Eco. Env. & Cons. 28 (3) : 2022; pp. (1478-1484)
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ISSN 0971–765X

DOI No.: http://doi.org/10.53550/EEC.2022.v28i03.053

Development and Quality Assessment of Probiotic Ice-cream using Stevia as Sweetener

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(Received 13 November, 2021; Accepted 20 December, 2021)

ABSTRACT

Probiotics is defined as a live microorganism that, when consumed in adequate quantities, confers a health benefit to the host. Stevia is a zero-calorie sweetener does not increase the blood glucose level, making it a possible alternative for diabetics and replacing sugar with different concentrations. Stevia incorporated at four different levels (0.1%, 0.2%, 0.3% and 0.4%) and sugar reduction levels (6.5%, 13%, 19% and 25%). Based on the organoleptic evaluation highest overall acceptability for the Ice-cream incorporated with 0.2% stevia and sugar replacement (13%) was finalised. The 12 hrs incubated milk with Probiotic bacteria cell count of 10^7cfu/ml was added to Ice-cream (T_1). After inoculation of purified probiotic into the Ice-cream does not affect the sensory properties of probiotic Ice-cream with stevia (T_1). Final product (T_1) contains viable cell count of 10^7cfu/ml remained same count thus proving the probiotic bacterial viability. The selected sample was analysed for Physico-Chemical properties. Overrun percentage was high in T_1 sample (35.8%) and T_1 (72.5 min) had significantly higher meltdown time than control (55.4 min). The selected sample contains the moisture of 61.8%, Protein-5.6%, Fat-11.0%, Ash-0.92%, carbohydrate-20.7% and total solids of 38.2%. Energy values of selected Ice-cream sample were found to be 217.1 kcal/g for control sample and 204.2 kcal/g for T_1 sample.

Key words : *Ice-cream, Stevia, Lactobacillus caseishirota, Sensory evaluation and Proximate analysis*

Introduction

The health virtues and growing consumer consciousness about probiotics have grabbed the interest of the food industry. Some of the main health benefits associated with probiotics includes: Antimicrobial activity, Prevention and treatment of diarrhea, Relief of symptoms caused by lactose intolerance, Anti-mutagenic and anti-carcinogenic activities, and Stimulation of the immune system. The dairy industry, in particular, has found probiotic cultures as valuable component for the development of new functional products like dahi, yoghurt, Ice-cream, probiotic drinks, etc.. The seven core genera of microbial organisms most often used in probiotic products are "*Lactobacillus, Bifidobacterium, Saccharomyces, Streptococcus, Enterococcus, Escherichia*, and *Bacillus*. In general, these lactobacilli are presently the most frequent "probiotic" representatives in commercial probiotic products and are followed by bifid bacterium spp.

Stevia is a zero-calorie sweetener, extracted from the leaves of *Stevia rebaudiana*. It is 250–300 times sweeter than sucrose. Stevia has a negligible effect on blood glucose level, it is attractive to people on carbohydrate-controlled diets / Diabetic diets and health-conscious people (Khattab *et al*., 2015). Stevia would be accepted as a sugar substitute in various

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food products since stevia has a sugar –like taste and often a somewhat bitter taste (Alizadeh *et al*., 2014). Stevia has different health benefits like; Stevia (*Stevia rebaudiana*) extract had a hypolipidaemic effect used to reduce the resistance of cardiovascular disease, cholesterol, triglyceride and low-density lipoprotein cholesterol, stevia significantly reduces postprandial glucose levels and postprandial insulin levels compared to other artificial sweeteners, Stevia is safe for diabetes, stevia does not have the neurological or renal side effects as other artificial sweeteners. Ice-cream is considered as a suitable vehicle for delivering functional ingredients such as probiotics. Functional foods contribute a positive effect on health in addition to their basic nutritional value. Several categories of functional foods such as probiotic, and low-calorie products are being developed to meet the needs of health-conscious consumers. The demand for functional foods is growing rapidly all over the world due to the increased awareness of the consumers on the impact of food on health (Stoon *et al*., 2002).

