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Effect of Milk Supplementation with Fructooligosaccharides and Inulin on Viable Counts of Probiotic Bacteria in Goat and Cow Milk Yoghurts

Sirimu Celestin [1], S.S Thorat[2], R.J Desale[3]and U.D Chavan[4]

[1]*M.Sc Graduate, Intake 2013-14, Department of Food Science and Technology, Post Graduate Institute, Mahatma PhuleKrishiVidyapeeth, Rahuri -413722 (India).*
[2]*Professor, Department of Food Science and Technology, Post Graduate Institute, Mahatma PhuleKrishiVidyapeeth, Rahuri-413722(India).*
[3]*Assistant Professor, Department of Animal science and Dairy science, Post Graduate Institute, Mahatma PhuleKrishiVidyapeeth, Rahuri-413722(India).*
[4]*Professor, Department of Food Science and Technology, Post Graduate Institute, Mahatma PhuleKrishiVidyapeeth, Rahuri -413722 (India).*

***Abstract**: The main aim of this study was to examine the performance of probiotic bacteria (Lactobacillus acidophilus NCDC-291 and BifidobacteriumbifidumNCDC-232) as sole starter culture in fermentation of goat and cow milk supplemented with Fructooligosaccharides (FOS) and Inulin as prebiotics. Sixteen batches of set yoghurts were conventionally formulated from both types of milk fortified with 3% Skim Milk Powder (SMP) and supplemented with 1.5, 2, and 3 % FOS and Inulin separately. During 14 day refrigerated storage, enumeration of viable cells using Pour Plate technique was carried out.The results showed that the supplementation of both goat and cow milk with either FOS or Inulin improved both the growth and survival of the probiotic cultures in the resulting yoghurts. The highest cell count of Lactobacillus acidophilus NCDC-291 recovered was 8.62 log cfu/ml, obtained in FOS-enriched goat milk yoghurt (GF_3). Likewise the highest count of BifidobacteriumbifidumNCDC-232 was 8.58 log cfu/ml, recovered from Inulin-containing cow milk yoghurts (CI_3) which also sustained stable better than in FOS-enriched yoghurts. It was found that addition of FOS or Inulin to either milk gave better results regarding the growth and survival of probiotic bacteria than in control sample.*

***Key Words**:fructooligosaccharides, Inulin, prebiotics, probiotics.*

I. Introduction

Yoghurt is defined as a product resulting from milk by fermentation with a mixed starter culture consisting of Streptococcus thermophilus and Lactobacillus delbrueckii spp. bulgaricus. Different countries or even different parts of the same country developed their own fermented milks. The best-known product is the thermophilic fermented milk, yoghurt, which has enjoyed increased popularity in the last three decades [1]. Because of the claims made in favour of probiotic bacteria, various fermented milk products have been formulated [2]. [3] defined probiotics as a "live microbial food supplement which beneficially affects the hosts by improving their intestinal microbial balance". While bifidobacteria are difficult to propagate in food due to oxygen sensitivity and low acid tolerance, the addition of prebiotics to dairy foods may lead to promising results to ensure the presence of high numbers of bifidobacteria during normal shelf life of the dairy products [2]. The most recent definition by [4] states that "A dietary prebiotic is a selectively fermented ingredient that results in specific changes, in the composition and/or activity of thegastrointestinalmicrobiota, thus conferring benefit(s) upon host health."Synbiotics is where probiotics and prebiotics are used in combination to manage microflora[5].

Despite their popular use in dairy industry [6], market surveys have revealed poor viability of probiotic bacteria in commercial yoghurt preparations [7].Several works have been done to improve the growth and viability of probiotic bacteria by adding supplements to the milk base [8];[9] but presently information is not sufficient on the effects of specific prebiotics on growth and survival of a particular probiotic strain.Therefore an interest to promote the growth and viability of probiotics in yoghurt through enrichment of milk with Fructooligosaccharides and Inulin motivated the author to undertake the present study. Selected probiotic organisms (Lactobacillus acidophilus NCDC-291 and Bifidobacteriumbifidum NCDC-232) were used as starter culture for milk fermentation coupled with Inulin and FOS addition in the manufacture of bovine milk yoghurt and goat milk yoghurt.

II. Materials And Methods

The present investigation was conducted in the Laboratory of the Department of Food Science and Technology, Mahatma PhuleKrishiVidyapeeth (MPKV), Rahuri; DistrictAhmednagar (MS), India.This study examined, but not exclusively, the probiotic bacterial count in yoghurt samples made with whole cow and goat milks separately and enriched with prebiotics (Fructooligosaccharides/FOS and Inulin).Sixteen batches (16 treatment combinations) of set yoghurts were produced from both types of milk and analyzed as described below.

