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Research Article
Probiotic Ice Cream Made with Tiger-nut (*Cyperus esculentus*) Extract

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Abstract
Objective: This study has been done to investigate/evaluate the effect of incorporation different ratios of tiger-nut extract, together with two probiotic bacterial strains in ice cream. **Methodology:** Substitution of aqueous phase by 25, 50, 75 and 100% tiger-nut extract (TNE) in the manufacture of probiotic ice cream, using two probiotic bacterial strains namely *Lactobacillus acidophilus* La-5 and *Bifidobacterium bifidum* Bb-12 was evaluated. **Results:** The results indicated that all treatments had no significant effect ($p>0.05$) on the pH, surface tension and whipping ability of ice cream mix, while the mix viscosity increased as the percentage of TNE was increased. Moreover, no significant effect ($p>0.05$) on the overrun, meltdown and sensory properties of resultant substituted ice cream were observed. Over storage period of 90 days at -20°C, the viability/survival of the two probiotic bacterial strains was higher in all ice cream treatments, made with TNE than in control. The same was true for the total bacterial counts. **Conclusion:** The results indicated that substitution of aqueous phase by 50% TNE was the most superior for production of ice cream with no adverse effect on the physical and sensory properties. In the same time, it also enhanced the survival of the incorporated probiotic bacterial strains.

Key words: Tiger-nut, probiotic bacterial strains, *Lactobacillus acidophilus*, *Bifidobacterium bifidum*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Scientific progress in understanding the relationship between nutrition and health has an increasingly profound impact on consumer's approach to nutrition which has resulted in the development of the concept of functional foods[1]. Functional foods can be defined as foods containing significant levels of biologically active components that provide specific health benefits beyond the traditional nutrients they contain[2]. 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[3]. The functional foods comprise conventional foods containing naturally occurring bioactive substances (e.g., dietary fiber) or foods enriched with bioactive substances (e.g., probiotics and antioxidants) or synthesized food ingredients introduced to traditional foods (e.g., prebiotics).

Probiotics are viable microorganisms that are beneficial to the host when administered in appropriate quantities[4]. They have been incorporated into a wide range of foods, including dairy products, such as yogurt, cheese, ice cream and dairy desserts, but also in non dairy products, such as chocolate, cereals and juices[5]. In fact, probiotic products are important functional foods as they represent about 65% of the world functional food market[6]. The probiotic bacteria used in commercial products are mainly members of the genera *Lactobacillus* and *Bifidobacterium*[7]. Supplementation of milk with *L. acidophilus* and/or *Bifidobacterium* spp. has been very popular as both these species are resistant to intestinal bile salt[8].

Two important criteria are considered to determine the efficacy and successful use of a product containing probiotic microorganisms: (i) Consumers acceptance and (ii) The survival of probiotic microorganisms during processing and storage[9,10]. Survival of LAB, including probiotic strains, in foods depends on many factors such as pH, temperature, oxidation/reduction potential, water availability and the presence of inhibitors and competing microorganisms[11]. In general, the food industry has applied the recommended level of the 10^6 CFU g^{-1} at the time of consumption for *L. acidophilus*, *Bifidobacterium* and other probiotic bacteria[12,13].

Ice cream is considered to be of the most popular frozen milk products consumed widely in our country and all over the world. It is highly appreciable not only by adults but also by the children[14]. It has nutritional significance but possesses no therapeutic properties. The growing interest of consumers in therapeutic products has led to the incorporation of probiotic cultures into ice cream to result in dietetic ice cream[15].

Cyperus esculentus (Tiger-nut in English, Habelaziz in Arabic and Chufa in Spanish) has long been recognized for its health benefits as they are rich in fiber, protein, natural sugars, minerals such as phosphorous and potassium and in vitamins, such as E and C. They have a high content of soluble glucose and oleic acid, along with high energy content (starch, fats, sugars and proteins[16,17]. Tiger-nut contributes to the reduction of cholesterol, it reduces the risk of coronary heart disease, arteriosclerosis and is recommended for those who have heavy digestion, flatulence and dysentery and cancer especially of the colon[18]. They are through to be beneficial to diabetics and those seeking to reduce cholesterol or lose weight. The very high fiber content combined with its delicious taste make tiger-nut ideal for healthy eating[19]. Tiger-nut has of favor and texture reminiscent of coconut. Tiger-nut is rich in oil which can be extracted for urinary industrial use[17].

