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Probiotics,Organoleptic and Physicochemical Properties of Vegetable Milk Based Bio-ice cream Supplemented with Skimmed Milk Powder

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Abstract: The aim of the present study was to evaluate non fermented probiotic ice cream made from vegetable (lupine and peanuts) milk, and to evaluate its probiotics, organoleptic and physicochemical characteristics. Ice cream samples were produced from blends of vegetable milk and skimmed milk powder together with the added Probiotics bacteria of *Lactobacillusacidophilus* and *Bifidobacterium. lactis*. The changes of Bio–ice cream qualities and survival of added microbes were evaluated at 0, 1, and 30 days. The viable of *L. acidophilus* decreased by 1.62 and 2.05 log cycles in lupine and peanuts milk ice creams throughout 30 days storage respectively. The counts of *B. lactis* reduced by 1.32 and 2.22 log cycles in lupine and peanuts ice creams respectively. Regardless of ice cream color, incorporation of vegetable milk ice cream significantly ($P \leq 0.05$) enhanced the taste, texture, flavor, and overall acceptability of ice cream. The highest total solids and protein were found in ice creams containing peanuts milk and lowest fat and total acceptability were found in ice creams containing lupine milk. The produce of ice cream with vegetable milks developed the growth and viability of *B. lactis* and *L. acidophilus*.

Keywords: Bio- ice cream, Lupine, Peanut Milk, Probiotic, Bacteria

1. Introduction

Consumer is looking for the functional foods can offer more health benefits. Full-fat milk is rich in saturated fatty acids, which has been shown to significantly elevate total, Low-density lipoprotein (LDL), and high-density lipoprotein (HDL)-cholesterol) [4] and [3] which has long been associated with increased risk of cardiovascular disease. Functional food that contains viable probiotics in particular addresses the improvement in intestinal micro flora activation of the immune system, reduction in serum cholesterol and inhibition of potential pathogens [12]. However, these probiotics must be present in adequate amount (10^6–10^7cfu/g) at the time of consumption in order to render it being effective [13].Fermented yogurt and milk can effectively be used to deliver probiotic bacteria. The former unfortunately may cause loss of viable probiotic due to pH reduction and accumulation of organic acids as a result of fermentation [14]. In this regard ice cream, due to its neutral pH, may be used to deliver the probiotics [15]. Partial replacement cow milk with vegetable milks such as soy milk could improve the pH of probiotic ice cream resulting increased the survival of probiotics [16]. On the other hand, Ice cream is a delicious, wholesome, nutritious frozen dairy product, which is extensively consumed in different parts of the world. Ice cream has nutritional significance but encompasses no therapeutic properties [17]. Ice cream is traditionally made from cow s milk and thus contains about 16% (w/w) lactose [18]. The demand for alternatives to cows' milk is growing due to problems with its lactose (Lactose intolerance) and cholesterol contents and desire for vegetarian alternatives. Ice cream can be made a functional food by adding fruits, protein rich ingredients and also using vegetable milk such as coconut and soy milks and the addition of probiotics. Moreover, the lupine and peanut milks contain unique nutrient compositions in peanut and lupine milks can support the growth and survival of the lactic acid bacteria in ice cream and thus could improve the health benefits and nutritional components and health benefits of probiotic ice creams.

In particular, lupine proteins are receiving attention in terms of health benefits, particularly in relation to a number of conditions now known as ‘metabolic syndrome’ which includes a cluster of factors such as, obesity, high blood pressure, insulin resistance and elevated blood cholesterol (5).

Lupine enriched foods have the potential to: beneficially influence satiety (appetite suppression) and energy balance [10], beneficially influence glycaemic control [6], improve blood lipids [7], reduce hypertension [8] and improve bowel health [9]. Partial replacement of cow milk with vegetable milks may affect the physical properties of ice creams. Fortification of yoghurt ice cream with soy protein has been shown to improve the textural quality of the product including firmness and viscosity [19]. Lecithin in the soy also acts as emulsifiers which increase the viscosity and stability of ice cream, refined texture and extend the melting time [20]. Abdullah et al. [21] improved the quality of ice cream by using different ratios of skim milk in soymilk blend and found that large quantity of skim milk in soymilk decline beany flavour of soy beans and increased quality of ice cream. Fatemeh and Other authors [22] Effects of Vegetable Milk on Survival of Probiotics and Rheological and Physicochemical Properties of Bio-ice cream, studied the Physicochemical and Rheological properties of soy and coconut milk ice cream by adding soy oil and sugar contents and found the addition of sugar and replacing skim milk powder with in Vegetable milk ice cream increased ice cream mix viscosity and reduced melting rate of ice cream. The present study was carried out to investigate the effect of lupine and peanut milks on the survival of probiotic in ice cream during storage and the physicochemical and organoleptic properties of non fermented Bio-ice cream. There has been a shortage of milk production. Thus, the supplementation and/or substitution of cow milk with lupine and peanut milk would improve the yield and nutritional quality of ice cream at a comparatively lower cost. Up to now, no work has been published on producing ice cream using cow milk supplemented with Vegetable milk (lupine and peanut milk). Therefore, the aim of the present study was to investigate the effect of incorporation of Vegetable milk on physicochemical, microbiological and organoleptic characteristics of Bio-ice cream.

