

PROBIOTIC YOGURT PRODUCTION IN HOMOGENISED PASTEURISED MILK AND SKIMMED MILK

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Abstract

Yogurt is a fermented product that obtained from inoculation of yogurt starter to milk. The aim of this study was to investigate the effect of different concentration of probiotic culture *viz. Lactobacillus acidophilus, Lactobacillus casei* (1 %, 5% and 10%) inoculated to Homogenised ,pasteurised milk and skimmed milk along with starter culture. Fermentation was stopped at pH 4.5 - 4.7. Then the samples were kept at 4°C. Physicochemical characteristics(pH ,titratable acidity,syneresis) and viability of probiotic bacteria in this sample were evaluated during 21-days of refrigerated storage. pH of both yogurt inoculated with *L.acidophilus* and *L.casei* were found to be decreased as the concentration of probiotic increased throughout the storage period. Probiotic bacteria count decreased significantly in homogenised milk than skimmed milk yogurt . There was increase in acidity by the addition of both probiotic cultures, but it was within the limit(0.9) upto 14 days except T4(10% *L.Acidophilus*). At 21st day in all samples including control, acidity goes beyond limit. So 1% and 5% inoculums of *L.acidophilus* and *L.casei* can be safely applied to yogurt without producing a drastic change in acidity. When using skimmed milk for yogurt production, 1% and 5 %inoculum of *L.acidophilus* produces no significant increase in acidity even after 21 days of refrigerated storage. Yogurt prepared with skimmed milk showed more syneresis than pasteurised milk. At the end, the sample containing probiotics showed the highest syneresis. Probiotic bacteria count decreased significantly in homogenised milk than skimmed milk yogurt.

Keywords : *L.acidophilus* , *L.casei*, probiotic yogurt, refrigerated storage, pasteurised homogenised milk ,skimmed milk .

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Materials and Methods

Lyophilized cultures of Probiotic bacteria, *Lactobacillus acidophilus*, *Lactobacillus casei* and commercial yogurt culture containing *Streptococcus thermophilus* and *L. delbrueckii ssp. Bulgaricus* were obtained from National Institute of Dairy, Karnal, Haryana. Pure culture of *Lactobacillus acidophilus* (ATCC 4356) *Lactobacillus casei* (NCDC 018) and *L. delbrueckii ssp. Bulgaricus* propagated in MRS agar media and broth and *Streptococcus thermophilus* were in M17 broth and agar.

Preparation of Probiotic yogurt

Homogenised, standardized and pasteurized milk (3.62% protein, 3.61% lactose, 1.6% fat and 9.70% total solid) and Skimmed milk were used for preparation of probiotic yogurt. All yogurt samples were produced in hygienic conditions. Both milk were heated up to 85°C for 30 min followed by cooling down to 40°C. The yogurt starter culture was then added at a concentration of 1:1 in all samples. Experimental preparations of yogurt including control plain yogurt in Homogenised pasteurized milk (T1), yogurt containing 1% *Lactobacillus acidophilus* (T2), yogurt containing 5% *Lactobacillus acidophilus* (T3), yogurt containing 10% *Lactobacillus acidophilus* (T4) probiotic yogurt containing 1%, 5% and 10% *Lactobacillus casei* (T5, T6, T7 respectively). Same was repeated for Skimmed milk. control plain yogurt in Skimmed milk (S1), yogurt containing 1% *Lactobacillus acidophilus* (S2), yogurt containing 5% *Lactobacillus acidophilus* (S3), yogurt containing 10% *Lactobacillus acidophilus* (S4) probiotic yogurt containing 1%, 5% and 10% *Lactobacillus casei* (S5, S6, S7 respectively). The mixtures were subsequently poured into 250-mL plastic cups and incubated at 43°C. Fermentation was stopped at pH 4.5 - 4.7. Then the samples were kept at 4 °C. At the second part of experiments, the sample with the most general acceptability was selected. Physicochemical characteristics (pH, titratable acidity, syneresis) and viability of probiotic bacteria in this sample were evaluated during 21-days of refrigerated storage.

