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Kirti Verma
Department of Dairy Microbiology, college of Dairy Technology, C.G.K.V. Raipur, Chhattisgarh, India

Manorama
Department of Dairy Microbiology, college of Dairy Technology, C.G.K.V. Raipur, Chhattisgarh, India

Correspondence
Kirti Verma
Department of Dairy Microbiology, college of Dairy Technology, C.G.K.V. Raipur, Chhattisgarh, India

Development of soya milk based pro-biotic frozen desert and its subsequent sensory, chemical and microbial analysis

Kirti Verma and Manorama

Abstract
The current global burden of chronic diseases necessitates the introduction of functional foods, including probiotic ones. Thus, the aim of the present study was to develop a soya based probiotic frozen dessert and for this five type of soya milk blends comprising of T_1 (Soya milk - 84 per cent, SMP - 0 per cent), T_2 (Soya milk - 83 per cent, SMP - 1 per cent), T_3 (Soya milk - 82 per cent, SMP - 2 per cent), T_4 (Soya milk - 81 per cent, SMP - 3 per cent) and T_5 (Soya milk - 80 per cent, SMP - 4 per cent) were prepared. Two controls standard control (Buffalo milk kulfi) and product control (Soya frozen dessert without culture) were also prepared. *Lactobacillus rhamnosus* GG was added at the rate of 10^9 as probiotic culture in all except the product control. Their chemical, microbiological, sensory and statistical analysis was done for 24 days at - 20°C±1°C. Results showed that T4 comprising of 81 per cent soya milk and 3 per cent SMP was best among all having fat and total solids of 9.37 per cent and 45 per cent, respectively. The product has a shelf life of 18 days as the probiotic count started to decrease from 10^8 to 10^5 after that, which is below the recommended value for a probiotic food. Statistical analysis showed significance differences for pH, sensory, probiotic count and total viable count during storage.

Keywords: chronic diseases, *Lactobacillus rhamnosus,* probiotic

Introduction
Lactose intolerance, one of the most common food intolerance, is estimated to affect about three quarters of the world's population. Almost 60 to 70 per cent of people in India are lactose intolerant and thus has lead to the demand for alternatives to cows' milk. In this regard, soy milk has been recognized as cheapest and nutritionally best alternative for animal milk. It contains unique nutrient composition comprising of protein, indigestible fiber and polysaccharide, amino acids, lecithin and unsaturated fat, minerals and vitamins, as well as bioactive polyphenols such as isoflavones, phenolic acids, saponins and tannins. Also offering health benefit such as lowering the risk of getting cancers, diseases associated with cardiovascular, hypercholesterolemia, diabetes as well as bone and kidney diseases. Due to this reason use of soymilk in various products is being increasing.

Increasing awareness among consumers to ensure good health coupled with changing life style has led to the concept of functional foods. Functional food that contains viable probiotics in particular is getting much popular among people due to its various health benefits like-improvement in intestinal micro flora, activation of the immune system, reduction in serum cholesterol and inhibition of potential pathogens. Fermented yogurt and milk can effectively be used to deliver probiotic bacteria but it may cause loss of viable probiotic due to pH reduction and accumulation of organic acids as a result of fermentation. In this regard kulfi/ice cream due to its neutral pH, can be used to deliver the probiotics.

Frozen products are obtained by freezing a pasteurized mix prepared with milk fat or edible vegetable oils and fat in combination and milk protein alone or in combination or vegetable protein products singly or in combination with the addition of nutritive sweetening agents eg. Sugar, dextrose, fructose, liquid glucose, dried liquid glucose, maltodextrin, high maltose, corn syrup, honey, fruit and fruit products, eggs and egg products, coffee, cocoa, chocolate, condiments, spices, ginger and nuts. The said product may also contain bakery products such as cake or cookies as a separate layer/or coating, it may be frozen hard or frozen to a soft consistency. It shall have pleasant taste and flavor free from off flavor and rancidity (FSSAI, 2011) [3].

Various kulfi/ice cream are being made using different probiotic organisms like *Lactobacillus acidophilus*, L. *rhamnosus*, L. *paracasei* ssp. *paracasei, Bifidobacterium lactis*.

Lactobacillus rhamnosus GG shows extremely good intestinal wall adhesion and thus considered a good probiotic. Various health benefits have also been reported for the strain like- Diabetes management, immunity boosting effects, cancer fighting and preventing, helps in weight loss, shows antimicrobial effect against *Salmonella, Staph. aureus* and *E.coli* etc.

