Journal of Food Biosciences and Technology,
Islamic Azad University, Science and Research Branch, Vol. 4, No. 1, 21-30, 2014

The Effect of Milk Supplementation on the Growth and Viability of Starter and Probiotic Bacteria in Yogurt during Refrigerated Storage

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Received 8 October 2012; Accepted: 24 April 2013

ABSTRACT: In the present work, the effect of milk supplementation on viability of yogurt bacteria (*Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus*) and probiotic bacteria (*Lactobacillus acidophilus* and *Bifidobacteria*) was studied during yogurt manufacture and thirty three days storage. Incubation time to reach pH value of 4.5 was greatly affected by the addition of casein fraction of milk proteins. The viable counts of *L. delbrueckii subsp. bulgaricus* and *Bifidobacteria* were increased in yogurt supplemented with tryptone and milk powder plus five fold starter culture. Addition of 500 mg L^{-1} of cysteine, promoted the growth of *L. acidophilus* until three weeks from the date of production. *Bifidobacteria* counts remained more than 10^5 cfu ml^{-1} in yogurt supplemented with 2% milk powder and inoculated with five fold starter culture. Using a high level of inoculums promoted the viability of *L. acidophilus* and *Bifidobacteria* significantly ($p< 0.05$) in the first week of storage.

Keywords: *Probiotic, Supplementation, Yogurt.*

Introduction

Probiotics are defined as viable microorganisms that exhibit a beneficial effect on the health of the host upon ingestion by improving the properties of indigenous microflora (Gomez & Malcata, 1999).

Probiotics enhance the population of beneficial bacteria in the human gut, suppress pathogens and build up resistance against intestinal diseases. The modulation of immunity, alleviation of lactose intolerance, prevention of some forms of cancers and the lowering of serum cholesterol by these bacteria has also been reported (Talwalker & Kialasapathy, 2003; Prado *et al*., 2008).

Probiotic microorganisms can not affect the intestinal environment unless their population reaches a certain minimum level. There is no general agreement on the minimum concentration for probiotic bacteria, while some researchers suggest concentration level of above 10^5-10^6 viable cell per ml or gram of product (Dave & Shah, 1997), other stipulate more than 10^7 and 10^8 as satisfactory level (De Vuys, 2000).

Several members of the lactic acid bacteria have gained recognition as probiotic bacteria, amongst them, *Lactobacillus acidophilus* and *Bifidobacteria* are more significant.

Yogurt has long been recognized as a product with many desirable effects for consumer. In recent years, there has been a significant increase in the popularity of yogurt as a food product (Lourens-Hattingh

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& Viljoen, 2001).

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 objectional taste imported by some of the probiotic bacterial strains (Dave & Shah, 1997; Lourens-Hattingh & Viljoen, 2001; Tamime, 2005).

Most of the probiotic yogurts include live strains of *L. acidophilus* and species of *Bifidobacterium* in addition to the conventional yogurt organisms, *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. Bulgaricus* (Tamime, 2000; Tamime, 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; Nighswonger *et al*., 1996; Vinderola *et al*., 2000).

Several works have been done to improve the growth and viability of probiotic bacteria by adding supplements to milk base. Milk supplementation by adding dairy ingredients (Dave & Shah, 1997; Oliviera *et al*., 2001; Sodini *et al*., 2002), Oxygen scavengers (Dave & Shah, 1997; Dave & Shah, 1998) and carbohydrates (Chick *et al*., 2001) have been reported.

In this regard, most of the efforts have been done using ABT (*L. acidophilus, Bifidobacteria and S. thermophilus*) starter cultures that are devoid of *L. delbrueckii subsp. bulgaricus* (Dave & Shah, 1998; Oliviera *et al*., 2001).

L. delbrueckii subsp. bulgaricus produces lactic acid during refrigerated storage. This process is known in the industry as "post-acidification" and, if it occurs, it causes a loss in viability of the probiotic bacteria (Tamime, 2005).

L. delbrueckii subsp. bulgaricus has a critical role in lactic acid and flavor production in yogurt and when excluded from the starter culture, it profoundly affects the texture, acidity and the aroma of the final product.

Therefore, in recent years some yogurt products have been made using AB-yogurt cultures (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 & Viljoen, 2001).