This study has been done to investigate/evaluate the effect of incorporation different ratios of tiger-nut extract, together with two probiotic bacterial strains, on the physico-chemical and sensory properties of the manufactured ice cream when stored at -20°C for 90 days. Evaluation of the viability of the used probiotic bacterial strains, during these storage conditions was another goal of the study.

MATERIALS AND METHODS

Materials source: Skim milk powder (low heat, USA), commercial grade granulated sugar cane (Sugar and integrated industries Co., Egypt) and *Vanilla* (Boehringer Manheim GMB, Germany) were purchased from the local markets of Cairo, Egypt. Anhydrous milk fat was obtained from the dairy processing unit, Faculty of Agriculture, Cairo University, Egypt. Stabilizer/emulsifier called lacta 9050 was obtained from the Egyptian office for Trading and Agencies (eta), Cairo, Egypt. Tiger-nut (*Cyperus esculentus*) was purchased from Al Azhar market, Cairo, Egypt. *Lactobacillus acidophilus* La-5 (DVS) and *Bifidobacterium bifidum* Bb-12 (DVS) were obtained from Chr-Hansen's Lab., Denmark. The two probiotic strains had previously been shown to demonstrate probiotic properties[20].

Methods

Preparation of the tiger-nut extract: The dry tiger-nuts were sorted, washed and soaked in tap water at a ratio of 1:3 (w/v) at room temperature of 30°C for 24 h to obtain the hydrated or fresh-like nuts of 45-50% moisture content. The hydrated tiger-nuts were blanched by boiling in 0.2% solution of sodium bicarbonate to eliminate the nutty flavor, which may be objectionable to some consumers and then washed twice in tap water. The hydrated tiger-nuts were blended with distilled water (1:3 w/v) using a Philips kitchen blender (model HR 2848/AB) at the maximum speed for 5 min[21]. The homogenous slurry was filtered using a muslin cloth by squeezing until virtually no extract was recovered and centrifuged at 2500xg for 5 min. The supernatant obtained and heated at 70°C for 20 min, cooled to 4°C and refrigerated for further processes[21]. The average composition of tiger-nut extract was 96.86, 0.34, 0.95, 1.65 and 0.20 for moisture, protein, fat, carbohydrates and ash, respectively.

Activation of the bacterial strains: *Bifidobacterium bifidum* and *Lactobacillus acidophilus* were activated individually by three successive transfers in modified MRS followed by three successive transfers in sterile 10% reconstituted skim milk powder and incubated at 37°C for 48 h under anaerobic conditions[22,23], cultures were prepared 24 h before used.

Manufacture of ice cream: One batch of ice cream mix was prepared by dispersing the required amount of skim milk powder (11 g/100 g mix), anhydrous milk fat (10 g/100 g mix), sucrose (15 g/100 g mix) and lacta 9050 (0.4 g/100 g mix) in 63.6 g distilled water (aqueous phase); served as a control ice cream mix. Aqueous phase was substituted by tiger-nut extract at ratio 25, 50, 75 or 100 to create four treatments. All ice cream mixes were heated to 70°C, homogenized at 13.6/3.5 MPa (stage 1/stage 2), pasteurized at 81.5°C and the aged overnight at 5.0±2°C. Just before freezing in a batch freezer (Taylor, model 103), 2.0% probiotic bacteria (*Lactobacillus acidophilus* La-5 and *Bifidobacterium bifidum* Bb-12, 1:1) and 0.04% *Vanilla* were added to each mix. The resultant ice cream was filled into plastic cups, covered and hardened in a deep freezer at -30°C for 24 h before analysis. Three replicates were made from each treatment.

