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Formulation And Quality Analysis Of Probiotic Ice- Cream Prepared Using *Lactobacillus* Strains

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1. ABSTRACT

A study was undertaken to develop a probiotic ice cream using Lactobacillus strain isolated from curd. Ice-cream is the commonly consumed dessert by all age grouped population and hence, the benefit

A study was conducted to develop a probiotic ice cream using Lactobacillus strains separated from curd. Today, the demand for functional foods with health benefits is growing rapidly due to the increased awareness of the consumers on impact of food on health around the world. Probiotic ice cream was developed ice cream can be stored for a long time without losing its properties, and it is a highly popular product all over the world. The formulated ice cream was evaluated for its proximate composition, quality parameters and microbiological aspects. The storage stability was also evaluated to check the viability of probiotics on storage. Lactobacillus strains are effective in treating various gastrointestinal disorders, adding them in ice cream can make it accessible for a large population. The findings revealed that the probiotic viability was constant for first 15 days and later on decreased gradually. The sensory quality was acceptable and the viability can be increased by some effective processing methods like encapsulation

Key words:

Probiotics, *Lactobacillus,* curd, Ice-cream, sensory quality

2. INTRODUCTION

The significant role of probiotic in enhancing the gut health and overall human well-being has increased the demand for probiotic-based nutriment tremendously. Since studies have proved benefits of fermented milk and its effect on improved gut microbiota, many extensive studies have been conducted on the beneficial effect of probiotics on human; and its relation in preventing and treating gut – related diseases such as

infectious and antibiotic- associated diarrhoea, irritable bowel syndrome, lactose intolerance, indigestion and stomach bloating has been established. **(M.L. Ritchie & T.N.Romanuk, 2012)**

Etymologically, the word probiotic appears to be a composite of the Latin predeposition *pro* ("for" or "in support") and the Greek adjective (biotic)from the noun *bios meaning* "for life" or " in support of life"**(C.Ezema, 2013)**. The definition of probiotic has evolved over time and today probiotics are defined as "*live microorganisms that, when ingested in adequate amounts, exert a health benefit to the host"* retaining the previous definitions by Food and Agriculture Organization of the United Nations and world Health Organisations(FAO/WHO) with a minor grammatical changes **(Hill et al., 2014).**

Most probiotics are commonly known as Lactic acid bacteria (LAB) due to their ability to produce lactic acid when fermented on sugar- rich substrate. Based on its morphological and phenotypic characteristics, the LAB was initially split into the genera *Betbacterium, Thermobacterium, Streptobacterium, Streptococcus, Betacoccus, Tetracoccus* and *Microbacterium.* Only Streptococcus remains in use presently. Majority of others have been renamed into *Lactobacillus,Bifidobacterium* and *Enterococcus* **(D. Harzallah and H. Belhadj, 2013; W. H. Holzapfel et al., 2001).** LAB (Lactic Acid Bacteria) is commonly found as resident microflora of the gastro-intestinal and genitor- urinary tract of vertebrates **(Dessalegn & Ashenafi, 2010)** More specifically, the metabolic activities of selected strains belonging to the *Lactobacillus* genus allow the body to enhance the digestion of lactose, decrease the cholesterol level in the blood serum, prevent intestinal disorders, and stimulate immune function **(Havenaar *et al.*, 1992; Gilliland and Walker, 1989)**. Nowadays, the interest in probiotic research and industrialization is on developing combination of different probiotics species and strains as many studies have proven that it delivers superior impact on human health compared to the use of single probiotic strain **(Holzapfel *et al.*, 2001).** The most common species used as probiotics in the manufacturing of fermented milk and other dairy products are *Lactobacillus* and Bifidobacterium **(McFarland and Elmer, 2006)**.

Probiotic food is described as "a food product containing live probiotic bacteria in appropriate populations integrated into a suitable matrix"**(Gibson & Roberfroid, 1995; Saxelin *et al.*, 2003).** This means that their viability and metabolic activity must be maintained throughout the whole food processing process, from manufacture to consumer consumption, as well as their capacity to survive in the gastrointestinal system **(Sanz, 2007).**Based on the existing data, the concentration of probiotic bacteria required for biological effects varies depending on the strain and the desired health impact **(Champagne *et al.*, 2005).** Nonetheless, populations of 106–107 CFU/g in the final product have been established as therapeutic quantities of probiotic cultures in processed foods **(Talwalkar *et al.*, 2004)**, with daily consumption of 100 g or 100 mL of food providing 108–109 CFU and thus benefiting human health **(Jayamanne and Adams, 2006).**

Probiotic bacteria have been linked to a variety of health advantages, and more than 90 probiotic products containing one or more probiotic organism categories are available globally **(Tharmaraj and Shah, 2003).**