The effect of milk supplementation on growth and viability of probiotic and yogurt bacteria in such fermented products have not been studied precisely.

Therefore 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. These results would be applicable to the development of probioric containing fermented dairy products.

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

Materials and Methods

- *Starter culture and dairy ingredients*

A commercial AB-yogurt starter culture was used in this study. The starter culture was in freeze dried DVS (Direct Vat System) form.

The storage and maintenance of the culture was carried out according to the recommendation of the manufacturer.

Six ingredients and their combinations have been tested (table 1).

- *Yogurt production and storage*

Pasteurized and homogenized milk containing 2.5% fat and 10.4% total solid non-fat, was tempered to 50ºC and fortified with supplements as table 1. The mixtures were heated to 85ºC for 30 min, cooled to 43ºC, and the starter culture was added (as recommended by the supplier). The inoculated fortified milk was dispensed in 100 ml polystyrene cups. The cups were heat-sealed with aluminum foil (thickness. 80 µm).

Incubation was carried out at 43 ± 0.5ºC and fermentation was terminated at pH of 4.5.

The time taken to reach the pH of 4.5 was recorded for each group in order to study the effects of added supplements.

When the fermentation was terminated, yogurt cups were stored at 4ºC for 33 days.

- *Chemical analysis (pH and titrable acidity determination)*

The pH values of the inoculated dairy mixtures and yogurts were measured using a pH electrode and meter (Hach pH meter, Hach company, USA) after calibration.

The titrable acidity (TA) was determined by the AOAC method and expressed as % lactic acid (AOAC, 1984).

The pH and TA values of samples were measured at 30 min intervals, 45-min after incubation at 4ºC, until the pH value of 4.5 was reached.

During the refrigeration storage, samples were taken at pre-determined intervals to determine the pH and TA as well as the viable counts of starter and probiotic bacteria in yogurt samples.

The one day period represents the analyses carried out after the samples pH reached the value of 4.5 and periods of 6 to 33 days represent analyses of yogurt samples after 6, 14, 23, 33 days of storage at 4ºC, respectively.

- *Microbiological analyses*

Viable counts of *S. thermophilus*, *L. delbrueckii subsp. bulgaricus, L. acidophilus* and *bifidobacteria* were monitored during manufacturing and storage period for 33 days at 4ºC.

One gram of each yogurt sample was diluted with 9 ml of 0.09 sterile normal saline and was mixed uniformly with a vortex mixer.

Appropriate dilutions were made and subsequently pour-plate method was applied in duplicate order onto the selective media.

The counts of *S. thermophilus* were enumerated on M17 agar (Merck, Darmstadt, Germany) and by incubating the plates aerobically at 37ºC for 24 ± 3h (Dave & Shah, 1998).

Differential enumeration of *L. delbrueckii subsp. bulgaricus* was performed on MRS agar (Merck, Darmstadt, Germany) adjusted to the pH of 5.2 and anaerobic incubation at 43ºC for 72h (Dave & Shah, 1998).

MRS - clindomycin - ciprofloxacin agar (MRS-CL/CIP Agar) was used for selective enumeration of *L. acidophilus* by incubating the plates anaerobically at 37ºC for 72h ± 3h (ISO / DIS 20128 IDF 192).

Selective enumeration of *Bifidobacteria* was performed on MRS agar supplemented with 0.5 mg L^{-1} dichloxallin (Sigma Chemical Co., St Louis, USA), 1 gr L-1 Lithium chloride (Merck, Darmstadt, Germany) and 0.5 gr L-1 cysteine hydrochloride (Merck, Darmstadt, Germany). The anaerobic incubation at 37ºC

for 72h ± 3h was performed by modification of the method described by Favaro-Trindade & Grosso (2004).

- *Statistical analyses*

For each condition, three independentreplicates of the experiments were carried out. Before statistical analysis, the populations of bacteria were converted to log CFU g^{-1}. All data were analyzed using the one way ANOVA procedure of the SPSS, version 11.5 (SPSS, Chicago, Ill.). Duncan's multiple range test was used to determine if significant differences existed among logs CFU g^{-1} of bacteria

Results and Discussion

- *Effects of milk supplementation on pH and titrable acidity*

Changes in pH and TA during incubation at 45ºC for the yogurt mixtures are shown in table 2 and table 3 respectively.