2. Materials and Methods

2.1. Bacteria Strain

Lactobacillus acidophilus and Bifidobacteriumbifidum were obtained as pure freeze-dried probiotic culture from CHR-Hansen (Horsholm, Denmark).

2.2. Preparation of Starter Cultures

Each one gram of strain was cultured in 100 mL of sterilized skimmed milk (10 w/v) , simplified by the addition of 0.05% (w/v) L-Cys-HCl, 1% (w/v) yeast extract and 2% (w/v) glucose. The incubation was carried out under aerobic condition in a water bath (40°C) until pH was reached to 5.0 [23].

2.3. Preparation of Vegetable Milk

Lupine and peanuts milk were extracted from the seeds by the methods according to [1: 2].

2.4. Ice Cream Production

Fresh pasteurized and homogenized milks (lupine and peanuts milk), skimmed milk powder, butter, sugar, and vanilla was purchased from local super market. Stabilizer (Danisco AS, Copenhagen, Denmark) containing cellulose gum, guar gum, mono- and diacyl- glycerol’s of fatty acid, was used as stabilizer. Ice cream mix formulated according to some properties of mixture milks (Table 1) such that it had 38.7%–40.8% total solids and 10 - 10.5% fat for a total batch of 100kg (Table 5).

The vegetable milk (lupine and peanuts milk) was initially heated to 50°C followed by the other additives. The mixture was mixed at 65°C in two stages by a homogenizer with 16000 rpm for 5 min followed by pasteurization (10 min heating at 80°C followed by cooling to 4°C). The mixture was aged overnight at 4°C prior to the addition of each probiotic culture (4% w/w) followed by immediate freezing in batch ice cream maker (1.5 L, Baumatic Gelato1SS). The frozen mixture was packed in 100 mL plastic cups and then stored in a freezer (−18°C). Three different batches of ice cream per treatment were manufactured.

2.5. Physicochemical Analysis

The vegetable Milk and ice creams were analyzed for pH by a digital pH meter and for titratable acid (TA) by titrating a sample (10g) with NaOH (0.1N)using phenolphthalein (0.1w/v) as an indicator. The dry matters were measured by drying samples at100±1°C for 3.5h using an air oven [24]. The protein and fat contents of milk and ice creams were measured by the Kjeldahl and Soxhlet methods respectively [24]. All chemical analyses were carried out in triplicate.

Table 1. *Physicochemical compositions of lupine and peanuts milk*

Parameters	pH	T.S(g/100g	T.A(%lactic acid)	M.C(g/100g)	Protein(g/100g)	Fat(g/100g)
Peanuts Milk	7.03±0.10	14.67±0.10	0.10±0.10	85.33±0.12	5.40±0.12	5.00±0.12
Lupine Milk	6.30±0.10	12.14±0.07	0.60±0.01	87.86±0.12	5.10±0.12	3.30±0.12

Values are means of triplicate samples (±SD).
T.S: Total solids
T.A: Titritable acidity
M.C: Moisture content

Table 2. *The content of components used in Bio-ice cream made from vegetable milk (%by weight).*

Ice cream mixture	Milk	Skimmed Milk Powder	Sugar	butter	water	Stabilizer	Vanillin
Lupine	50.0	10.0	15.0	4.4	20.0	0.5	0.1
Peanuts	50.0	10.0	15.0	4.4	20.0	0.5	0.1

2.6. Bacteriological Analysis

The viability of probiotics was measured immediately after inoculating the probiotic cultures and after 1 and 30 days of frozen storage at −20°C. The samples (10 g) were decimally diluted with sterile peptone water (1 g L-1; Merck). One mL aliquot dilutions were pour plated in triplicate on MRS agar for *L. acidophilus* and MRS agar supplemented with 0.05% (w/v) L-Cys-HCI (Merck) for *B. lactis*. The plates were incubated at 38±1°C for 72 h under aerobic condition with 5% CO_2 (v/v) for *L. acidophilus* and anaerobic condition (Anaerocult A) for *B. lactis*. The bacterial viability was represented as survival rate [23].