Materials and Methods

The present study was carried out in Department of Food Science and Technology and Department of Food Safety and Quality Assurance at College of Food Technology, Rudrur. Materials used and methods adopted for the present investigations are presented under the following headings.

Raw Materials

The main raw materials like milk, cream, skim milk powder, sugar, guar gum, stevia, Yakult milk were procured from local market, Bodhan, Nizamabad District.

Methods

Preparation of Ice-cream by using stevia in varying proportions

Preliminary trials were conducted to evaluate the acceptability of Ice-cream samples with different levels of incorporated stevia powder as low-calorie sweetener. During the preliminary trials, incorporation of stevia beyond 0.4 per cent was found to result in unacceptable sensory quality. Four treatment formulations were evaluated during the final trials in which stevia was incorporated at 0.1% (F_1), 0.2% (F_2), 0.3% (F_3) and 0.4% (F_4) by partial replacement of sugar at 6.5% (F_1), 13 % (F_2), 19% (F_3), 25% (F_4).

Isolation of Probiotic (*Lactobacillus casei*s hirota) from Yakult probiotic milk

Preparation of Nutrient agar and MRS medium

The nutrient agar media ingredients viz., NaCl-5 g, peptone- 5 g, Beef extract-3 g, Agar agar-18 g was mixed in 1000 ml double distilled water. The ingredients of MRS agar viz., Protease peptone-10 g, HM Peptone B-10 g, Yeast extract-5 g, Dextrose (Glucose)-20 g, Tween 80-1 g, Ammonium citrate-2 g, Sodium Acetate-5 g, Magnesium sulphate-0.1g, Manganese sulphate-0.050 g, Dipotassium hydrogen phosphate-2 g, Agar Agar-18 g, pH-6.5±0.2 was mixed in 1000 ml double distilled water. Then both the media were sterilized in autoclave at 121 °C for 15mins and cooled at room temperature. The autoclaved media was poured in the sterilized Petri plates for solidification in laminar air flow. Then all the plates were incubated in upright position at 30 °C for 24 hours for checking sterility.

Isolation of Probiotic (*Lactobacillus casei* Shirota) from yakult probiotic milk

Probiotic milk was used for the isolation of *Lactobacillus* spp. on MRS agar and Nutrient Agar plates by using spread plate method. Initially, 1 ml of probiotic milk sample was added to 9 ml of sterile saline water (0.85% NaCl) and further serially diluted up to 10^{-9}. Then, 0.1 ml aliquots of the diluted

Table 1. Formulations of Ice-cream by using stevia in varying proportions

Ingredients (g)	Control	F_1	F_2	F_3	F_4
Milk (ml)	63.4	64.3	65.1	66	66.8
Cream (g)	17.8	17.8	17.8	17.8	17.8
SMP (g)	3.7	3.7	3.7	3.7	3.7
Sugar (g)	15	14.0	13.1	12.1	11.2
Guar gum (g)	0.1	0.1	0.1	0.1	0.1
Stevia (g)	0	0.1	0.2	0.3	0.4

samples from 10^{-3} to 10^{-5} were spread plated on MRS agar and Nutrient agar plates with the replications. After proper spread plating, all the plates were incubated in up down position at 37 °C for 24-48 hrs in incubator. After incubation the colonies on MRS and Nutrient Agar plates were enumerated for the growth of bacteria from the taken sample. All experiments were performed in triplicate. After enumeration, individual common colonies were selected and purified with streak plate technique. The purified bacteria were identified based on its colony morphology and presence of common load in the sample and further used for experimentation. Meanwhile stock and working cultures of Lactobacillus spp. were maintained in slant agar tubes (Goyal *et al.*, 2012).

Formulation of Ingredient Minimal Media (MM) broth tubes for observation of *Lactobacillus casei* shirota

All four formulations of ingredient Minimal Media (MM) broth tubes were prepared by diluting the ingredients with five times dilution compare to the original ingredient's composition in each with sterile distilled water and sterilized in autoclave at 121 °C for 15 min and cooled in aseptic conditions. Above all broth tubes are clearly labelled and inoculated with 1 loopful of isolated and purified probiotic (*Lactobacillus casei* strain shirota) and incubated at 10, 20, 30, 37 °C. While inoculation and incubation shaking conditions were provided by proper vortex mixing and maintained under aseptic conditions. Then observation of growth was observed by turbidity in broth tubes, then taken for spectrophotometer readings after 12 hrs and 24 hrs of incubation and noted down the readings.