The times taken to reach the pH value of 4.5 in the yogurt samples indicate the significant effect of milk supplementation on the incubation time. The shortest time (3.7 h) was observed in the milk supplemented with yeast extract and the longest time (6.8 h) was observed in the milk without supplementation (control group).

Table 2. Changes in pH during the fermentation of yogurt (to reach pH ~ 4.5)

Treatment Groups		Time (min)												
		45	**75**	**105**	**135**	**156**	**195**	**225**	**255**	**285**	**315**	**345**	**375**	**405**
Control	X	6.48	6.41	6.36	6.35	6.32	6.18	5.98	5.76	5.32	5.05	4.90	4.75	4.57
	S	0.02	0.06	0.07	0.05	0.02	0.16	0.25	0.32	0.14	0.11	0.14	0.17	0.09
TRY	X	6.50	6.41	6.21	6.00	5.60	4.99	4.76	4.62					
	S	0.00	0.05	0.05	0.06	0.05	0.10	0.04	0.08					
WP	X	6.49	6.41	6.32	6.25	5.92	5.37	5.05	4.77	4.66	4.54			
	S	0.02	0.04	0.07	0.08	0.14	0.10	0.04	0.01	0.04	0.01			
WPC	X	6.51	6.42	6.38	6.33	6.15	5.89	5.16	4.85	4.71	4.60			
	S	0.01	0.05	0.01	0.04	0.01	0.28	0.25	0.13	0.05	0.10			
MP	X	6.37	6.20	5.88	5.36	4.98	4.68	4.54						
	S	0.04	0.11	0.25	0.40	0.21	0.07	0.01						
YE	X	6.18	6.05	5.82	5.54	5.25	5.00	4.78	4.68	4.53				
	S	0.06	0.09	0.21	0.20	0.13	0.00	0.04	0.08	0.04				
Suc	X	6.15	6.03	5.80	5.52	5.24	5.00	4.85	4.65					
	S	0.01	0.08	0.21	0.20	0.09	0.00	0.06	0.15					
TC	X	6.38	6.29	6.23	6.20	5.82	5.46	5.22	4.86	4.62				
	S	0.04	0.00	0.01	0.01	0.47	0.66	0.57	0.29	0.12				
TCS	X	6.38	6.30	6.18	5.77	5.30	4.90	4.72	5.06					
	S	0.01	0.01	0.04	0.34	0.21	0.00	0.01	0.67					
MP-SC	X	6.41	6.34	6.23	5.91	5.29	4.92	4.72	4.58					
	S	0.01	0.01	0.01	0.03	0.04	0.03	0.02	0.02					

* X and S are mean values and standard deviation respectively.

Table 3. Changes in titrable acidity during the fermentation of yogurt (to reach pH ~ 4.5)

Treatment Groups		Time (min)											
		45	75	105	135	156	195	225	255	285	315	345	375
Control	X	14.8	16.1	16.6	16.8	17.3	20.1	27.8	31.3	39.5	45.0	51.5	49.8
	S	0.8	1.4	0.8	1.1	0.7	2.0	9.6	11.0	3.5	0.0	2.1	15.9
TRY	X	16.1	17.8	20.5	24.8	34.1	51.3	58.0	62.0				
	S	0.1	1.0	1.4	3.9	1.5	1.8	2.8	0.0				
WP	X	15.2	16.1	17.8	19.7	29.4	37.8	46.3	55.5	62.0	68.0		
	S	0.6	0.9	1.1	1.9	4.7	3.2	1.8	3.5	2.8	8.5		
WPC	X	14.8	16.4	16.7	18.1	23.2	26.6	40.5	52.3	57.5	61.5		
	S	0.3	0.5	0.4	0.6	6.6	6.9	6.4	1.8	2.1	2.1		
MP	X	16.9	20.5	25.9	39.5	52.5	58.5	64.2					
	S	0.6	3.5	7.3	14.4	13.4	9.2	5.9					
YE	X	20.0	24.6	25.9	33.5	44.1	48.0	61.0	55.5				
	S	0.0	4.4	5.4	8.1	10.0	4.2	15.6	0.7				
Suc	X	20.8	23.5	26.6	33.3	41.2	48.0	59.0	57.5				
	S	0.4	3.5	4.4	8.1	7.1	7.1	14.1	0.7				
TC	X	15.1	16.4	17.8	21.0	28.3	33.5	49.0	50.6	56.0			
	S	0.1	0.9	2.5	5.4	13.8	16.3	33.)	15.0	8.5			
TCS	X	17.2	18.9	21.5	30.4	44.0	57.5	66.6	72.0				
	S	1.2	2.3	4.2	10.1	10.6	3.5	3.7	2.8				
MP-SC	X	18.5	19.5	22.5	27.5	44.7	59.5	65.0	71.0				
	S	0.7	0.7	0.7	0.7	0.5	0.7	1.4	1.4				

