yogurt cultures on the survival of bifidobacteria in fermented milks. J. Soc. Dairy Technol., 47: 58-60.

Shah, NP; Lankaputhra, WEV; Britz, ML and Kyle, WSA (1995). Survival of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in commercial yoghurt during refrigerated storage. Int. Dairy J., 5: 515-521.

Sodini, I; Lucas, A; Oliveira, MN; Remeuf, F and Corrieu, G (2002). Effect of milk base and starter culture on acidification, texture, and probiotic cell counts in fermented milk processing. J. Dairy Sci., 85: 2479-2488.

Tamime, AY and Robinson, RK (1999). Yogurt: science and technology. 2nd Edn., Cambridge, UK, CRC Press. P: 640.

Tamime, AY; Saarela, M; Korslund Sondergaard, A; Mistry, VV and Shah, NP (2005). Production and maintenance of viability of probiotic micro-organisms in dairy products. In: Tamime, AY (Eds.), *Probiotic dairy products*. Oxford, Uk, Blackwell Publishers. PP: 39-72.

Wang, YC; Yu, RC and Chou, CC (2002). Growth and survival of bifidobacteria and lactic acid bacteria during the fermentation and storage of cultured soymilk drinks. Food Microbiol., 19: 501-508.