2.1 Materials

2.1.1 Milk

a) The composite cow's milk was obtained from Research Cum Development Project on Cattle (RCDP), MPKV, Rahuri.

b) The composite goat's milk was procured from All India Coordinated Research Project (AICRP) on Goat project, MPKV Rahuri.

c) Skim Milk Powder (SMP: EveryDay-Nestle, Manufactured by Nestle India Ltd, New Delhi-110 001) was procured from a local market.

2.1.2 Starter cultures

Pure probiotic strains of Lactobacillus acidophilus NCDC-291 and Bifidobacteriumbifidum NCDC-232were obtained in freeze dried form from National Collection of Dairy Cultures (NCDC), Karnal- Haryana (India).

2.1.3 Prebiotics

Fructooligosaccharides (FOS) was supplied on gratis basis by Rashesh& Co.-Mumbai, India.
Inulin was obtained from HIMEDIA Laboratories, Mumbai (India).

2.2 Methods

2.2.1 Activation and propagation of cultures

Pure strains of Lactobacillus acidophilus NCDC-291 and Bifidobacteriumbifidum NCDC-232 were used as starter cultures. Skim Milk Powder (SMP) was dissolved in distilled water at 12 g/100 ml and heated to boiling. The cultures were prepared by inoculating 10 ml aliquots of cooled (to 37^0C) reconstituted skim milk (RSM) with freeze dried probiotic cultures.
The activated organisms after three successive transfers were used for the preparation of inocula and production of yoghurt.

2.2.2 Yoghurt preparation and storage

The yoghurt was prepared by blending separately raw whole cow's milk and whole goat's milk with 3% SMP and warming the mix at 65 °C for 5 minutes. The yoghurt mix (1litre) was divided into four equal (250 ml) portions three of which were supplemented with either Fructooligosaccharides (FOS) or Inulin at 1.5, 2 and 3 per cent level and the fourth portion was without prebiotics (control sample). All the yoghurt milk blends were homogenized and heat treated at 85°C for 30 min (in a temperature-controlled water bath), followed by cooling to 37°C and aseptically inoculated with 2% (v/v) of eachof Lactobacillus acidophilus NCDC-291 and Bifidobacteriumbifidum NCDC-232 inocula. All batches were held at 37°C in incubator for fermentation until a coagulum was formed at about pH 4.6, the step at which fermentation process was terminated. The fermentation time ranged from 10 to 12 hours. Yoghurts were immediately cooled and stored at 4°C for subsequent analyses. The procedure referred in Fig.1 was used for yoghurt preparation.

2.2.3 Treatment combinations and yoghurt manufacture

Table 1. Treatment combinations

Type of Milk	Treatment group	Prebiotic Supplement
Cow milk + SMP =C	C_0 (Control)	-
	CF_1	1.5% W/V
	CF_2 CF_3	2% W/V 3 % W/V
	C_0 (Control)	-
	CI_1	1.5 % W/V
	CI_2	2% W/V
	CI_3	3 % W/V
Goat milk +SMP=G	G_0 (Control)	-
	GF_1	1.5% W/V
	GF_2 GF_3	2% W/V 3 % W/V
	G_0 (Control)	-
	GI_1	1.5 % W/V
	GI_2	2% W/V
	GI_3	3 % W/V

SMP: skim milk powder, F or FOS: Fructooligosaccharides, I: Inulin

Sixteen batches of set yoghurts were formulated from both types of milk supplemented with 1.5%, 2% and 3% FOS and/or Inulin (i.e. 8 combinatinations for each type of milk). All treatments were replicated three times.

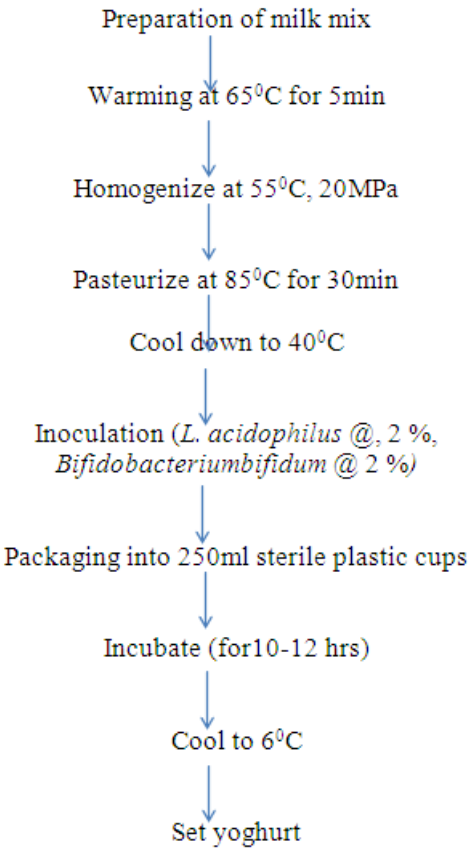

Fig 2.1 Yogurt preparation flow diagram.

