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Enrichment of probiotic ice-cream with prebiotic green banana flour (Resistant starch)

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Abstract
Base ice cream mix was standardized to contain 10% fat, 11% milk solids not fat MSNF), 15% sugar (66 % sucrose + 33% glucose), 0.3% Carageenan gum as a stabilizer and 0.2% and emulsifier. The required amounts of sugar, stabilizer, skim milk powder (97% DM), cream (30% fat) and full cream buffalo milk (6% fat) were calculated. To the base ice cream mix green banana flour at 2%, 4% and 6% level was added and one sample kept as a control without adding green banana flour. Then after homogenization, and sterilization followed by cooling, probiotic strains were mixed in prebioticice cream mix, followed by aging and freezing. The probiotic ice cream with prebiotic component was evaluated for chemical properties, cultures survival and sensory characteristics during 8 week of frozen storage at-26°C. It could be concluded that, value addition of green banana into unripe banana flour minimizes the post harvest losses and also effectively used to develop functional probiotic ice creams rich in fiber and resistant starch. Therefore, could be used as good source of dietary fiber or prebiotic component for making synobiotic ice cream with good nutritional and functional properties. The ice cream obtained by added unripe banana flour to the level of 4 percent was rated higher sensory score for overall acceptability by the consumers and also has moderate viable counts (log cfu/g) of probiotic organisms.

Keywords: green banana flour, post harvest losses, ice cream, prebiotic, probiotic, microbial count, resistant starch

Introduction
Ice cream has good potential for use as a probiotic vehicle because of its composition as well as its pleasant taste and attractive texture. Furthermore, probiotics are able to survive over long storage periods in frozen systems such as ice cream matrix that includes nutritive constituents such as milk proteins, fat, and lactose (Cruz *et al*., 2009) [7]. Ice cream is a complex colloidal food system that in its frozen state consists of ice crystals, air cells, and partially coalesced fat droplets dispersed in a continuous freeze-concentrated aqueous (serum) phase containing polysaccharides such as galactomannans, carrageenans, cellulosics, sugars (sucrose and lactose), proteins, and minerals (especially calcium, but also sodium and potassium) (Goff 2008) [8]. Ice cream's structure and colloidal design, together with its low-temperature storage, renders it a very promising carrier for the stabilization and in vivo delivery of bioactive compounds and beneficial microorganisms. In this sense, the nutritional/nutraceutical potential of banana starch has been claimed by several authors (A. Aparicio-Saguila´ n *et al*., 2007) [3]. Banana (*Musa Cavendish*) is the fourth most important crop after rice, wheat and corn. Over thirty million tons of Bananas are produced every year in India from the total land area of 0.82 million ha. During banana commercialization and post-harvest handling in India, large quantities of this commodity are lost due to the fact that the banana is a climacteric fruit and the Indian habit of consuming ripe fruit. About one-fifth of all bananas harvested become culls. When banana bunches arrive at central collection stations, bananas too small for shipping are removed, along with those that have damaged or spoiled areas that could cause microbial contamination of the bunch. Rejected bananas are normally disposed of improperly. A successful industrial use of the culled bananas would alleviate the problem while offering employment and financial return to the inhabitants. Most likely, the first practical application of culled bananas would be use of the pulp for starch production or production of a low-cost banana flour ingredient (P. Zhang *et al*., 2005) [11]. Banana cultivars are a potential source of complex carbohydrates, such as β-glucans and resistant starch, are known to be physiologically beneficial and are associated with disease prevention. Resistant starches are not digested in the human small intestine and are fermented by bacterial microflora in the large bowel, affecting a number of physiological functions and thus having different effects on health, e.g., reduction of the glycemic and insulinemic responses to food, hypocholesterolemic action and protective effects against colorectal cancer (Asp *et al*., 1996).

The minimum necessary concentration of probiotic bacteria to cause abeneficial result has been generally accepted as 10^6 viablecells/g-ml product at the moment the product is consumed (Blanchette *et al*. 1996) [6]. Also, many authors suggest thatingestion of 10^8–10^9 viable cells per day is needed todevelop beneficial effects for humans (Reza Mohammadi *et al*., 2011) [12].

The objective of this study is to use unripe banana flour as a food ingredient to enrich probiotic ice cream, which is low in fat content and high in resistant starch with viable bacterial culture.

Materials and Methods

Materials

Commercial hard green (unripe) pre-climacteric bananas (*Musa paradisiaca* L.) in the stage 2 (color index) were purchased from the local farm of Rangareddy, India. Freshly harvested unripe banana were peeled, cut into 1 cm slices and immediately rinsed in citric acid solution (0.3 g/100 ml). The slices were dried at 50°C and subsequently ground using a lab scale emery flour mill (Kalasi, India) to pass a BSS mesh size of 60 (0.25 mm) and stored at 27°C in sealed plastic containers for further analysis. The process of production of unripe banana flour is depicted in flow chart in Figure 1.

