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REGULAR ARTICLE

VIABILITY OF *Lactobacillus acidophilus* AND SYNERESIS OF PROBIOTIC YOGHURT PRODUCED FROM RECONSTITUTED SKIM AND WHOLE MILK POWDER DURING 35 DAYS REFRIGERATED STORAGE AT 4±2 ºC

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ABSTRACT

Currently, the food industry wants to expand the range of probiotic yogurts but each probiotic bacteria offers different and specific health benefits. This study examined the viable counts of *Lactobacillus acidophilus* and percentage syneresis of probiotic yoghurt produced from reconstituted skim and whole milk powder stored for 35 days at 4±2 ºC. Skim milk probiotic yoghurt (SMPY) and Whole milk probiotic yoghurt (WMPY) were produced by reconstituting dry milk powder (130 g/900 ml w/v), which was pasteurized at 85 ºC for 15 mins, cooled to 43 ºC and inoculated with freeze dried probiotic yoghurt mixed starter culture containing *Streptococcus thermophilus* (ST), *Lactobacillus bulgaricus* (LB) and *Lactobacillus acidophilus* (LA). The yoghurt samples were analyzed for viability of ST, LB, LA and also syneresis. During storage, the viable counts of ST in SMPY decreased from $5.43x10^8$ to $5.18x10^6$ cfu/ml, LB ($2.47x10^8$ to $8.10x10^5$ cfu/ml) and LA ($1.83x10^8$ to $5.78x10^5$ cfu/ml). Similarly, the viable counts of ST in WMPY decreased significantly from $5.40x10^8$ to $5.15x10^6$ cfu/ml, LB ($2.43x10^8$ to $7.82x10^5$ cfu/ml) and LA ($1.80x10^8$ to $5.84x10^5$ cfu/ml). Although the mean viable counts of the LA decreased during storage, both SMPY and WMPY still contained an average of $1.48x10^6$ cfu/ml of probiotic cells up to 28 days of storage, which is above the "therapeutic minimum" of 10^6 cfu/ml. The percentage syneresis of SMPY and WMPY increased significantly during the 35 days of storage, from 24.4-32.0 % and 24.8-32.7 % respectively. There was a positive correlation between storage time and syneresis thus affecting the texture. In conclusion, yoghurt made from either skim or whole milk powder can be used as an adequate carrier of LA (probiotic bacteria) up to a period of 28 days at 4±2 ºC and a stabilizer should be used to reduce the separation of whey and thus maintain the texture.

Keywords: Lactic acid bacteria, probiotic bacteria, plain stirred yoghurt, syneresis, storage time

INTRODUCTION

Lactic acid bacteria (LAB) that are basically used in the dairy industry for the fermentation of a wide variety of food products are used primarily for their preservative and therapeutic effects (**Gourama and Bullerman, 1995**). However, during the past two decades, there has been renewed interest in the study of the nutritional and therapeutic aspects of dairy products (**Sinha *et al.*, 1989**). *Streptococcus thermophilus* and *Lactobacillus delbruekii* ssp. *bulgaricus* are used for manufacturing of yogurt. *Bifidobacteria spp*, *Lactobacillus acidophilus* and *Lactobacillus casei* are widely used as probiotic bacteria in human and animal health (**Gilliland, 1990**). They are called probiotic bacteria and are defined as living microorganisms, which upon ingestion in sufficient quantity exert health benefits beyond inherent basic nutrition (**Grosso and Trindade, 2004**). Also, with the advent of functional foods (foods with additional health benefits), the trend towards healthier eating has continued to grow (**Farooq and Haque, 1992**) and emphasis has been placed on developing new fermented milks containing these microorganisms called probiotics (**Banon and Hardy, 1991**). *Lactobacillus spp*. (probiotic microorganisms) constitutes a major part of the human intestinal microflora and plays an important role in maintaining good health (**Tamime and Robinson, 1985**). *Lactobacillus spp*. are increasingly being incorporated into fermented dairy products such as yoghurt and acidophilus milk. However, they grow slowly in milk because they lack proteolytic activities and the usual practice is to add yoghurt starter bacteria culture to enhance the fermentation process in the production of probiotic yoghurt (**Dave and Shah 1996**). In order for *Lactobacillus spp*. to provide therapeutic benefits, it has been recommended that they be viable and ingested in numbers greater or equal to 1 million cells per gram of yoghurt (**Kailasapathy and Chin**). But, several factors such as pH, hydrogen peroxide, oxygen content, lactic and acetic acids concentration and temperature of storage have been postulated to affect the viability of probiotic bacteria in yoghurt (**Shah *et al.*, 1995**). Thus, maintaining the viability of *Lactobacillus spp.* until yoghurts are consumed in order to ensure the delivery of live organisms has been of much interest. Foods containing probiotics are sold in many countries, although their survival rate in foods is doubtful since some of the strains are extremely sensitive to a series of factors (**Tamime and Robinson, 1985**), and one of the requirements for microorganisms to be used as dietary adjuncts is the need to maintain viability and activity in the carrier food before consumption (**Fuller, 1999**).