2.2.4 Enumeration of viable counts of the probiotic cells in yoghurt samples

The viable counts of Lactobacillus acidophilus NCDC-291, and Bifidobacteriumbifidum NCDC-232 were enumerated using pour plate technique on MRS agar. Yoghurt samples (1.0 ml) were decimally diluted in 9 ml of sterile peptone water (at pH 7.0) up to 10^{-6} dilution. From 10^{-6} dilution tube, 1.0 ml aliquots were plated applying the pour plate technique. Bile-MRS agar duplicate plates incubated aerobically were used

for estimation of Lactobacillus acidophilus counts while on the other hand MRS agar duplicate plates prepared for enumeration of Bifidobacteriumbifidum were supplemented with Lithium chloride and sodium propionate (LP-MRS) and then incubated under anaerobic conditions in GasPak System (HIMEDIA Laboratories, Mumbai, India). Incubation temperature and time for both bacterial strains were set at 37 ^{0}C for 48 to 72 hours [10]; [11]. At the end of incubation period, enumeration was done for plates containing 25-250 colony forming units each, with the help of colony counter (Model Digital colony counter, VCC2; VSI Electronics Pvt. Ltd, Akola, India).

2.2.5 Statistical analysis

Three independent replicates of each experimental treatment were carried out in this work.The data obtained were statistically analyzed by two way ANOVA using Excel and ($P<0.05$) was considered statistically significant.Least Square Difference –LSD (referred to as CD) was used for mean comparison.

III. Results And Discussion

Enumeration of viable counts of Lactobacillus acidophilus NCDC-291 and Bifidobacteriumbifidum NCDC-232 was conducted after 12 hours, 7 days and 14 days from the time of yoghurt manufacture.

3.1 Viable counts ofLactobacillus acidophilus NCDC-291at 10^{-6} dilution in goat milk yoghurt

The counts of Lactobacillus acidophilus NCDC-291in goat milk yoghurts are presented in Table 2. It is obvious that the cell counts in FOS-enriched samples were higher than the counts in control sample whereby the highest cell count of Lactobacillus acidophilus NCDC-291 among all supplemented samples was 8.62 log cfu/ml obtainedfromGF_3 on day-7 and the lowest cell recovery was 6.04 log cfu/mlrecovered fromG_0 on day-14. The cell growth was found to be fairly satisfactory after 12 hours and it reached the maximum in the first 7 days of storage time (this phenomenon could be ascribed to possible capability of Lactobacillus acidophilus NCDC-291to grow in acidic medium).After 12 hours, the highest count among FOS-enriched samples was recovered from GF_2 sample. However, after 7 days the maximum count was obtained from GF_3. In a similar manner, the cell counts in Inulin-enriched samples were higher than those in control sample (i.e. after 12 hours, 7 days and 14 days), and it was trivially observed that Inulin-added yoghurt samples contained relatively less Lactobacillus acidophilus NCDC-291 cell counts than their counterparts, FOS enriched samples. Furthermore, it was noticed that the Lactobacillus acidophilus NCDC-291 experienced a decline in growth at the end of storage time (day-14). Supplementation of goat milk yoghurts with FOS and/or Inulin caused a significant ($p<0.05$) difference in Lactobacillus acidophilus NCDC-291 cell growth with respect to the controls samples (G_0) right from day-0 till the end of storage time (day-14). Similar observations were reported by [12].

Table 2 Viable counts of Lactobacillus acidophilus NCDC-291in goat milk Yoghurts.