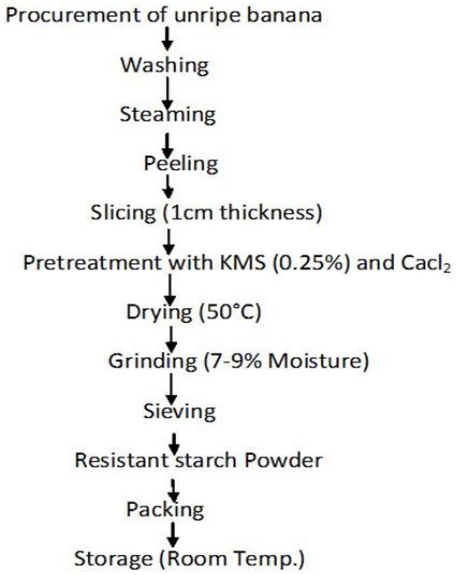

Fig 1: Flow chart of production of unripe banana flour

The ingredients of ice cream mix included full cream Buffalo milk with the fat content of 6%, skim milk powder (MSNF in 11 – 11.5% wt.), Light cream with 25% fat were procured within the dairy unit of Heritage Foods Pvt Ltd, Hyderabad. Stabilizer and emulsifier (mono- and diglycerides of fatty acids) were procured from the Food Ingredients speciality Ltd, Chennai and Sugar purchased from local market. Cultures of Bifidobacterium bifidum and Lactobacillus acidophilus were purchased from DSM, India were activated individually by three successive transfers in modified MRS followed by three successive transferes in sterile 10% reconstituted skim milk powder and incubated at 37°C for 48hr under anaerobic conditions (A. S. Akalın *et al*., 2018) [5] cultures were prepared 24 hr before used. The chemicals used in the investigation are of analytical grade. Sodium Hydroxide, Hydrochloric acid, Sulphuric acid, Sodium carbonate, Copper sulphate and Petroleum ether were obtained from Molychem, India. Bromocresol green, Phenolphthalein, Boric acid and Methyl red indicator were obtained from Qualigens, India. Locally manufactured skid mounted dairy unit with scrapped surface heat exchanger was used to make ice creams.

Methods

Blending of ingredients for preparation of probiotic ice cream

Base ice cream mix was standardized to contain 10% fat, 11% milk solids not fat (MSNF), 15% sugar (66 % sucrose + 33% glucose), 0.3% Carageenan gum as a stabilizer and 0.2% and emulsifier. The required amounts of sugar, stabilizer, skim milk powder (97% DM), cream (30% fat) and full cream buffalo milk (6% fat) were calculated. To the base ice cream mix green banana flour at 1%, 4% and 6% level was added and one sample kept as a control without adding green banana flour. The mix was heated at 85 ºC for 5 min, then cooled to 5 ºC. Then the starter culture of L. acidophilus and B. lactis Bb12 were added to all mixes to achieve approximately 10^8 cfu/g, mixed well. Then the probiotic ice cream mixes were aged at 4°C for 20 h. Each mix was frozen in an experimental ice cream batch freezer (Automatic ice cream maker, Delonghi, II Gelatic, Italy). The resultant products were packed into PVC cups (ca. 200 ml) covered and hardened at -26 ºC 24 hr before analyses. Three replicates were carried out for every treatment. The formulations for the ice creams are presented in Table 1. The ice cream samples were analyzed for their physic-chemical, microbial count and sensory attributes.

Table 1: Formulation of Ice creams added with unripe banana flour.

Ingredient	Control	A	B	C
Milk fat (%)	10	10	10	10
Milk solids not fat (MSNF) (%)	11	11	11	11
Sugar (%)	15	15	15	15
Stabilizer	0.3	0.3	0.3	0.3
Emulsifier	0.2	0.2	0.2	0.2
Green banana flour (Resistant starch) (%)	-	2	4	6
Vanilla flavour (%)	0.01	0.01	0.01	0.01