* X and S are mean values and standard deviation respectively.

Based on these findings, the decrease in the pH value was faster in yogurts that were supplemented with casein fraction of milk proteins than that observed in yogurt that was supplemented only with whey proteins.

This is in agreement with the report of Sodini *et al*. in 2002, who showed that milk supplementation with casein hydrolysate and milk protein concentrate decreases the fermentation time required to reach the pH value of 5 for milk when incubated with single culture of *S. thermophilus* ST7*, L. delbrueckii subsp. bulgaricus* LB12*, L. acidophilus* LA5 and *L. rhamnosus* LR35.

The decrease in the pH was faster in yogurt containing WP, WPC, acid hydrolysate of casein (ACH), or tryptone than that of the control group. An increase in the concentration of cysteine from 50 mgl^{-1} to 500 mgl^{-1} caused a pronounced increase in the time taken to reach the pH value of 4.5 (Dave & Shah, 1998).

- *Effects of milk supplementation on viability of starter and probiotic bacteria*

Changes in viable counts of starter and probiotic bacteria in yogurt supplemented with various ingredients during 33 of refrigerated storage are shown in table 4 to table 7.

Statistical analysis showed, when the pH reached the value of 4.5, counts of *L. delbrueckii subsp. bulgaricus* were higher in yogurt supplemented with tryptone, WP,

WPC and MP-SC5, than other groups.

During storage for 33 days, the counts of *L. delbrueckii subsp. bulgaricus* declined gradually in all yogurts, except those supplemented with TC and yeast extract, which showed the highest counts of this bacterium at 23rd day and 14th day respectively. At the 14th day, counts of *L. delbrueckii subsp. bulgaricus* were highest in yogurt supplemented with tryptone and MP-SC5 while the counts of this bacterium, didn't have meaningful different between yogurt samples ($p< 0.05$) for other days.

The viability of *L. delbrueckii subsp. bulgaricus* Lb1466 was enhanced significantly ($p< 0.05$) in the presence of probiotic organisms during storage at 4^{oC} for 28 day (Donkor *et al*., 2006).

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. This is in agreement with the report of Dave & Shah, 1998, that the counts of *S. thermophilus* were highest in yogurt supplemented with tryptone.

The counts of *S. thermophilus* increased slightly until the 14th day of storage and then declined for the control, WP, WPC, yeast and MP-SC5 groups. The incorporation of cysteine at 250 or 500 mg L^{-1} adversely affected the growth of *S. thermophilus*. In contrast, cysteine at 50 mg L^{-1} was found to promote the growth of *S. thermophilus* (Dave & Shah, 1997; Dave & Shah, 1998).

Our results did not show significant effect of milk supplementation with 500 mg L^{-1} of cysteine on the viable counts of *S. thermophilus*, while marked reduction (3-4 log) of counts related to *S. thermophilus* occurred when fermented soymilk drinks were held at 25ºC for 10 days. In contrast the viable counts remained high in drinks held at 4ºC (Wang *et al*., 2002).

The survival rate of *S. thermophilus* was better as compared to the yogurt containing probiotic bacteria.

Counts of *L. acidophilus* showed a constant decline in all yogurt products during refrigerated storage.

From 0 day until the 14th day, the counts of *L. acidophilus* were significantly higher.

Addition of 500 mg L^{-1} of cysteine, promoted the growth of *L. acidophilus* until three weeks from the date of production. This confirmed the findings of Dave & Shah (1997 and 1998) who observed improved viability of *L. acidophilus* in yogurt supplemented with 250 or 500 mgl^{-1} of cysteine.