Syneresis occurs when whey separates from yoghurt due to contraction of the coagulum. (**SAS, 2004**) reported that the cause of whey separation is not clear. In addition to the protein network formed as a result of the acidification in yoghurt production, yoghurt culture bacteria such as *Streptococcus thermophilus* are known to produce a polysaccharide slime. Which is thought to have a stabilizing effect on the protein gel that helps to prevent syneresis and responsible for enhancing the rheological properties of yoghurt (**Farooq and Haque, 1992**). Therefore, in the commercial manufacture of yoghurt, particularly stirred type, it is common practice to add additional stabilizers like gelatin, pregelatinized starch, agar, guar gum, pectin and carrageenan. Such additives are used to prevent syneresis, improve viscosity and body, as well as playing a cost-saving role in reducing the amount of extra milk solids required and enhanced mouth-feel in low-fat-reduced varieties (**Farooq and Haque, 1992**).

Although a lot of research work has been done on probiotic yoghurt made from fresh milk and skim milk fortified fresh milk, literature is scanty on the viability of *Lactobacillus acidophilus* in probiotic yoghurt produced from reconstituted whole or skim milk powder. Hence, a practical approach towards creating awareness among manufacturers, food regulatory bodies and other consumers of probiotic yoghurt is to study the percentage syneresis and viability of *Lactobacillus acidophilus* in probiotic yoghurt produced from reconstituted whole and skim milk powder.

Therefore, the objective of this study was to assess the effect of storage on percentage syneresis and viability of *Lactobacillus acidophilus* in probiotic yoghurt produced from reconstituted whole and skim milk powder in a mixed culture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* stored at 4±2 ºC for 35 days to ascertain the extent to which the probiotic activity can be sustained in both types of milk .

MATERIALS AND METHODS

Substrates and Starter Culture

The substrates used for the study were reconstituted whole and skim milk powder. Freeze-dry mixed yoghurt starter culture containing *Streptococcus thermophilus*, *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Lactobacillus acidophilus* bacteria was purchased from Geomex Industries Ltd. Canada.

Production of Whole and Skim Milk Probiotic Yoghurt

This was done according to the method of (Shah *et al*., 1995) and as described by (**Lee and Lucey, 2004**) in Fig 1. After production, the yoghurt samples were subjected to determination of viable counts of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Lactobacillus acidophilus* bacteria and examined for syneresis (Day 0) and weekly for a period of 35 days. All analyses were done in triplicates**.**