Physical properties of ice cream mix: The pH value was measured using a laboratory pH meter with glass electrode ((HANNA, instrument, Portugal). The surface tension of the ice milk mix was measured according to Arbuckle[24]. A tube of uniform bore is used and the number of drops of the sample falling per time is compared with that of water. The surface tension of water is 72-73 dynes. Flow time of the ice cream mix was measured as the time to empty a 50 mL pipette at 5°C under atmospheric pressure[24]. Thixotropy behavior of ice cream mix was determined using a Brookfield Synchro-lectric viscometer (Model LVT; Brookfield Engineering Inc., Stoughton, MA). Readings (CP) were taken at the shear rate of 10 sec^{-1} for 5 min, 30 sec apart, using spindle -0 at 7°C. Three readings were recorded for each mix. Whipping ability of the ice milk mix was determined as mentioned by Baer *et al.*[25] using a mixer (Heidolph No. 50 111, Type RZRI, Germany) with some modification as follows: The mix (150 mL) was placed at 4°C in a 600 mL glass mixing bowl, calibrated with known volumes of water and placed inside a 2 L bowl. Between the bowls, a mixture of ice and salt was used to cool the mix during whipping by 3 blades of 2.6 cm. A mixer speed setting of 10 was used for whipping the ice milk mix. Changes in volume were recorded at 5, 10, 15 and 20 min.

Determination of the physical properties: The overrun percentage of resultant ice cream was calculated for all treatments using the weight-volume method described by Adapa *et al.*[26]. Meltdown of frozen ice milk was determined according to Arndt and Wehling[27] by carefully cutting the foamed plastic cups from the ice milk samples (~50 g), placing the samples onto wire mesh over a glass funnel fitted on conical flask and weighing the amount of ice milk drained into the conical flask at 30°C every 10 min. Hardness of frozen ice cream was measured using fruit pressure tester (Penetrometer, mod., FT 327). Samples were tempered to -19°C in chest-type freezer for 18 h before testing. The pounds (kg cm^{-2}) of force required for a cylindrical probe (diameter = 0.8 cm and length = 2.65 cm) to penetrate the sample are a function of the hardness of frozen ice cream. Three measurements were recorded for each cup.

Bacteriological analysis: All samples of mixes of ice cream were taken prior to freezing and the resultant of ice cream when fresh and during storage for 90 days for bacteriological analysis according to the methods described in standard methods for examination of dairy products[28]. Samples of ice cream were taken prior and after freezing then after 15, 30, 60 and 90 days. Samples were left to be melted in thermostatically controlled water bath at 40°C for not more than 15 min. Each sample was thoroughly mixed before being examined. Total bacteria count was determined by aerobic plate count method on plate count agar (Oxoid)[29]. Spore forming was counted on standard plate count agar (Oxoid) after activating spores by heating samples at 80°C for 10 min. Viable count of *Lactobacillus acidophilus* was determined according to Dave and Shah[30], using MRS-sorbitol agar. The plate were incubated anaerobic at 37°C for 72 h. *Bifidobacterium bifidum* was enumerated on MRS agar (Oxoid) supplement with L-cysteine and lithium chloride

(Sigma chemical Co., USA) and anaerobic incubation at 37°C for 72 h as described by Dave and Shah[30]. The results expressed as log colony forming unit CFU g^{-1} of sample and survival percentage at the end of freezing storage period was also calculated according to Desai *et al.*[31] and Nebesny *et al.*[11] as flow:

$$\text{Survival (\%)} = \frac{\text{Log CFU g}^{-1} \text{ in ice cream after 90 days}}{\text{Log CFU g}^{-1} \text{ of ice cream at day}} \times 100$$

Sensory evaluation: Frozen ice cream was sensory evaluated according to Magdoub *et al.*[32], using a scheme of 10 points for appearance 10 points for melting quality, 30 points for body and texture and 50 point for flavour.

Statistical analysis: Analysis of variance (ANOVA) and Duncan's test were conducted using a statistical analysis system[33]. A probability to $p \leq 0.05$ was used to establish the statistical significance.