Sample	Log CFU (after12 hr)	Log CFU (Day-7)	Log CFU(Day-14)
G_0	8.20	8.23	7.07
GF_1	8.30	8.47	7.17
GF_2	8.34	8.54	7.23
GF_3	8.28	8.62	7.30
SE ±	0.006	0.006	0.007
CD at 5%	0.019	0.018	0.021
G_0	7.74	8.07	6.04
GI_1	8.11	8.20	6.07
GI_2	8.07	8.11	6.25
GI_3	8.04	8.14	6.28
SE ±	0.009	0.008	0.010
CD at 5%	0.027	0.025	0.030

3.2 Viable counts ofLactobacillus acidophilus NCDC-291at 10^{-6} dilution in cow milk yoghurt

The counts of Lactobacillus acidophilus NCDC-291in cow milk yoghurts are presented in Table 3. The maximum cell growth was not observed at the end of bovine yoghurt manufacture (i.e. after 12hrs) rather it was reached at the end of the first 7 days of storage. The cell count of 8.50 log cfu/ml(for GF3) emerged as the highest Lactobacillus acidophilus NCDC-291 cell recovery in all bovine yoghurt samples (slightly lower than 8.62 log cfu/mlwhich was obtained in goat milk yoghurts) on the day-7.The lowest Lactobacillus acidophilus NCDC-291 cell counts were obtained in the control samples. It was found that the cell growth substantially decreased in 7 days after day-7 of refrigerated storage.Addition of either FOS or Inulin to cow milk yoghurts caused a significant ($p<0.05$) increase in the cell growth with respect to the controls (C_0) and the difference maintained throughout the storage time. Another striking observation was that relatively high cell counts were recovered from FOS-enriched yoghurt samples as compared to the inulin-supplemented samples. In consistence

with these results, the studies of [13] showed that Lactobacillus acidophilus NCDC-291growth was significantly more satisfactory with oligofructosethan with inulin.

Table 3 Viable counts of Lactobacillus acidophilus NCDC-291in Cow milk Yoghurts.

Sample	Log CFU (after12 hr)	Log CFU (Day-7)	Log CFU(Day-14)
C0	8.15	8.31	7.04
CF_1	8.23	8.39	7.11
CF_2	8.30	8.43	7.30
CF_3	8.32	8.50	7.28
SE ±	0.008	0.006	0.007
CD at 5%	0.023	0.018	0.021
C_0	7.69	7.79	6.07
CI_1	7.65	7.95	6.25
CI_2	8.20	8.32	6.30
CI_3	8.17	8.38	7.28
SE ±	0.011	0.008	0.009
CD at 5%	0.033	0.026	0.029

3.3 Viable counts ofBifidobacteriumbifidum NCDC-232 at 10^{-6} dilution in Goat milk yoghurt

The mean counts of Bifidobacteriumbifidum NCDC-232 in goat milk yoghurts are presented in Table 4. Unlike Lactobacillus acidophilus NCDC-291 growth, the multiplication phase forBifidobacteriumbifidum NCDC-232 cell seemed to occur during milk fermentation (incubation time) with the highest cell count of 8.53 log cfu/ml which was obtained at the end of yoghurt manufacture (after 12 hr) in GI_3. Relatively low Bifidobacteriumbifidum NCDC-232 counts were obtained from the control samples, G_0 (i.e. 8.11 and 8.25 log cfu/ml after 12 hr; 8.07 and 7.65 log cfu/ml on day-7; 7.04 and 7.23 log cfu/ml on day-14) in comparison to their

Table 4 Viable counts of BifidobacteriumbifidumNCDC-232in goat milk Yoghurt

Sample	Log_{10}CFU (after 12 hr)	Log_{10}CFU (Day-7)	Log_{10}CFU(Day-14)
G_0	8.11	8.07	7.04
GF_1	8.23	8.17	7.30
GF_2	8.30	8.15	7.36
GF_3	8.28	8.20	7.34
SE ±	0.006	0.008	0.007
CD at 5%	0.017	0.024	0.021
G_0	8.25	7.65	7.23
GI_1	8.34	8.23	7.32
GI_2	8.47	8.30	7.30
GI_3	8.53	8.25	7.28
SE ±	0.008	0.012	0.009
CD at 5%	0.025	0.035	0.029

Corresponding supplemented samples (i.e. samples made with FOS and Inulin enrichment respectively). Under storage, Bifidobacteriumbifidum NCDC-232 cell count underwent a gradual decline but yet the growth was maintained above 7.00 log cfu/ml of goat milk yoghurts through the end of storage time. Another interesting feature was that the Inulin-added samples contained relatively high cell counts as compared to their counterparts, FOS-goat milk yoghurts.This situation implies that Inulin could be a better substrate for Bifidobacteriumbifidum NCDC-232 growth than FOS. Nevertheless, addition of either FOS or Inulin imparted a significant ($p<0.05$) difference in cell growth between control samples and enriched samples. A similar tendency was observed in the study performed by [14] on acidophilus bifidus yogurt, which showed that inulin improved the growth of bifidobacteria, but showed no positive effect on the growth of L. acidophilus. Also [15] highlighted the ability of Bifidobacteriumbifidum in fermentation (degradation) of Inulin.