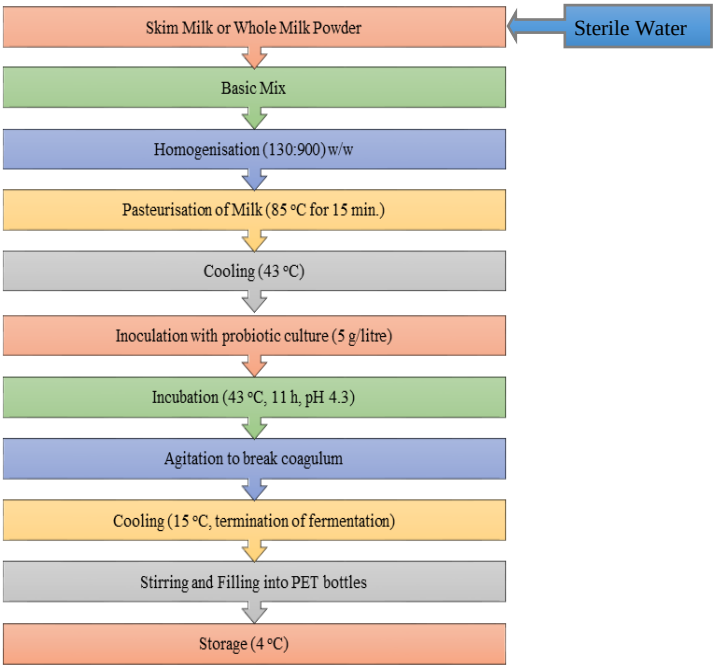

Figure 1 Flow Chart for the Production of Probiotic Yoghurt
Source: (Shah *et al.*, 1995)

Microbiological Analyses

Approximately 1 ml of each yoghurt sample (SMPY AND WMPY) was diluted with 9 ml of sterile 0.1 % (w/v) peptone water and mixed uniformly with a vortex mixer. Subsequent serial dilutions were made and viable counts of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Lactobacillus acidophilus* enumerated using the pour plate technique.

Enumeration of *Streptococcus thermophilus* bacteria

The counts of *Streptococcus thermophilus* were enumerated on *Streptococcus thermophilus* (ST) agar after incubating the plates aerobically at 37 ºC for 24 h. The colonies of *Streptococcus thermophilus* on the plates were counted using a colony counter and recorded as colony forming units per ml of sample (**Dannenberg and Kessler, 1988**).

Enumeration of *Lactobacillus delbrueckii* ssp. *bulgaricus* bacteria

The counts of *Lactobacillus bulgaricus* were differentially enumerated on MRS agar adjusted to pH 5.2 and incubated anaerobically (using the BBL Gas Pak system) at 37 ºC for 72 h. The colonies of *Lactobacillus bulgaricus* were counted using a colony counter and recorded as colony forming units per ml of sample (**Dannenberg and Kessler, 1988**).

Enumeration of *Lactobacillus acidophilus* bacteria

The counts of *Lactobacillus acidophilus* were selectively enumerated on MRS-Maltose agar with pH adjusted to 6.2 and incubated anaerobically (using the BBL Gas Pak system) at 37 ºC for 72 h. Plates containing the colonies of *Lactobacillus acidophilus* were counted using a colony counter and recorded as colony forming units per ml of sample (**Dannenberg and Kessler, 1988**).

Determination of percentage syneresis

Syneresis was determined using the modified method of **Gilliland (1990**). Approximately 50 ml of yoghurt was filtered with a funnel for 2 h at 10 ºC. The resultant whey was collected in 100 ml graduated cylinder and used as an index of syneresis. The % syneresis was thus calculated from the formula:

$$\%\ Syneresis = \frac{Vol.of\ Separated\ Whey}{Vol.of\ Sample} \times 100$$

Statistical analyses

All data were subjected to Analysis of variance (ANOVA) to determine any significant difference at 5% level using the method of **Shah et al., (1995**) and was reported as means of three replicates. Means were separated by Duncan's multiple range tests to establish if there were significant differences between the samples (**SAS, 2004**).