Influence of temperature on different MM broth tubes with lactobacillus cultures

All the tubes were taken for spectrophotometer readings after 12 hrs and 24 hrs of incubation and recorded the readings of density of inoculated Probiotic bacteria.

Table 4. Formulation of Ingredient broth tubes

Formulation	Ingredients used
MM-1	Milk (6.51%) + sugar (1.31%)
MM-2	Cream (1.78%) + Guar gum (0.01%)
MM-3	Milk powder (0.37%) +Stevia (0.02%)
MM-4	All ingredients

Probiotic inoculation in selected formulation of Ice-cream

Before inoculation 100 ml of pasteurized milk was taken in 7 conical flasks with replications and these flasks were sterilized in Autoclave at 121 °C for 15 min. Above all the conical flasks were inoculated with 2 loopful of isolated, purified probiotic organism and incubated at 37 °C for 24 hrs under shaking cum incubator. After 6 hrs of incubation, the *lactobacillus casie*shirota count was taken through plate count method in already inoculated milk hourly up to 12 hrs. Meanwhile all the plates inoculated with every hour probiotic culture were incubated at 37 °C for the growth of lactobacillus and done the cfu/ml count for every plate after 24 hrs of incubation. Finally, 10^7cfu/ml cell milk was used for preparation of final probiotic Ice-cream product.

Formulation of probiotic Ice-cream using stevia as a sweetener

Formulation of probiotic Ice-creamusing stevia as sweetener was prepared by using selected *Lactobacillus casei* shirota inoculated milk with cell count of 10^7 cfu/ml was added to Ice-cream mix (T_1) just before freezing.

Table 5. Formulation of probiotic Ice-cream using stevia as a sweetener

Ingredients	Control	T_1
Milk (ml)	63.4	65.1
Cream (g)	17.8	17.8
SMP (g)	3.7	3.7
Sugar (g)	15	13.1
Guar gum (g)	0.1	0.1
Stevia (g)	0	0.2
Probiotic culture (*Lactobacillus caseishirota*)	0	10^7 cfu/ml

Ice-cream Analysis

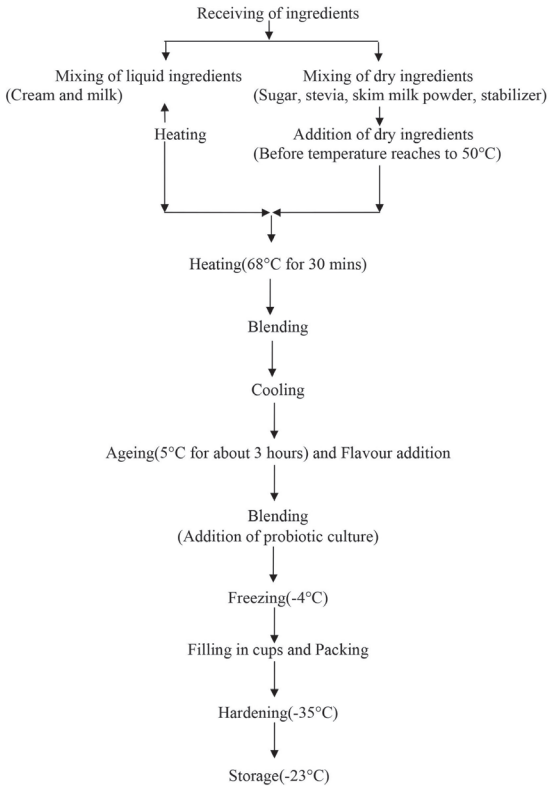
Organoleptic evaluation

Organoleptic evaluation was carried out by 10 sensory panel members to evaluate the multigrain bar samples. Evaluation was made for the sensory parameters like appearance, colour, taste, texture, flavor and overall acceptability. Organoleptic evaluation was done by 9-point hedonic scale.