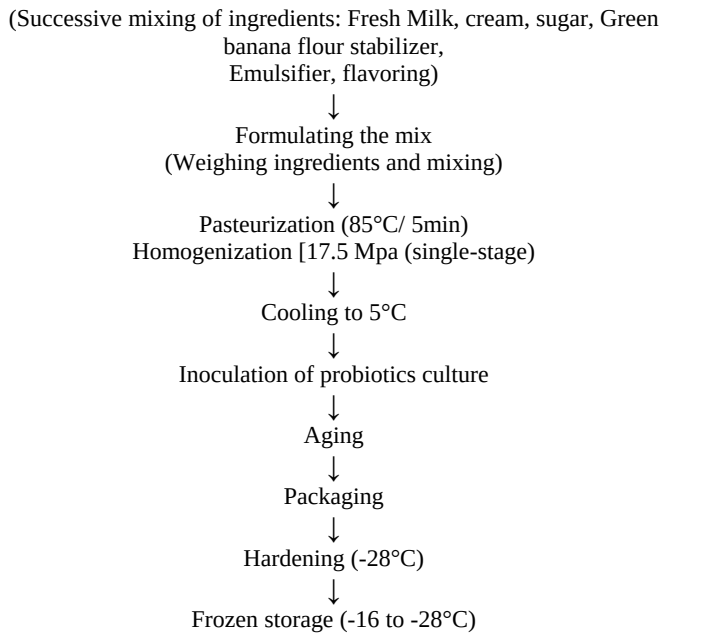

Fig 2: Flow chart for production of Prebiotic enriched Probiotic Ice cream

Determinations of pH, Acidity, Overrun and Melting rate

For the pH measurement, about 100 g ice cream samples were melted at 5°C then the pH was measured using a pH meter

(model Shimadzu 331). Acidity measured by titrating against 0.1N NaoH. The overrun, was measured as described by Arbuckle (1986) [4]. To determine the melting resistance (Meltdown), the blocks of frozen ice cream weighting 200 g at -18 °C allowed melting at 20-25 °C for 45 min. The melting rate was determined according to the method described by Prindiville *et al*. (2000) [10] with some modifications. The ice cream samples were weighed in plastic cups and stored at -18 °C for 2 weeks were evaluated. Pre-weighed ice cream samples (70 g) were carefully removed from the plastic cups and laced on a wire mesh (1 cm2), and a previously weighed beaker was placed beneath the mesh to collect the melted ice cream. The system was kept at 24 °C ± 1 °C. After 30 min, the beaker with the melted ice cream was replaced with another one and weighed. This process was repeated at 10-min intervals up to 70 min. The assay was performed in triplicate for all samples.

Proximate analysis

Moisture, Crude protein, Total fat (Gerber metod), Crude fiber and Resistant starch were determined using approved AOAC methods (2002) [2].

Enumeration of Probiotic Bacteria

10 g of each ice cream was aseptically transferred into a sterile stomacher bag, diluted with 90 mL of Ringer's solution (Qualigens: India) and homogenised for 1 min in bag mixer. Subsequent serial dilutions were made and viable cell numbers enumerated using the pour plate technique. One millilitre of the first dilution was used to obtain 10-fold serial dilutions, which were used for microbial counts (Ahmet Ayar *et al*., 2017) [1]. Before microbiological analysis, the samples were kept at 4 °C for 30 min. The colony counts of Bifidobacterium bifidum and Lactobacillus acidophilus were enumerated in each ice cream sample using de Man, Rogosa, and Sharpe (MRS)-sorbitol and MRSNNLP (where NNLP = nalidixic acid, neomycin sulfate, lithium chloride, and paromomycin sulfate) agar, respectively (Tharmaraj and Shah, 2003). The inoculated plates were incubated anaerobically at 37°C for 72 h using stability chamber (Pelican, India). Counts were expressed as log10 CFU/g. All analyses were conducted in triplicate.

Sensory Evaluation

The sensory assessments were conducted in quality control lab of dairy plant located at Uppal. The panel of 25members consisted of staff and trainee students. The panelists were naive to project objectives. The samples (Control, samples (A, B and C)) were coded with three digit-numbers and served with the order of presentation counter-balanced. Panelists were provided with a glass of water and, instructed to rinse and swallow water between samples. They were given written instructions and asked to evaluate the products for acceptability based on its appearance, flavour, texture, taste, color and overall acceptability using nine-point hedonic scale (1 = dislike extremely to 9 = like extremely; Meilgaard *et al*., 1999) [9].

Results and Discussion

Mean values of pH decreased with the addition of more green banana flour. The decline of pH may be due to contain the various organic acid compounds in banana flour. Similar kind of results was reported by Sun-Waterhouse *et al*., (2013) [13]. Mean values of Acidity increased with the addition of increased levels of green banana flour. The elevated acidity may be due to contain the various organic acid compounds in banana flour. Commercially all of the probiotic ice cream samples normally within the overrun values compared to the values reported in literature (80 – 120 %); although the increased concentration of green banana flour in ice cream decline the overrun values of the ice creams compared to control sample. The results shown in Table 2 indicate that the addition of green banana flour decreased the melting rate especially at concentration of 6% green banana flour in the mix when compared to control sample. This result may be due to green banana flour containing higher percentage of resistant starch. Fiber and resistant starch components has the ability to deabsorb water, thus decreasing the viscosity and reducing the melting rate. Cottrell *et al*. (1979) reported that the rate of meltdown in ice cream must be controlled without producing excessively small meltdown products. A meltdown between 10 – 40% was considered to be acceptable when the block was allowed to melt for two hours at 25 °C.