RESULTS

Table 1 Changes in Viable Counts (cfu/ml) of *Streptococcus thermophilus* during refrigerated storage at 4±2 ºC for 35 days

Storage Time (days)	*Streptococcus thermophilus*	
	SMPY	**WMPY**
0	$5.43x10^{8}{}_{a}$	$5.40x10^{8}{}_{a}$
7	$5.23x10^{8}{}_{a}$	$5.19x10^{8}{}_{a}$
14	$4.90\ x10^{8}{}_{b}$	$4.88\ x10^{8}{}_{b}$
21	$8.42x10^{7}{}_{c}$	$8.40x10^{7}{}_{c}$
28	$7.63x10^{7}{}_{d}$	$7.60x10^{7}{}_{d}$
35	$5.18x10^{6}{}_{e}$	$5.15x10^{6}{}_{e}$

Legend: SMPY – Skim milk probiotic yoghurt; WMPY- Whole milk probiotic yoghurt

Values in the same column and type of yoghurt with different subscripts are significantly different at ($p<0.05$)

Table 2 Changes in Viable Counts (cfu/ml) of *Lactobacillus bulgaricus* during refrigerated storage at 4±2 ºC for 35 days

Storage Time (days)	*Lactobacillus bulgaricus*	
	SMPY	**WMPY**
0	$2.47x10^{8}{}_{a}$	$2.43x10^{8}{}_{a}$
7	$2.13x10^{8}{}_{b}$	$2.07x10^{8}{}_{b}$
14	$6.01x10^{7}{}_{c}$	$5.98x10^{7}{}_{c}$
21	$9.40x10^{6}{}_{d}$	$9.37x10^{6}{}_{d}$
28	$3.52x10^{6}{}_{e}$	$3.50x10^{6}{}_{e}$
35	$8.10x10^{5}{}_{f}$	$7.82x10^{6}{}_{f}$

Legend: SMPY – Skim milk probiotic yoghurt; WMPY- Whole milk probiotic yoghurt

Values in the same column and type of yoghurt with different subscripts are significantly different at ($p<0.05$)

Table 3 Changes in Viable Counts (cfu/ml) of *Lactobacillus acidophilus* during refrigerated storage at 4±2 ºC for 35 days

Storage Time (days)	*Lactobacillus acidophilus*	
	SMPY	WMPY
0	$1.83x10^{8}{}_{a}$	$1.80x10^{8}{}_{a}$
7	$1.57x10^{8}{}_{b}$	$1.50x10^{8}{}_{b}$
14	$4.73\ x10^{7}{}_{c}$	$4.68\ x10^{7}{}_{c}$
21	$3.60x10^{6}{}_{d}$	$3.53x10^{6}{}_{d}$
28	$1.50x10^{6}{}_{e}$	$1.47x10^{6}{}_{e}$
35	$5.78x10^{5}{}_{f}$	$5.84x10^{5}{}_{f}$

Legend: SMPY – Skim milk probiotic yoghurt; WMPY- Whole milk probiotic yoghurt

Values in the same column and type of yoghurt with different subscripts are significantly different at ($p<0.05$)

Syneresis and Storage Time

Syneresis values for both samples of probiotic yoghurt stored at 4º±C for thirty-five days are shown in Fig 2. There was a significant increase in syneresis ($P< 0.05$) for both samples of probiotic yoghurts during the storage period. The percentage syneresis of SMPY and WMPY increased significantly during the 35 days of storage, from 24.4% to 32.0% and 24.8% to 32.7% respectively This finding is in agreement with the report of (**Dave and Shah, 1996**). Furthermore, there was a good correlation between syneresis and storage time. The increase in syneresis values could be due to the probable absence of stabilizers which could have made the yoghurt samples firm and whey separation minimal. The stabilizers would have in addition improved the water binding capacity of the yoghurt samples. The increase could also be due to the denaturation of beta-lactoglubin in the processed milk and the aging process of the curd as storage progressed (**Collier, 2004**). Moreover, the slower rate of syneresis in skim milk probiotic yoghurt (SMPY) could be as a result of higher total solids as reported by (**Varga *et al*., 2002**) who observed that increases in total solids reduce the rate of syneresis.