They concluded that, these results could be due to the adverse effect of cysteine on *S. thermophilus* that caused prolonged fermentation time and perhaps favored the multiplication of *L. acidophilus* in yogurt supplemented with cysteine (Dave & Shah, 1997b).

In the present study, improved viability of *L. acidophilus* was neither due to the adverse effect of cysteine on *S. thermophilus* nor longer fermentation time.

There were not significant ($p< 0.05$) differences in the counts of *S. thermophilus* between yogurts supplemented with cysteine and other yogurt samples and the counts of *L. acidophilus* yogurts with shorter fermentation time.

All the products showed a decline in viable counts of *Bifidobacteria* during refrigerated storage except the yogurt supplemented with milk powder and inoculated with fivefold starter culture (MP-SC5).

The counts of *Bifidobacteria* were higher considerably between 15 to 33 day of storage in yogurt supplemented with sucrose and MP-SC5 than other yogurt samples. Only, *Bifidobacteria* counts remained more than 10^5 cfu ml^{-1} in yogurt supplemented with MP-SC5 throughout the 33 days of refrigerated storage.

When the time to reach the pH value of 4.5 was taken into consideration, the counts of *Bifidobacteria* were significantly ($p<0.05$) higher in the yogurt that was supplemented

with tryptone.

The use of 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 & Robinson, 1994).

In the present study, five fold increase in inoculum caused significant ($p< 0.05$) increase in the survival of *Bifidobacteria* during storage period. This is in contrast with Dave & Shah, (1997 b) findings who showed that increased inoculum did not improve the viability of *Bifidobacteria* in yogurt made with ABT starter culture.

Table 4. Changes in the number of *Lactobacillus delbrueckii ssp. Bulgaricus* during storage of yogurt supplemented with various ingredients

Treatment Groups	Storage time (days)				
	0	7	14	23	33
Control	6.45 (0.00)	6.40 (0.40)	6.38 (0.17)	5.79 (0.24)	5.98 (0.16)
TRY	7.89 (0.41)	6.28 (0.28)	6.73 (0.22)	5.98 (0.41)	5.25 (0.95)
WP	7.43 (1.16)	6.50 (0.00)	6.49 (0.02)	5.73 (0.58)	6.19 (0.11)
WPC	6.77 (0.10)	6.39 (0.52)	6.30 (0.24)	6.39 (0.60)	5.95 (0.10)
MP	6.37 (0.00)	6.09 (0.12)	6.23 (0.00)	6.25 (0.18)	5.82 (0.12)
YE	6.36 (0.16)	6.22 (0.09)	6.52 (0.17)	6.21 (0.03)	5.95 (0.01)
Suc	6.56 (0.20)	6.20 (0.48)	6.32 (0.32)	5.95 (0.04)	5.37 (0.13)
TC	6.27 (0.00)	6.50 (0.22)	6.65 (0.12)	6.71 (0.02)	5.93 (0.04)
TCS	6.55 (0.30)	6.17 (0.30)	6.40 (0.06)	6.23 (0.06)	6.07 (0.37)
MP-SC	8.95 (0.21)	6.00 (0.02)	6.86 (0.15)	6.70 (0.08)	5.06 (0.31)

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)	9.26 (0.31)	8.56 (0.15)	8.81 (0.13)	9.06 (0.15)
TRY	9.63 (0.15)	8.66 (0.29)	8.84 (0.06)	8.80 (0.10)	8.45 (0.28)
WP	8.81 (0.31)	8.88 (0.24)	9.20 (0.45)	8.84 (0.25)	8.52 (0.07)
WPC	8.70 (0.74)	8.67 (0.37)	9.44 (0.55)	8.65 (0.19)	8.45 (0.10)
MP	8.72 (0.46)	8.67 (0.45)	8.18 (0.67)	8.26 (0.19)	8.22 (0.22)
YE	8.45 (0.08)	8.70 (0.44)	8.56 (0.40)	8.64 (0.34)	8.22 (0.05)
Suc	8.76 (0.12)	8.76 (0.52)	8.80 (0.05)	8.69 (0.08)	8.48 (0.16)
TC	8.40 (0.45)	8.89 (0.29)	8.55 (0.03)	8.36 (0.11)	8.12 (0.16)
TCS	8.90 (0.40)	8.49 (0.54)	8.55 (0.02)	8.36 (0.07)	8.20 (0.12)
MP-SC	8.85 (0.40)	7.71 (0.40)	9.17 (0.05)	8.59 (0.20)	8.24 (0.12)