3.4 Viable counts ofBifidobacteriumbifidum NCDC-232 at 10^{-6} dilution in cow milk yoghurt

The mean counts of Bifidobacteriumbifidum NCDC-232 in cow milk yoghurts are presented in Table 5. In a similar manner, relatively low counts of the strain were obtained from the control samples (8.17 and 8.27; 8.04 and 7.63; 7.08 and 7.28 log cfu/ml in FOS and Inulin enriched yoghurt samples respectively after 12 hrs, 7 days and 14 days).It was found that Bifidobacteriumbifidum NCDC-232cell multiplied exponentially during cow milk fermentation (yoghurt manufacture) where the highest cell number of 8.58 log cfu/ml was obtained at the end of yoghurt manufacture (after 12 hr) in CI_3, revealing the less acidic tolerance nature of Bifidobacteria. The Bifidobacteriumbifidum NCDC-232 cell counts decreased during storage time but still the cell count was maintained above 7.00 log cfu/ml of bovine yoghurt till day-14. Again the Inulin-containing samples manifested higher cell counts than FOS-cow milk yoghurts. Enrichment of bovine yoghurts with either

FOS or Inulin caused a significant ($p<0.05$) difference in Bifidobacteriumbifidum NCDC-232cell counts between control samples and treated samples.

Table 5 Viable counts of Bifidobacteriumbifidum NCDC-232in cow milk yoghurt.

Sample	Log_{10}CFU (after 12 hr)	Log_{10}CFU (Day-7)	Log_{10}CFU(Day-14)
C0	8.17	8.04	7.08
CF_1	8.25	8.11	7.25
CF_2	8.28	8.14	7.30
CF_3	8.30	8.15	7.28
SE ±	0.006	0.008	0.009
CD at 5%	0.019	0.023	0.026
C0	8.27	7.63	7.28
CI_1	8.34	8.20	7.25
CI_2	8.47	8.17	7.28
CI_3	8.58	8.23	7.30
SE ±	0.009	0.011	0.012
CD at 5%	0.027	0.033	0.037

Ultimately, the findings from enumeration of viable probiotic cells prompted the author to draw the following inferences: First, Goat milk supplemented with FOS could be a better treatment combination for the maximum growth of Lactobacillus acidophilus NCDC-291 strain; second, Cow milk enriched with inulin would constitute the better combination for maximum proliferation of Bifidobacteriumbifidum NCDC-232 cell; third, Goat milk may support the growth of both strains better than cow milk could do. Similar observation was reported by [16]. The analysis showed that the number of probiotic bacteria were at the required level, between 10^6-10^9cfu/ml from thetime of yoghurt production to the fixed end (14 days) of refrigerated storage. In general, the survival rate of Bifidobacteriumbifidum NCDC-232 during the storage was more satisfactory in Inulin-yoghurts than in FOS-supplemented and control yoghurts.Similar tendencies of prebiotic effect on probiotic growth were found by [16]. Also in consistence with these findings, [17] studied the growth of yoghurt bacteria and bifidobacteria in yoghurts containing chicory fructooligosaccharideduring storage at 4°C for 28 days. The decrease in all bacteria recovery was observed during storage period, but bifidobacteria were affected by strain type and the presence ofFOS. Thatwassupposedly due to prebiotic effects of this oligofructose.Also, comparable effect of Inulin was observed by [18] in their investigation into the viability of bacteria in the probiotic ice cream which indicated that addition of inulin stimulated the growth of B. lactisand L. acidophilus.

IV. Conclusion

This investigation proved that supplementation of either goat or cow milk with FOS and/or Inulin improvesgrowth and survivalof the used probiotic bacteria during and after milk fermentation. It was found that when Lactobacillus acidophilus NCDC-291 and Bifidobacteriumbifidum NCDC-232 fed with FOS or Inulinwere able to ferment the yoghurt milk mix and survive throughout storage period with sufficient count of viable cells at the end of storage time. Both prebiotics proved to be potential and selective growth-promoting substances for the used probiotic bacteria. It is worthwhile to conclude that functionalsynbiotic yoghurt can be made by combining goat or cow milk with FOS or Inulin at the appropriate concentrations. Further research with different probiotic strains is suggested to find out their performance and to broaden their use in dairy industry.

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