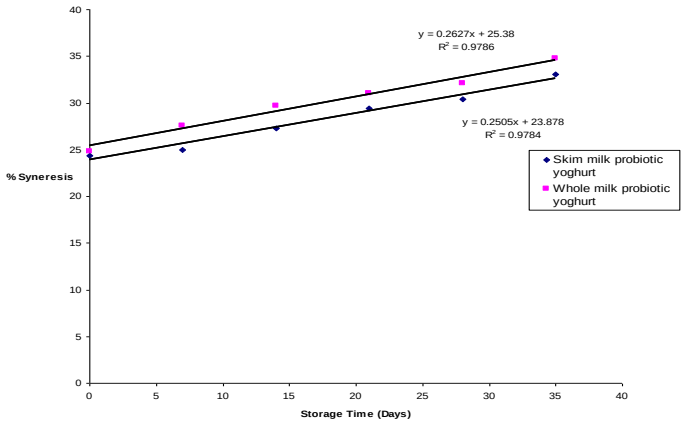

Figure 2 Changes in Syneresis of Skim and Whole Milk Probiotic Yoghurt during storage

DISCUSSION

Tables 1, 2 and 3 show the changes in the viable counts of *Streptococcus thermophilus* (ST) *Lactobacillus* ssp. *bulgaricus* (LB) and *Lactobacillus acidophilus* (LA) both samples of probiotic yoghurt (SMPY and WMPY). The viable counts of *S. thermophilus, L. bulgaricus* and *L. acidophilus* decreased throughout the storage period irrespective of the type of milk used. The viable counts of ST in SMPY decreased from $5.43x10^{8}$ to $5.18x10^{6}$ cfu/ml, LB ($2.47x10^{8}$ to $8.10x10^{5}$ cfu/ml) and LA ($1.83x10^{8}$ to $5.78x10^{5}$ cfu/ml). Similarly, the viable counts of ST in WMPY decreased significantly from $5.40x10^{8}$ to $5.15x10^{6}$ cfu/ml, LB ($2.43x10^{8}$ to $7.82x10^{5}$ cfu/ml) and LA ($1.80x10^{8}$ to $5.84x10^{5}$ cfu/ml). However, viable counts of *S. thermophilus* were more numerous than that of *L. bulgaricus* and *L. acidophilus* in both types of yoghurt. The decrease in viable counts was more pronounced in *S. thermophilus* followed by *L. acidophilus* and *L. bulgaricus*. This result is in agreement with the findings of (**Dave and Shah, 1996**). However, the viable counts of LA in both sample of yoghurt remained well above the recommended therapeutic minimum of 1 million cells per ml of yoghurt up to 28 days of storage. But, after 28 days, the decrease in the viable count of LA fell below the recommended therapeutic minimum. The decrease in viability of the lactic acid bacteria especially *L. acidophilus* could be as a result of the decrease in pH of the yoghurt samples. This finding is in agreement with the report of (**Tamime and Robinson, 1995**) who reported one log cycle decrease in viable count of LA with decrease in pH. Also, (**Sakai *et al*., 1987**) reported that the final pH of yoghurt can also affect the viability of *L. acidophilus* and *Bifidobacteria* spp. It has also been reported that pH values of 4.5 or lower can jeopardize the viability of probiotic organisms in yoghurt stored at 5 ºC (**Banon and Hardy, 1991**). Furthermore, (**Yeganehzad *et al*., 2007**) reported that the pH of fermented milks may decrease considerably during storage, which can affect the growth and viability of *L. acidophilus* and *Bifidobacterium* spp. Furthermore, (**Kurman and Rasic, 1991**) reported that the most important factor in probiotic mortality was the low pH of the yoghurt and any drop in pH below 4.3 greatly affected their viability.

CONCLUSION

This study showed that the viability of *L. acidophilus* was sustained above the 'therapeutic minimum' of 10^{6} cfu/ml up to the 28th day of storage and that the changes in the viability of *Lactobacillus acidophilus* in skim and whole milk probiotic yoghurt did not differ significantly. Thus, yoghurt made from either skim or whole milk powder can be used as an adequate carrier of probiotic bacteria like fresh milk. The positive correlation between syneresis and storage time of the yoghurts showed that stabilizers should be used to prevent whey separation during storage.

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