Physico-chemical analysis

Physico-chemical analysis of Ice-cream samples

Flow sheet 1:Preparation of probiotic Ice-cream using stevia as a sweetener



(Ref : -Deshmukh *et al*., 2014)

were carried out. Over run was determined by BIS (1983) method, the meltdown time (Rajor, 1980), pH by using pH meter, Titratable acidity (BIS, 1981).

Proximate Analysis

Proximate analysis of Ice-cream samples were carried out. Moisture content was determined by AOAC (1990), Fat by using Gerber Method outlined by Nielsen (2010), Protein by micro-kjeldahl method, total ash (AOAC, 2016) method, Carbohydrate by difference method, total solids (AOAC, 2000), Energy value of Ice-cream samples were computed from proximate composition of the Ice-cream by taking the value of 4, 4 and 9 kcal for carbohydrates, protein and fat respectively.

Theoretical determination of energy value of Probiotic Ice-cream with Stevia

Energy value of Ice-cream samples were computed from proximate composition of the Ice-cream by taking the value of 4, 4 and 9 kcal for carbohydrates, protein and fat respectively. Based on this Energy value of selected Ice-creams was calcuted (Amala *et al.*, 2017).

Probiotic count /viability

The probiotic count of Ice-cream was performed as per the procedure described by Inoue *et al*., (1998).

Results and Discussion

Organoleptic evaluation of selected Ice-cream by using stevia in different proportions

Based on organoleptic evaluation, the colour and appearance score of F_3 and F_4 were significantly lower than control, whereas, F_1 and F_2 did not show any significant difference. The significant difference in Texture scores was observed between control and all the four treatments. The flavour scores of treatments F_4 was significantly lower from that of control. F_2 did not differ significantly from control. The Overall acceptability F_3 of and F_4 were significantly lower from that of control, whereas F_2 did not show any significant difference from that of control. From the results of sensory evaluation, 0.2 per cent stevia (F_2) was selected because it had obtained the highest overall acceptability scores among the four treatment groups.

Influence of temperature on different MM broth tubes with lactobacillus culture

All four formulations of ingredient with 10 ml broth tubes were prepared and inoculated with 1 loopful of isolated and purified probiotic (*Lactobacillus casei* shirota) culture and incubated at 10, 20, 30, 37 °C. Observation of growth was seen in the form of turbidity in few broth tubes. For all the tubes, spectrophotometer readings were taken after 12 hrs and 24 hrs of incubation and observed that the ingredients were consumed at 37 °C comparing at other temperatures. Hence we concluded from above that our Ice-cream should be preserved at below 30 °C.

Table 6. Influence of temperature on different MM with lactobacillus culture after 12 hrs of incubation

Formulations	10°C	20°C	30°C	37°C
F_1	0.210	0.252	0.271	0.662
F_2	0.329	0.330	0.336	0.693
F_3	0.190	0.192	0.200	0.512
F_4	0.390	0.395	0.400	0.721

Organoleptic evaluation of probiotic Ice-cream using stevia as sweetener

Assessed the quality and acceptability of the

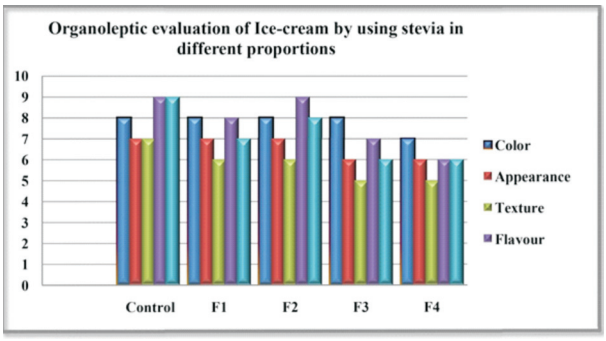


Fig. 1. Organoleptic evaluation of selected Ice-cream by using stevia in different proportions

Table 7. Influence of temperature on different MM with lactobacillus culture after 24 hrs of incubation