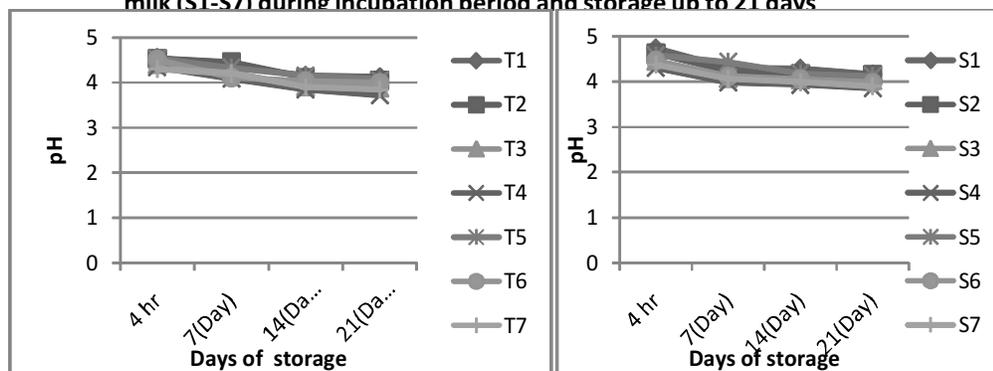
Determination of pH and titratable acidity

pH of the milk and yogurt samples was determined with a pH meter at room temperature. pH was determined in a single cup of yogurt per replication 1, 2, 3, and 4 h after inoculation, and in three cups (*Lactobacillus acidophilus* and *Lactobacillus casei*) of yogurt per replication 1, 7, 14 and 21 days of storage. Titratable acidity was determined in yogurt samples at room temperature according to the methods described in AOAC (2002). Yogurt samples (10 g) were diluted with 10 ml distilled water and titrated with 0.1 N NaOH in the presence of phenolphthalein. Titratable acidity was expressed as the percent of lactic acid based on the sample weight.

Table 1: pH of various yogurt samples Homogenized, pasteurized milk(T1-T7) and Skimmed milk (S1-S7) during incubation period and storage up to 21 days

Samples	pH of yogurt for various incubation periods				
	4 hr	7(Day)	14(Day)	21(Day)	
T1	4.55	4.35	4.15	4.12	(T1), Homogenised pasteurized milk (T2) -yogurt containing 1% <i>Lactobacillus acidophilus</i> , (T3),yogurt containing 5% <i>L.acidophilus</i> (T4) yogurt containing 10 % <i>L.acidophilus</i> T5,T6,T7 probiotic yogurt containing 1%,5% and 10% <i>L. casei</i> .
T2	4.53	4.45	4.11	4.05	
T3	4.41	4.21	3.92	3.89	
T4	4.34	4.09	3.85	3.72	
T5	4.53	4.33	4.15	4.06	
T6	4.51	4.11	4.03	3.99	
T7	4.30	4.20	3.90	3.84	
S1	4.74	4.31	4.29	4.16	(S1) plain yogurt in Skimmed milk, (S2),yogurt containing 1% <i>L. acidophilus</i> (S3)yogurt containing 5% <i>L. acidophilus</i> (S4)yogurt containing 10 % <i>L. acidophilus</i> S5,S6,S7-probiotic yogurt containing 1%, 5% and 10% <i>L. casei</i> (respectively)
S2	4.62	4.23	4.17	4.15	
S3	4.46	4.08	4.04	4.03	
S4	4.30	3.98	3.93	3.86	
S5	4.58	4.43	4.18	4.12	
S6	4.46	4.11	4.05	3.97	
S7	4.37	4.06	3.99	3.90	

Figure 1: pH of various yogurt samples Homogenized, pasteurized milk(T1-T7) and Skimmed milk (S1-S7) during incubation period and storage up to 21 days