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)	6.23 (0.06)	6.03 (0.01)	5.81 (0.11)	5.39 (0.55)
TRY	6.07 (0.07)	5.77 (0.17)	6.06 (0.04)	5.74 (0.20)	5.42 (0.54)
WP	6.74 (0.05)	6.19 (0.19)	6.03 (0.04)	5.59 (0.24)	4.56 (0.68)
WPC	6.32 (0.32)	5.70 (0.36)	6.00 (0.03)	5.84 (0.13)	4.93 (0.17)
MP	6.34 (0.06)	6.56 (0.13)	6.15 (0.03)	5.35 (0.59)	4.33 (0.28)
YE	5.70 (0.28)	6.50 (0.27)	6.20 (0.03)	4.81 (0.02)	4.37 (0.17)
Suc	6.18 (0.00)	6.56 (0.38)	5.98 (0.20)	4.95 (0.04)	4.72 (0.13)
TC	6.23 (0.35)	6.80 (0.08)	6.47 (0.04)	4.92 (0.02)	4.36 (0.11)
TCS	7.50 (0.14)	6.68 (0.15)	6.15 (0.25)	4.87 (0.02)	4.48 (0.00)
MP-SC	6.49 (0.06)	6.60 (0.15)	6.80 (0.02)	6.45 (0.08)	5.86 (0.17)

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)	5.58 (0.01)	4.85 (0.02)	4.43 (0.25)	4.65 (0.09)
TRY	6.89 (0.01)	5.82 (0.34)	4.93 (0.19)	4.73 (0.34)	4.81 (0.13)
WP	5.26 (0.20)	5.67 (0.12)	5.18 (0.07)	4.44 (0.36)	4.80 (0.15)
WPC	5.44 (0.03)	5.17 (0.05)	4.87 (0.17)	4.80 (0.03)	5.20 (0.43)
MP	5.34 (0.39)	4.69 (0.08)	4.70 (0.01)	4.65 (0.04)	4.15 (0.15)
YE	4.88 (0.41)	5.69 (0.15)	4.74 (0.06)	4.33 (0.01)	4.60 (0.18)
Suc	4.88 (0.41)	5.12 (0.25)	5.30 (0.38)	5.37 (0.56)	4.64 (0.39)
TC	4.73 (0.73)	5.82 (0.07)	5.01 (0.24)	4.71 (0.01)	4.48 (0.40)
TCS	5.25 (0.21)	4.99 (0.14)	4.97 (0.20)	4.73 (0.03)	4.23 (0.06)
MP-SC	5.30 (0.17)	5.40 (0.08)	5.53 (0.24)	5.04 (0.02)	5.40 (0.18)

Conclusion

Milk supplementation by adding various dairy and non-dairy ingredients showed different patterns of decrease or increase in pH or titrable acidity during manufacture and refrigerated storage of probiotic yogurt.

The viable counts of *S. thermophilus, L. delbrueckii subsp. bulgaricus, L. acidophilus* and *Bifidobacteria* were considerably affected by the added ingredients.

The time taken to reach the pH value of 4.5 decreased considerably by addition of 2 gr L-1 of yeast extract, tryptone, milk powder and 500 mg L-1 cysteine, but the incubation time increased in yogurt mixes supplemented with 2 gr L-1 of whey protein and whey protein concentrate.

The viability of *L. delbrueckii subsp. Bulgaricus* was improved in yogurt supplemented with tryptone, milk powder, WP and WPC.

The addition of 500 mg L-1 of cysteine promoted the viability of *L. acidophilus* up to 15 days of refrigerated storage, while the viability of *L. acidophilus* was adversely affected on addition of cysteine from 21 to 33 days of storage.

The use of high level of inoculums significantly increased viability of *L. acidophilus* and *Bifiodobacteria* from 15 to 33 days of storage. Furthermore, the addition of 2 gr L-1 tryptone increased the viable count of *Bifidobacteria* for the first week of the storage. While the addition of growth promoting substances affected the growth and viability of probiotic bacteria in yogurt but based on related researches it is clear that the proper selection and combination of probiotic strains has a profound effect on the growth and survival of probiotic bacteria in fermented milks.

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