Materials and Method

Probiotic Culture

Lactobacillus rhamnosus GG was procured in freeze dried form from the National Collection of Dairy Cultures (NCDC), Dairy Microbiology Division, National Dairy Research Institute, Karnal-132001 (Haryana). It was propagated in reconstituted skim milk powder (10-12 per cent solid not fat). 10 ml of this milk was taken in a test tube and the mouth of it was capped by cotton plug and was sterilized by autoclaving it at 121°C at 15 psi. After this it was cooled to room temperature and the ampoule of freeze dried lactic culture was broken aseptically and transferred to the sterilized skim milk. The contents were shaken properly, then the inoculated test tubes containing *Lactobacillus rhamnosus* GG were incubated at 37°C for 48 h. After incubation these cultures were preserved at 5-7°C in refrigerator and transferred once in a week.

Pathogenic Cultures

Escherichia coli 249, *Salmonella enteritidis* ssp. *Typhimurium* 113, *Staphylococcus aureus* 111 was obtained from the National Collection of Dairy Cultures (NCDC), Dairy Microbiology Division, National Dairy Research Institute, Karnal-132001 (Haryana) and the media used were from Himedia Laboratories Pvt. Ltd., 23, Wadhani Road, Ind. Est., LBS Marg., Mumbai-400086, India. *Escherichia coli* 249 in freeze dried form was propagated by inoculating it in nutrient broth medium and incubated at 37°C for 48 h. The activated culture was sub cultured in the same broth once in a week and preserved at 5-7°C. *Salmonella enteritidis* ssp. *Typhimurium* 113 was propagated in nutrient broth medium and incubated at 37°C for 48 h. The activated culture was sub gcultured in the same broth once in a week and preserved at 5-7°C. *Staphylococcus aureus* 111 was propagated in nutrient broth medium and incubated at 37°C for 48 h. The activated culture was sub cultured in the same broth once in a week and preserved at 5-7°C.

Preparation of soy milk

Soy milk was prepared according to the method given by Maity *et al*. (1998) [4] in the laboratory by soaking soyabean in a mixture of water and 0.5 per cent sodium bicarbonate ($NaHCO_3$) for 10-12h and then washed twice with water. It was then blanched for 30 min. at 100°C in 0.5 per cent $NaHCO_3$ solution and after that cooled and dehulled under tap water. The dehulled soyabean were then grinded along with warm water (80 – 90°C) in 4:1 ratio and the resulting soymilk was filtered through muslin cloth. It was finally sterilized by autoclaving and stored in refrigerator at 4°C.

Preparation of frozen dessert

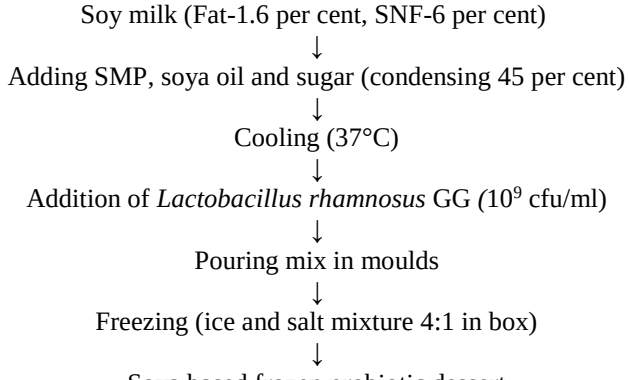
Lactobacillus rhamnosus GG 347 was subcultured in skim milk and MRS broth and incubated. The skim milk and MRS broth tubes was subcultured daily. Negative and gram staining was done to check the growth in both skimmed milk and MRS broth tubes. The revived probiotic culture was streaked in MRS plate and was in if there is enough is enough if half-life we are one off payment of and minor cubated at 37°C for 48h. Then one colony of probiotic culture was picked with the help of sterile toothpick and was transferred to the skim milk tube which was also incubated at 37°C for 48h. One ml of skim milk culture was pour plated in MRS agar to enumerate the probiotic culture.

Probiotic soya based frozen dessert was prepared as per methodology given by Rajor (1998) [5] with slight modification. There were two control out of which one was standard control (Buffalo milk kulfi) and the other was the product control (Soya frozen dessert without culture).

Table 1: The treatment details are given below

Treatments	Soya Milk (per cent)	Soya oil (per cent)	Sugar (per cent)	*Lactobaccilus rhamnosus* GG (cfu/g)	Skim Milk Powder (per cent)
T_1	84	5	11	10^9	0
T_2	83	5	11	10^9	1
T_3	82	5	11	10^9	2
T_4	81	5	11	10^9	3
T_5	80	5	11	10^9	4

Schematic diagram for preparation of soy milk based frozen dessert



For the selection of standard product all the five test products and two control were evaluated for probiotic count by pour plate method and sensory analysis as per 9- point Hedonic scale. The standard product storage stability was evaluated by storing the product at - 21°C for 24 days. The product was evaluated at the interval of three days for following chemical (pH and fat), microbiological (probiotic count, coliform count, total viable count) and sensory analysis.