Formulations	10°C	20°C	30°C	37°C
F_1	0.281	0.322	0.341	0.732
F_2	0.392	0.400	0.406	0.763
F_3	0.265	0.266	0.270	0.683
F_4	0.462	0.465	0.471	0.891

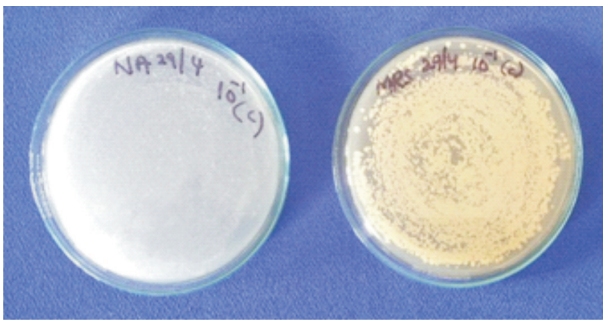


Fig. Isolation of *Lactobacillus casei*s hirota in NA and MRS plates

probiotic Ice-cream prepared by using stevia as sweetener were presented for organoleptic evaluation.

C-Control; T_1- Ice-cream sample with 0.2% stevia (sugar replacement of 13%) with 10^7 cfu/ml probiotic culture

By organoleptic evaluation, inoculation of isolated and purified probiotic culture in the Ice-cream does not affect the sensory properties of probiotic Ice-cream with stevia (T_1). The T_1 sample has same colour, appearance, texture, flavour as like control but in terms of flavour and overall acceptability was having high score than the control ice cream.

Physico-chemical parameters of probiotic Ice-cream using stevia as sweetener

Physico-chemical properties of Ice-cream samples were performed and tabulated in Table 3. Overrun percentage was high in T_1 sample (35.8%) and also T_1 (72.5 min) had significantly higher meltdown time than control (55.4 min). According to Flores and Goff (1999) the higher amount of air cells in stevia added Ice-cream could slow the meltdown because air cells reduce the rate of heat transfer across the Ice-cream. pH of control and T-$_1$ sample were 6.41 and 6.29 respectively. The Titratable acidity of control and T-$_1$ sample was 0.126% and 0.189% lactic acid respectively. There was no significant difference in pH and titratable acidity between control and T-$_1$ sample.

Table 9. Physico-chemical parameters of probiotic Ice-cream using stevia as sweetener

Sl No.	Physico-chemical parameters	Control	T_1 sample
1	Overrun (%)	32.7	35.8
2	Meltdown time (min)	55.4	72.5
3	pH	6.41	6.25
4	Titratable acidity (%)	0.126	0.189

Proximate composition of probiotic Ice-cream with stevia

Proximate composition of Ice-cream samples was performed and tabulated in Table 4. The moisture content in control and T_1 Ice-cream samples were 58.4% and 61.8% respectively. It can be observed that, the moisture in T_1 was higher than the control, it could be due to the low content of total solid. The fat content of control and T_1 sample were 10.7 % and 11.0% respectively, there was slight increase in fat content. The protein content of control and T_1 were 4.4 % and 5.6 % respectively. Amala *et al*. (2017)

Table 8. Organoleptic evaluation of probiotic Ice-cream using stevia as sweetener

Sample	Colour	Appearance	Texture	Flavour	OverallAcceptability
Control	8	7	7	8	7.5
T_1	8	7	7	9	7.7

have also reported similar findings that there was increase in protein content on replacement of stevia. In the present study also replacement of sugar with stevia in Ice-cream samples caused significant increase in protein content. It was seen that there was marked effect of stevia powder on carbohydrates. The carbohydrate content in control and T_1 Ice-cream samples were 25.7% and 20.7% respectively. Energy value of selected Ice-creams were found to be 217.1 kcal/g for control sample and 204.2 kcal/g for T_1 sample. The energy value decreased in T_1 comparing with control sample. The total solids content of T_1 were 41.6% and 38.2% respectively. Total solids content of T_1 sample was significantly lower than control.