Today, there has been a significant increase in the consumption of food products containing probiotic bacteria, including dairy products like fermented milk, ice cream, different desserts, whey-based drinks, sour cream, buttermilk, regular and flavoured liquid milk, and concentrated milk **(Akin *et al.*, 2007, Kailasapathy and Sultana, 2003).**

Ice cream is a tasty, healthy, and nutritious frozen dairy product that is enjoyed by people all over the world. It's a frozen blend of ingredients including milk, sweeteners, stabilizers, emulsifiers, and flavouring additives **(Marshall *et al.*, 2003).** Ice cream is a dairy product with a lot of potential as a probiotic bacteria food carrier. The addition of probiotic bacteria to ice cream is highly beneficial because, in addition to being a functional healthy food, ice cream contains beneficial substances such as dairy raw materials, vitamins, and minerals, and is widely consumed **(Reza *et al.*, 2011).**

The effectiveness of probiotic bacteria introduced at a higher dose is dependent on the dosage level. Their viability must be maintained throughout the shelf life of the product, as well as their capacity to live in the gut environment. They must establish themselves in certain quantities in the gastrointestinal tract to have beneficial health effects **(Kailasapathy & Sultana, 2003)**. Several food organizations throughout the world have developed a standard mandating a minimum of 106–107 colony forming units per gram (cfu g^{-1}) of *L. acidophilus* and/or *Bifidobacteria* in fermented milk products**(IDF, 1992).**

Probiotic ice cream is gaining popularity among probiotic dairy products: ice cream can be stored for a long time without losing its properties, and it is a highly popular product all over the world. In comparison to fermented dairy products, ice cream is an excellent vehicle for delivering probiotic organisms to the human body **(Haynes and Playne, 2002; Hekmat and McMahon, 1992).** However, during the production, processing, storage, and melting of the product, probiotic bacteria viability losses in ice cream are unavoidable. Probiotic cells degrade more quickly during the freezing process than during storage. The viability of probiotics in the finished product can be improved by using techniques like selecting and implementing oxygen-resistant probiotic strains, eliminating molecular oxygen, applying extreme heat treatment, using microencapsulation techniques, and modifying the product formulation (such as fortifying milk with nutrients and prebiotics). It has been discovered that adding probiotic bacteria to ice cream has minimal impact on its flavour, texture, or other sensory qualities. There are numerous approaches to enhance the product's sensory qualities in order to make up for any alterations that do take place (**Mohammadi*et al.*, 2011)**.

3. AIM

To prepare an ice cream using *Lactobacillus* strains and evaluate the quality parameters.

4. OBJECTIVE

- To isolate and identify *Lactobacillus* strains from curd.
- To Standardize and develop value added product from Lactobacillus strains.
- To study the physiochemical, microbial and organoleptic property of the product.
- To analyse shelf stability of the developed product

5. MATERIALS AND METHODS

5.1 RAW MATERIALS

Commercially available ingredients such as Skimmed milk (Good Life), Milk powder (Nestle), Fresh cream (Amul), icing sugar, vanilla essence (Bakers) and gelatin were procured from a local market of Kerala, India. Pure *Lactobacillus* culture was identified and isolated from a commercially available curd (Milma).

De Man, Rogosa, and Sharpe (MRS) agar medium was procured from SRL.

5.2 METHODOLOGY

5.2.1 IDENTIFICATION AND EXTRACTION OF Lactobacillus STRAINS FROM CURD

De Man, Rogosa, and Sharpe (MRS) agar was the medium selected. It was prepared by weighing 0.3 gm. The curd sample used for the incubation was obtained by three fold-serial dilution. It was streaked on the sterile MRS agar by quadrant streaking method under aseptic condition and incubated at 37°C for 48 hours. The colonies obtained as the result of incubation were studied, pure *Lactobacillus* strain was identified, isolated and restreaked on a fresh MRS agar medium followed by three-fold-serial dilution. The isolated *Lactobacillus* colonies were stored in MRS agar slants at 4°C until it was centrifuged to use in the preparation of ice-cream.

5.2.2 STANDARDISATION OF ICE-CREAM WITH *Lactobacillus* STRAIN

Pasteurized skimmed milk and milk powder were blended using a domestic blender, heated to 50°C and cooled to 5°C. Sugar and fresh cream were blended in another container for 10 minutes at room temperature. Blended sugar and cream mixture was added to the cooled milk mixture and homogenized for 10 min at room temperature using a blender. Four drops of vanilla essence was added to it.

The *Lactobacillus* inoculant culture was prepared by incubating it for 12 h at 37°C in sterilized- -skimmed milk. The inoculant culture was added to the ice cream mixture, homogenized and kept for ageing at 5 ±1°C for 12 hours. It was then stored at -20°C for storage. Standard ice cream (control) was prepared without the

inclusion of *Lactobacillus* strains and three variations of probiotic ice cream was made with varying amount of *Lactobacillus*(2 %, 5%, and 10%) Table 1.