Results and Discussion

Selection of treatment of soya based probiotic frozen dessert

Viable Probiotic Count

The initial viable probiotic organisms added to the product mix were 9 $\log_{10}$ cfu/g in all five treatments. The viable probiotic count was reported to decrease 8 $\log_{10}$ cfu/g after freezing in all treatments.

Sensory Evaluation

The five treatments and two controls were evaluated by sensory analysis on 9-point hedonic scale through a panel of five judges and their mean values are shown in Table 2. The lowest value for colour was gained by T_5 (6.75) and highest was gained by T_4 (7.83). A significant ($p<0.05$) difference was seen among T_1- T_5 and T_2-T_5, while C_1, C_2, T_1, T_3 and T_4 were found to be at par with each other. The lowest value in case of flavour was reported by C_2 (6.45) and highest was by T_4 (7.29) and showed significant ($p<0.05$) difference for T_3 and T_4, while C_1, C_2, T_1, T_2 and T_5 were at par with each other. C_2 and T_1 scored the lowest value of 6.5 for taste and highest was scored by T_4 (8.00). Significant ($p<0.05$) difference was seen among all except C_2, T_1 and T_2, which were at par with each other. The lowest value was scored by C_2 and T_1 of 6.7 for texture and highest was scored by C_1 (8.5). C_1, T_4 and T_5 showed significance ($p<0.05$) difference, while the rest were at par with each other. The lowest value for overall acceptability was reported by T_5 (6.5) and highest was gained by C_1 (8.5). C_1, T_3, T_4 and T_5 showed significant ($p<0.05$) difference, while the remaining was at par with each other.

Table 2: Sensory analysis of soya based probiotic frozen dessert

Treatments	Colour	Flavour	Taste	Texture	Overall Acceptability
C1	7.62^{B}	6.62^{A}	7.70^{B}	8.50^{C}	8.50^{D}
C2	7.29^{B}	6.45^{A}	6.50^{A}	6.70^{A}	6.50^{A}
T1	7.12^{B}	6.50^{A}	6.50^{A}	6.70^{A}	6.50^{A}
T2	7.25^{AB}	6.70^{A}	6.67^{A}	6.84^{A}	6.80^{A}
T3	7.45^{B}	6.91^{B}	7.12^{AB}	6.95^{A}	7.00^{AB}
T4	7.83^{B}	7.29^{C}	8.00^{BC}	7.54^{AB}	8.00^{C}
T5	6.75^{A}	6.58^{A}	7.83^{B}	7.60^{B}	7.50^{B}
CD (5%)	0.50	0.21	0.22	0.62	0.48

The superscript A, B, C,…… indicate the comparison of variables with respect to skim milk powder incorporation levels based on the CD values.

The purpose of present study was to determine if the product developed would have a comparable colour, flavour, taste and texture to control product.

The quality of soya based probiotic frozen dessert has been improved with the increase in addition of SMP and also resulted in decline of beany flavour.

The T_4 (Fig.2) was the most acceptable to the judges as the score for colour, flavour and taste were highest among treatments and controls, whereas the score for texture and overall acceptability was higher than the product control (C_2) (Fig.3). Whereas T_1 and T_2 melted immediately as taken out from refrigerated condition. The T_5 could not score much due to its colour and flavour as compared to T_4.

Fig.1: Photograph of Treatment (T_4) Sample of Soya Based Probiotic Frozen Dessert

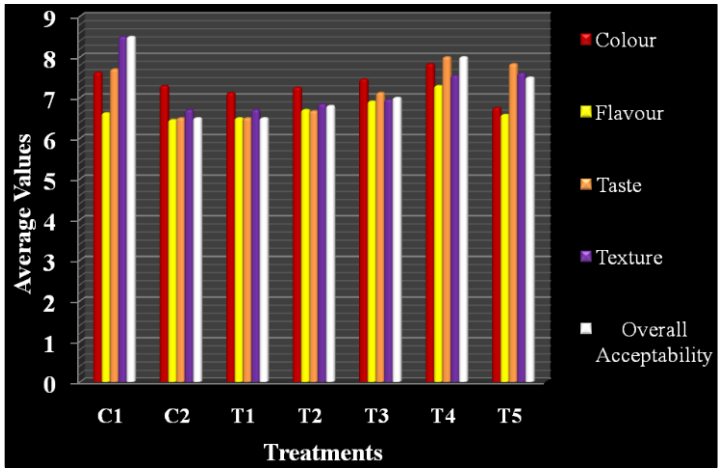


Fig 2: Graph showing mean sensory values of treatments samples of soya based probiotic frozen dessert