Table 3. *Probiotic bacteria count of Bio- ice creams with different milks.*

Bio-ice creams	Mixture(0days)(*Log10cfu/g*)	Icecream1days(*Log10cfu/g*)	Icecream30days(*Log10cfu/g*)	Survival Rate (%)
L.L	8.87±0.12	8.85±0.15	7.25±0.15	97.57±0.15
L.B	8.59±0.12	8.57±0.15	7.27±0.15	98.85±0.15
P.L	9.58±0.12	9.46±0.15	7.53±0.15	98.87±0.15
P.B	9.87±0.12	9.74±0.15	7.65±0.15	97.85±0.15

Values are means of triplicate samples (±SD).
L.L: ice creams inoculated with *Lactobacullusacidophilus* made using lupine milk or P.L: peanut milk
LB: ice creams inoculated with *Bifidobacteriumbifidum* made using lupine milk or P.B: peanut milk

2.7. Organoleptical Analysis

The organoleptically characteristics of the ice creams were evaluated following the IDF standards [11]. A trained panel of 12 members, composed of adult male (4,age ranged from 25 to 35) and female (8,age ranged from 25 to 40), was assigned to determine the quality of the ice creams (color, flavor, taste, texture and overall acceptability). Members were asked to score1-5 hedonic scale (1 = poor, 2 = acceptable, 3 = good, 4 = very good and 5 = excellent). The samples were randomized and presented using tag for each one. To determine the differences in judges ‘response, the means cores were analyzed by Duncan's multiple range tests.

Table 4. *Organoleptic property scores of ice creams with different milks.*

Bio-ice creams	Color	Texture	Taste	flavor	Overall acceptability
L.L	3.22±0.03^{a}	3.18±0.03^{a}	3.36±0.03^{b}	3.38±0.05^{a}	3.32±0.01^{a}
L.B	3.32±0.05^{a}	3.12±0.02^{b}	3.32±0.03^{b}	3.32±0.05^{b}	3.30±0.05^{a}
P.L	3.22±0.05^{a}	3.33±0.05^{b}	3.45±0.03^{a}	3.23±0.05^{c}	3.35±0.05^{a}
P.B	3.23±0.03^{a}	3.32±0.03^{b}	3.37±0.05^{b}	3.22±0.05^{c}	3.33±0.05^{a}

Values with different letters in the same column are significantly ($P<0.05$) different
L.L: Ice creams inoculated with *L.acidophilus* made using lupine milk
P.L: Ice creams inoculated with *L.acidophilus* made using peanut milk
LB: ice creams inoculated with *Bifidobacteriumbifidum* made using lupine milk
P.B: ice creams inoculated with *Bifidobacteriumbifidum* made using peanut milk

Table 5. *The physicochemical compositions of vegetable milk and probiotic ice creams, made using lupine and peanut milks.*

Vegetable milk/ Bio-ice creams	T.S(g/100g)	pH	T.A(%lactic acid)	Protein(g/100g)	Fat(g/100g)
L.M	12.0±0.1	7.00±0.5	0.72±0.2	4.40±0.5	3.30±0.1
P.M	14.67±0.1	7.03±0.3	0.91±0.1	5.20±0.5	5.00±0.3
L.L	39.0±0.3	7.11±0.1	0.72±0.4	2.13±0.1	10.0±0.2
L.B	38.7±0.1	7.15±0.1	0.70±0.3	2.21±0.3	10.3±0.1
P.L	39.9±0.2	6.71±0.1	0.12±0.1	2.8±±0.1	10.3±0.1
P.B	40.8±0.1	6.74±0.1	0.11±0.1	2.32±0.1	10.5±0.2

Values are means of triplicate samples (±SD).
L.M: lupine Milk
P.M: Peanut Milk
L.L: Ice creams inoculated with *L.acidophilus* made using lupine milk
P.L: Ice creams inoculated with *L.acidophilus* made using peanut milk
LB: ice creams inoculated with *Bifidobacteriumbifidum* made using lupine milk
P.B: ice creams inoculated with *Bifidobacteriumbifidum* made using peanut milk

2.8. Statistical Analysis

The viability of the probiotic micro-organisms were evaluated at three times of storage (0, 1 and 30 days), in triplicates and the results were expressed as mean ±S.E.M (standard mean error) values. The statistical analysis was performed using Minitab programmer (1998). Three separate samples were analyzed and mean values were calculated. The data were assessed by analysis of variance (ANOVA) and followed by Duncan's multiple range method for mean comparison. The mean values and the standard error were calculated from the data obtained with triplicate trials. The criterion for statistical significance was ($P<0.05$)[25].