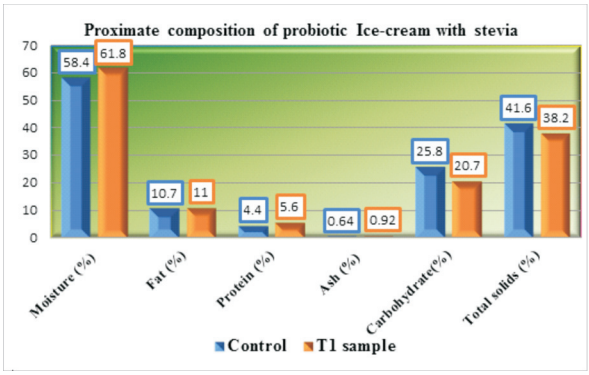


Fig. 2. Proximate composition of probiotic Ice-cream using stevia as sweetener

Checking the probiotic organism count in milk for incorporation into the Ice-cream

Probiotic culture was inoculated into the milk of different flask for checking the exact growth of organism and incubated at 37 °C. From 6 hours onwards growth of the Probiotic bacteria was checked through plate count method and after 24 hrs of incubation it was found that at 12 hrs of incubation bacterial cell growth reaching the 10^7 cell. Then this 12 hrs of multiplied bacteria in milk was used for preparation of final probiotic ice cream product.

Viability of probiotic organism in final Ice-cream using stevia as sweetener

After incorporation of *Lactobacillus casie* shirota with 10^7 cells into the best formulation of ice cream, it was again analysed for viability of probiotic organism through same plate count method. The viability of probiotic in Ice-cream using stevia as sweetener estimated by using plate count method and observed that final product contains viable cell count of 107 cfu/ml, since the probiotic viable count remained in the minimum limit. Finally, there is no change observed in cell no of probiotic organism because we were prepared under normal temperatures and there are no processing effects were observed in probiotic Ice-cream with stevia. It was observed that there was no change in the count of probiotic organism and even the product had the same properties as usual with original sensory characters.

Theoretical determination of energy value of Probiotic Ice-cream with Stevia

Energy value of Ice-cream samples were computed from proximate composition of the Ice-cream by taking the value of 4, 4 and 9 kcal for carbohydrates, protein and fat respectively and observed that Energy value of selected Ice-creams were found to be

Table 11. Viability of probiotic in Ice-cream using stevia as sweetener

S.No	Timings	Flask 1	Flask 2	Flask 3	Flask 4	Flask 5	Flask 6	Flask 7
1.	6 hrs	$2.9x10^1$	-	-	-	-	-	-
2.	7 hrs	-	$3.5x10^2$	-	-	-	-	-
3.	8 hrs	-	-	$3.7x10^3$	-	-	-	-
4.	9 hrs	-	-	-	$4.0x10^4$	-	-	-
5.	10 hrs	-	-	-	-	$4.2x10^5$	-	-
6.	11 hrs	-	-	-	-	-	$4.3x10^6$	-
7.	12 hrs	-	-	-	-	-	-	$4.5x10^7$

Table 12. Theoretical energy value of selected probiotic Ice-cream using stevia as sweetener

Samples	Carbohydrate	Protein	Fat	Energy value of the product Kcal/100 g
Control	25.8	4.4	10.7	217.1
T_1	20.7	5.6	11.0	204.2

217.1 kcal/g for control sample and 204.2 kcal/g for T_1 sample. The energy value decreased in stevia added probiotic sample (T_1) comparing with control sample.

Conclusion

In present investigation, it is stated that we have prepared probiotic Ice-cream using stevia as sweetener. Based on organoleptic evaluation the overall acceptability is high for Ice-cream using 0.2% stevia with 13% sugar replacement and that sample was finalized. Then added *Lactobacillus casei* shirota inoculated milk with cell count of 10^7 cfu/ml and viable count remained in minimal limit 10^7 cfu/ml thus it indicated that probiotic bacteria were viable. Having probiotic may provide so many health benefits and this can be said to be functional food. Frozen storage of the Ice-cream has no effect on culture survival and bacterial culture remained at levels sufficient to offer the suggested therapeutic effects. The current study shown that application of stevia as a natural sweetener decreased the calories and has a positive impact on physico-chemical and sensory parameters. Thus, making it suitable for health-conscious people.

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