Table 1: Standardization of ingredient composition for development of probiotic ice cream

	Skimmed milk(g)	Milk powder(g)	Fresh cream(g)	Sugar(g)	Stabilizer (%)	*Lactobacillus*culture (%)
Control	50	10	25	10	0.8	0
Variation 1	50	10	25	10	0.8	2
Variation 2	50	10	25	10	0.8	5
Variation 3	50	10	25	10	0.8	10

Figure 1: control ice cream

Figure 2: probiotic ice cream variations (before freezing)

5.2.3 SENSORY EVALUATION

The sensory evaluation of the probiotic ice cream samples were performed by 10 semi-trained panelists after 24 hours of storage at -20°C through the 9-point hedonic scale. The data were analysed for mean and standard deviation.

5.2.4 NUTRITIONAL ANALYSIS

5.2.4.1 ESTIMATION OF MOISTURE

The moisture content of the sample was analysed by AOAC method. The formula used was

$$\textbf{Moisture (\%)} = \frac{W1-W2}{W3} \times 100$$

Whereas W1 = empty weight of sample.

W2 = weight of Petri plate + weight of sample (initial weight)

W3= weight of dried sample + weight of petri plates (final weight).

5.2.4.2 TOTAL ASH

The ash content was obtained by incinerating the samples in a muffle furnace for 6 hours at temperature not more than 525ºC in crucibles to ensure complete carbon burning. After cooling to room temperature, the obtained ash was weighed and recorded.

Ash content = $\frac{W2-W1}{W3} \times 100$

Whereas W1 = empty weight of sample.

W2 = weight of Petri plate + weight of sample (initial weight)

W3= weight of dried sample + weight of petri plates (final weight).

5.2.4.3 TOTAL CARBOHYDRATE CONTENT AND PROTEIN ESTIMATION

Total carbohydrate content was estimated as per Anthrone method, using glucose as a standard. The values are determined colorimetrically. Protein content was estimated by Lowry‘s method.

5.2.4.4 ESTIMATION OF FAT CONTENT

Fat content was estimated as per standard procedure given by AOAC (Association of Official Analytical Chemists) (1995) using soxhlet apparatus as petroleum extractable fat (%).The results were expressed as fat % using

Fat% = $\frac{M1-M2\times100}{M}$

Where,

M1= mass in g, of the Soxhlet flask with the extracted fat

M2= mass in g, of the empty Soxhlet flask, and

M = mass in g, of the material taken for the test.

5.2.5 CHEMICAL ANALYSIS

The TSS of probiotic ice cream samples were measured by using Hand Refractometer and expressed in ºBrix. Titrable acidity of samples were determined by titrimetric method using NaOH.The pH of the given samples were determined by using handheld pH meter.

5.2.5.1 ESTIMATION OF PROXIMATE COMPOSITION

The prepared product was analysed for proximate composition using the standard techniques as indicated below: Moisture, ash, fat were analysed by AOAC method. Total carbohydrate content was estimated as per Anthrone method and Protein content was estimated by Lowry‘s method.

5.2.5.2 DETERMINATION OF MICRONUTRIENTS.

The calcium, sodium and potassium content in samples were analysed by flame photometry method and phosphorous concentration was determined by colorimetric method

5.2.5.3 TOTAL CALORIFIC VALUE

Total calories of the sample were calculated by the following formula.

Total Calories = (Fat × 9) + (Protein × 4) + (Total Carbohydrate × 4)

5.2.6 MICROBIAL ANALYSIS

5.2.6.1 PROBIOTIC BACTERIA COUNT

Probiotic bacteria count was done by plate count method after diluting the sample by 1: 10 ratio. The counts were performed in duplicate by using the pour plating technique.

5.2.6.2 COLIFORM COUNT

In probiotic ice cream, EMB agar was used for enumeration. Plates of $1:10^0$and $1:10^1$ dilutions were prepared. Counts were performed in duplicate by using the pour plating technique. The inverted plates were placed in incubator maintained at 37±1°C for 48 hours. After incubation period the bacterial colonies were counted with the help of colony counter and multiplicated by respective dilution factor to enumerate the total number of bacteria (E-coli) in probiotic ice cream.

6. RESULTS AND DISCUSSION

6.1 Sensory evaluation

Ten semi trained panellists evaluated the probiotic ice cream samples made with 2%, 5%and 10% lactobacillus strains and compared these with a control sample. Overall acceptability and sensory attributes (appearance, texture, flavour, taste and melting quality) were measured on a 9-point hedonic scale. Results of the sensory analysis are listed in Table 1. All the sensory parameters were significantly affected by the addition of lactobacillus strains in ice cream. The sensory properties of ice cream samples were scored between 7 and 8 (moderately-like very much). The lactobacillus strains-added ice creams received similar sensory scores compared to control group.