3. Results and Discussion

3.1. Viability of Probiotic Bactrian Ice Cream

Table3: shows the changes in bacterial counts in non-fermented ice creams made using vegetable milks. The survival rate of probiotic bacteria in ice creams after 30 days tend to be higher in *B.bifidum* (7.65 and 7.27 logcfug^{-1}for peanut and lupine milk ice cream respectively) than in the presence of *Lactobacullus acidophilus* (7.53 and 7.25 logcfug^{-1} peanut and lupine milk ice cream respectively) (Table3). These finding agreed with Fatemeh *et al*. [27] they found the survival rate of probiotic bacteria in ice creams after 30 days tend to be higher in *B. bifidum* (7.767 and 7.371 log cfug_^{1} for soy and coconut ice cream respectively) than in *L. acidophilus* (7.847 and 6.870 log cfug_^{1} for soy and coconut ice cream respectively) .However, the decline in viable bacterial counts due to freezing is associated with freeze injury on these cells. In addition the mechanical stresses associated with the mixing and freezing process which incorporates oxygen in to the mixture may be responsible in further reduction in bacterial count [23]. The survival of both probiotics in ice cream was high ($P<0.01$) in the presence of lupine and peanuts milk. This could be explained by the high pH of lupine and peanuts ice creams which are known to be conducive to probiotic survival since these organisms are susceptible to inactivation when stored in acidic conditions [28]. As a result probably, these proteins can cover probiotics as a capsule. The high survival rate of *L. Acidophilus* cells during the frozen storage in other studies was attributed to the protection provided to the cells by the solid ingredients and the high fat content of the ice cream in the form of emulsion [29].

Heenan *et al*.,[16] demonstrated that the survival of probiotics increased in the frozen soy dessert due to the prevailing neutral pH. The highest survival of both probiotics was in lupine milk ice cream. It is probably due to the lupine milk proteins which provide physical protection against freezing damage by encapsulating probiotics by form stable net work looks like a gel structure [26].

3.2. Organoleptic Property

Table 4: shows the organoleptic property scores in non fermented ice creams made using vegetable milks (Table4). The presence of either *L. Acidophilus* or *B.bifidum* had no significant influence on the Color and Overall acceptability properties of both probiotics ice creams. The highest ($P\leq0.05$) scores of overall acceptability was (3.35) seen in peanut milk ice cream than the lowest core (3.30) seen in lupine milk ice cream. It is probably due to the lupine milk beany flavors, which could be explained by the soy milk woody or beany off flavors [21].

3.3. Physicochemical Parameters of Milks and Probiotic Ice Creams Made Using Lupine and Peanut Milk

Table5: The physicochemical compositions of the probiotic ice creams, lupine and peanut milks are shown in Table5. The highest (7.15) pH value and lowest (0.70%) titritable acidity (TA) and fat content (10%) were in ice creams containing lupine milk. The protein contents varied between 2.51% to 2.32% in ice creams inoculated with *B.bifidum* made using lupine and peanut milk, respectively. The highest (40.8%) total solids, fat (10.5%) and protein (40.8%) were found in ice creams containing peanuts milk and lowest (10.0%) fat content and total acceptability (3.30)

score were found in ice creams containing lupine milk. The total solids content of 40.8g/100g obtained for sample containing peanut milk is higher (P≤0.05) than other samples (39.0g/100g and 38.8g/100g) for sample containing lupine milk. This may be due the higher content of protein and fat of peanut milk. Lupine milk based ice creams showed lower (P≤0.05) fat content than ice cream made with peanut milk. This may be due to that lupine milk has generally low fat (3.30g/100g) content compared to that of peanuts milk (5.0g/100g).

4. Conclusions

Probiotic Food is being one of the largest markets of functional foods represent a vast growth potential for the food industry and may be explored through the development of innovative ingredients, processes, and products. This research proves that the rear serious confirmations those supporting develop of new lupine and peanut ice cream formulation and application of growing probiotic culture have important role in design of various functional products including vegetable milk ice cream. Stability during storage is important characteristics that are proved observing Probiotic characteristics of produced samples of vegetable milk ice cream. Moreover, incorporation of lupine and peanuts milk in ice cream manufacturing resulted in cost saving and improvement of the nutritional value and organoleptic quality.

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