Selvarani *et al.* (2000) [6] prepared a kulfi by blending soy milk to cow milk in 30:70 ratio and obtained a rich creamy yellow colour kulfi with added richness of higher protein and total solids. Bharad *et al*. (2010) [2] studied the effect of different combinations of buffalo and soymilk on the quality of kulfi and concluded that addition of 25 per cent soymilk had no effect on body and texture and melting quality of kulfi. Ahanian *et al*. (2014) [1] also reported that 50 per cent addition of soymilk did not have significant effect on flavour, texture, colour and overall acceptability.

Evaluation of fresh soya based probiotic frozen dessert

Chemical analysis of soya based probiotic frozen dessert

The selected fresh product (T_4) was evaluated for per cent fat, total solids and pH. The average fat percentage of T_4 was 9.37, Whereas for C_1 and C_2 was 10 and 9.37 per cent, respectively (Table 3).

The total solids was maintained at 45 per cent during the product preparation for test and controls (Table 3).

The average pH of the test product (T_4) was 7.08 whereas, for C_1 and C_2 were 6.49 and 7.08, respectively (Table 3).

Table 3: Chemical Analysis of Fresh Soya Based Probiotic Frozen Dessert

Treatments	Fat (per cent)	Total Solids (per cent)	pH (per cent)
T_4	9.37	45	7.08
C_1	10	45	6.49
C_2	9.37	45	7.08

Analysis of soya based probiotic frozen dessert

The fresh test product and C_1 were evaluated for probiotic, total viable, coliform and *Salmonella and Shigella* counts. The product control (C_2) was prepared without addition of probiotic culture, therefore, C_2 was examined only for total viable count.

Effect of product manufacture on probiotic viability

The initial load of probiotic culture was $\log_{10}$ 9/g. During the manufacture process i.e mainly freezing, the decrease in viable count to $\log_{10}$ 8/g was reported (Table 4).

Effect of product manufacture on total viable count

The total viable count of T4 was 8.0/g, whereas total viable count for C_1 and C_2 were 8.0/g and respectively (Table 4).

Table 4: Microbiological analysis of fresh soya based probiotic frozen dessert

Treatments	Probiotic Count ($\log_{10}$cfu/g)	Total Viable Counts ($\log_{10}$cfu/g)
T_4	8	8
C_1	8	8
C_2	-	0

Effect of product manufacture on coliform count

The coliform count was absent in fresh test and control products.

Effect of Product Manufacture on *Salmonella and Shigella* Count

Salmonella and Shigella count was absent in fresh test and control products.

Antimicrobial activity of soya based probiotic frozen dessert

The antimicrobial activity of test product (T_4) was evaluated against *Escherichia coli* NCDC 249, *Staphylococcus aureus* NCDC 111 and *Salmonella enteritidis* ssp. *Typhimurium* NCDC 113 by using well assay method.

The diameter for zone of clearance (Table 5) was the highest against *Escherichia coli* NCDC 249, *Staphylococcus aureus* NCDC 111 i.e. 2 cm. whereas for *Salmonella enteritidis* ssp. *Typhimurium* NCDC 113 the diameter of zone of clearance was 1cm.

Table 5: Antimicrobial Activity (zone of inhibition in cm) of Fresh Soya Based Probiotic Frozen Dessert

Product	Diameter (cm) of zone of clearance against		
	E.coli	Staph. Aureus	Salmonella
Lactobacillus rhamnosus GG	2	2	1

Sensory evaluation of fresh soya based probiotic frozen dessert

The test product (T_4) and controls (C_1 and C_2) were evaluated for sensory parameters on the basis of 9-point hedonic scale by a panel of five judges.

The average scores (Table 6) for colour of T_4 was 8.0 as compared to C_1 and C_2 was 8.5 and 7.5, respectively. The scores for taste were highest for T_4 i.e. 7.87 as compared to standard control C_1 i.e. 7.58 and product control C_2 i.e 6.75. The scores for overall acceptability of T_4 and C_1 were 8.0 whereas, scores for C_2 was 6.54.

Table 6: Sensory evaluation of fresh soya based probiotic frozen dessert

Treatments	Colour	Flavour	Taste	Texture	Overall Acceptability
T_4	8	7.12	7.87	7.75	8
C_1	8.5	7.5	7.58	8.55	8
C_2	7.5	6.75	6.75	6.74	6.54

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