Among the samples analysed, these three samples (the control, sample with 2 %, and 10% lactobacillus) had almost similar appearance (8.2± 0.26, 8.2±0.25, 8.25±0.33). Control sample scored more than other samples for all attributes. Among the lactobacillus incorporated samples other than the control, sample with 10% lactobacillus got a high score in terms of flavor and melting point. Also samples with 5% and 10% lactobacillus strains had similar textures (7.66±0.28, 7.7±0.4). Taste quality obtained a high score in control compared to other samples. Overall, the acceptability was high for control. Among the lactobacillus strains-added ice cream samples, 10% lactobacillus strains received slightly higher scores for appearance, flavour

and overall acceptability. Based on the response obtained, ice cream with 10% lactobacillus strains was the final selected sample for further study.

Table 1: Mean scores of sensory attributes for different probiotic ice cream samples

Sensory attributes	Control	Ice cream with 2%Lactobacillus	Ice cream with 5% Lactobacillus	Ice cream with 10% Lactobacillus
Appearance	8.2±0.26	8.2±0.25	8.1±0.3	8.2±0.33
Texture	8.3±0.25	7.4±0.43	7.6±0.3	7.7±0.4
Flavour	8 ±0.1	7.1±0.24	7.5±0.24	7.6±0.35
Melting quality	8.21±0.6	7.1±0.24	7.4±0.31	7.8±0.24
Taste	8 ±0.04	7.4±0.5	7.4±0.44	7.5±0.4
OAA*	8.2±0.42	7.5±0.52	7.6±0.3	7.7±0.43

*** OAA- Overall Acceptability**

6.2 Chemical and microbial analysis

The results of the chemical analysis are summarised in Table 2.The compositional analysis of ice cream with 10% lactobacillus strains indicated following values: moisture: 51.8±0.21 %; Ash: 2.5±0.2%; Carbohydrate: 13.5±0.52%; Protein: 6.7±0.3%;Fat:10.8±0.32%; sodium:3±0.3% ;potassium: 3±0.2%; calcium: 9±0.32%; phosphorous: 3±0.3% (Table 3). The chemical analysis results indicated pH: 5.89; total soluble solids 0.03°Brix and titrable acidity: 0.693g/L. The microbial analysis indicate 3.525 (5 log CFU/g) of lactobacillus strain in the selected sample and absence of *Esheriae coli* on first day.

Table 2: Effect of storage on proximate, chemical and microbial content of probiotic ice-cream

Parameters	1st day	15 day	30 day
Proximate analysis			
Moisture	51.8±0.21	50.6±0.34	49.9±0.13
Ash	2.5±0.2	2.4±0.2	2.4±0.4
Carbohydrate	13.5±0.52	13.1±0.51	12.8±0.52
Protein	6.7±0.3	6.4±0.17	6±0.11
Fat	10.8±0.32	10.5±0.3	9.9±0.4
Chemical analysis			
Titrable acidity	0.693±0.02	0.688±0.03	0.682±0.03
P_H	5.89±0.2	5.65±0.3	5.42±0.4
Total soluble solids	0.03±0.02	0.035±0.03	0.038±0.03
Microbial analysis			
***Lactobacillus* (5 log cfu/g)**	3.525±0.4	3.502±0.3	3.250±0.3
Esheriae.coli	Nil	Nil	Nil

Table 3: Micronutrient composition of probiotic ice cream

Parameters	Ice cream with 10% Lactobacillus
Micronutrients (%)	
Sodium	3±0.3
Potassium	3±0.2
Calcium	9±0.32
phosphorous	3±0.3

6.3 Effect of storage study

The results of storage study are summarized in Table 2. The proximate analysis showed a decrease in nutrient content during the storage. The difference in carbohydrate, protein and fat from day 1 to day 30 were 0.7±0.52 %, 0.89±0.11%, 0.82±0.4% respectively. A slight increase in the total soluble solids content was observed after 30 days of storage ranging from 0.03±0.02-0.038±0.03°brix. The pH value decreased during the storage period. The storage of the 10% lactobacillus probiotic ice cream influenced the total number of microorganisms, the viability of the strains were constant for first 15 days, later a decrease in the viability of microorganisms was observed in the fermented ice cream after 30 days. However E.coli absence were noted during the 30 day storage period.

7. CONCLUSION

The results of the present study indicate that ice cream could be considered as a carrier for probiotic strains with health benefits. The result shows that the probiotic viability was constant for first 15 days and later on decreased gradually. The sensory quality was acceptable and the viability can be increased by some effective processing methods like encapsulation.

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