Table 3: Physical Properties of Probiotic Ice cream

Samples	pH	Acidity (%)	Over run	Melting rate (%)
Control	6.71±0.02	0.19±0.02	81.83±0.01	2.87±0.03
Sample –A	6.68±0.01	0.21±0.03	78.81±0.03	2.82±0.01
Sample-B	6.60±0.02	0.23±0.01	77.59±0.02	2.78±0.01
Sample-C	6.57±0.01	0.24±0.02	77.31±0.03	2.72±0.03

The Resistant starch content of the samples was increased on the addition of unripe banana flour and the high percentage of resistant starch is found to be 3.3 %.The resistant starch content in the accepted sample (sample B) is 2.2 %. An increase in resistant starch was observed when the amount of unripened green banana flour increased in the ice cream.

It is evident that there is an increased percentage of crude fiber and resistant starch gradually in the ice cream on the addition of unripe banana flour as compared to control sample. This increased amount of crude fiber and resistant starch in the ice cream may be attributed to the addition of unripe banana flour.

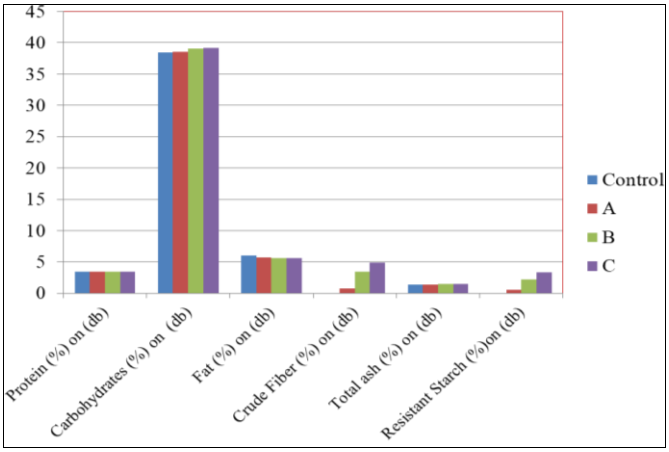

Fig 3: Nutritional evaluation of probiotic Ice creams

Table 4: Viable counts (log cfu/g) of probiotic ice creams enriched with green banana flour

Storage Period	Control	A	B	C
O day	7.52	7.56	7.54	7.55
30th day	7.02	7.34	7.46	7.50
60th day	6.12	6.32	6.66	6.91

Table 4 shows changes in the counts of Bifidobacterium bifidum and Lactobacillus acidophilus in prebiotic enriched ice cream samples throughout 60 days of storage. The viable counts of Lb. acidophilus ranged between 6.12 and 6.91 log cfu/g in the samples. Although slight fluctuations were seen throughout storage, viable counts decreased in all samples at the end of storage compared with the beginning. The highest count of *Lb. acidophilus* throughout storage was obtained in the sample-C probiotic ice cream containing 6% green banana flour, followed by sample-B, Sample –A and Control sample. This may due to the increased levels of resistant starch in probiotic ice-cream and acted as a protective mechanism during storage. Reza Mohammadi *et al.,* (2012) reported that apple fiber increased the viability of *Lb. acidophilus* and *B.* lactis in yogurt.

In sensory evaluation it was found that the mean values of overall acceptability control, A, B and C were found as 6.6, 6.7, 6.9 and 6.7 respectively on the Hedonic scale. However the sample 'B' has better value for over all acceptability as compared to other samples.

Statistically the sample B was found to be more significantly acceptable as -compared to other samples under investigation.

Table 5: Sensory evaluation of prebiotic enriched probiotic ice creams

Sample	Appearance	Colour	Flavor	Texture	Taste	Overall acceptability
Control	7.2	6.5	6.8	7.1	7.4	6.6
A	7.1	6.5	6.6	6.6	6.8	6.7
B	6.7	7	7	7.1	7.5	6.9
C	6.8	6.8	6.4	6.4	6.4	6.7

Conclusion

Physical, nutritional and organoleptic properties of probiotic ice cream enriched with green flour were evaluated. It could be concluded that, value addition of green banana into unripe banana flour minimizes the post-harvest losses and also effectively used to develop functional probiotic ice creams rich in fiber and resistant starch. Therefore, could be used as good source of dietary fiber or prebiotic component for making synobiotic ice cream with good nutritional and functional properties. The ice cream obtained by added unripe banana flour to the level of 4 percent was rated higher sensory score for overall acceptability by the consumers and also has moderate viable counts (log cfu/g) of probiotic organisms

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