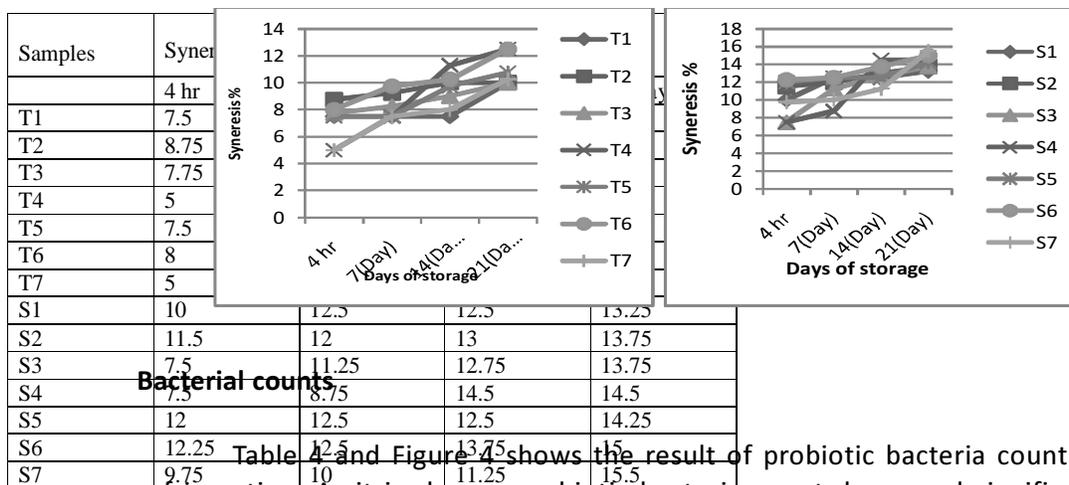


Titrateable Acidity (% Lactic acid)

Titrateable Acidity of Probiotic yogurt prepared using Homogenised Pasteurised milk is shown table 2. There was increase in acidity by the addition of both probiotic cultures, but it was within limit (0.9) upto 14 days except T4 (10% *L. Acidophilus*). At 21 day in all samples including control, acidity goes beyond limit. So 1% and 5% inoculums of *L. acidophilus* and *L. casei* can be safely applied to yogurt

Table 3 : Syneresis of Probiotic yogurt samples Homogenized, pasteurized milk(T1-T7) and Skimmed milk (S1-S7) during incubation period and storage up to 21 days

Figure 3 : syneresis of Probiotic yogurt samples Homogenized, pasteurized milk(T1-T7) and Skimmed milk (S1-S7) during incubation period and storage up to 21 days



Bacterial counts

Table 4 and Figure 4 shows the result of probiotic bacteria count during refrigeration. As it is shown, probiotic bacteria count decreased significantly in homogenised milk than skimmed milk yogurt. 1 % inoculums of *L.acidophilus* survived better in homogenised milk than *L.casei*,but 1 % inoculums *L.casei* survived better in skimmed milk than *L.acidophilus*.The main factors for loss of viability of probiotic organisms have been attributed to the decrease in the pH of the medium and accumulation of organic acids as a result of growth and fermentation (Shah and Jelen, 1990; Shah, 2000b).

end of storage period. The comparison of the probiotic batches to those of the control showed that the bulk of the organic acids was produced by the yoghurt cultures, but the selected probiotic organisms (*L.acidophilus* and *L. casei*) survived better in the acidic environment with viable counts above the legal requirement of 6.00 log cfu/g.

Conclusion

Different termination pH appeared to have no effect on the viability of both probiotic Organisms in two samples. Survival of probiotic organisms was strain dependant. The decline in pH during storage showed increased levels of organic acids resulting in low counts of *L.acidophilus* and *L. casei* . 1 % inoculums of *L.acidophilus* survived better in homogenised pasteurised milk than *L.casei*, but 1 % inoculums *L.casei* survived better in skimmed milk than *L.acidophilus* under acidic conditions.. On the other hand, increased lactic acid production by this organism, subsequently affected the survival of *L.acidophilus* and *L. casei*. In both type yogurt prepared, *L.acidophilus* and *L. casei* remained viable in population above 6 log CFU/ml during refrigerated storage. On storage there was decrease in pH ,increase in acidity and syneresis but the selected probiotic organisms (*L.acidophilus* and *L. casei*) survived better in the acidic environment with viable counts above the legal requirement of 6.00 log cfu/g.

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