RESULTS AND DISCUSSION

Properties of ice cream mixes: Table 1 shows pH value, surface tension (dyne) and flow rate of ice cream mixes made with or without different levels of tiger-nut extract (TNE). As a percentage of TNE increased in ice cream mix, the pH value and surface tension (dyne) decreased; the decrease being significant ($p<0.05$) only in the T_2 for pH and in the T_3 for surface tension compared with the control ice cream mix. Conversely, gradual increased was observed in flow time (sec) of ice cream mix when the percentage of TNE was increased ($p<0.05$). The increase in the flow rate could be attributed to the fact that TNE is rich in dietary fiber and polysaccharides[34], which hydrated with water and increase the viscosity of ice cream mix[35].

Figure 1 shows that substitution of aqueous phase with the TNE caused an increase in apparent viscosity, the increase was proportional to the addition rate of TNE. However, the increasing in apparent viscosity was significant only in T_3 and T_4 compared with the control mix ($p<0.05$). Also, the viscosity of all the ice cream mixes gradually decreased with the time of shear rate increased, which reflected that all the ice cream mixes show thixotropy properties. Soukoulis *et al.*[36] found the content of fibre in insoluble compounds increased significantly the viscosity and the shear thinning behaviour of the ice creams, due to the increase of total solids and the formation of networks comprised of hydrated cellulose and hemi-cellulose.

Table 1: Physical properties of the ice cream mixes made with or without different levels of tiger-nut extract

Ice cream mixes-base treatments	Physical properties of ice cream mixes pH	Surface tension (Dyne)	Flow rate (sec)
Cont	6.65^{A}	49.79^{A}	14.00^{D}
T_1	6.59^{A}	49.47^{A}	15.87^{C}
T_2	6.49^{B}	49.70^{AB}	16.59^{C}
T_3	6.47^{B}	48.53^{BC}	19.01^{B}
T_4	6.44^{B}	47.93^{C}	23.81^{A}

Means (n = 3) with the same letters in the same column are not significantly different at $p \leq 0.05$, Cont: Ice cream mix made without tiger-nut extract, T_1: Aqueous phase substituted with 25% tiger-nut extract, T_2: Aqueous phase substituted with 50% tiger-nut extract, T_3: Aqueous phase substituted with 75% tiger-nut extract and T_4: Aqueous phase substituted with 25% tiger-nut extract

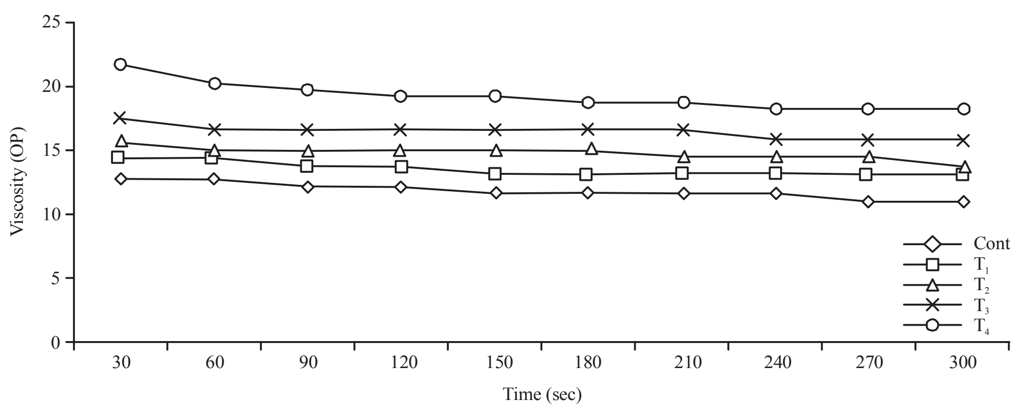

Fig. 1: Apparent viscosity and thixotropy behavior of the ice cream mixes made with or without different levels of tiger-nut extract at the shear rate of 10 (sec^{-1}) for 5 min, Cont: Ice cream mix made without tiger-nut extract, T_1: Aqueous phase substituted with 25% tiger-nut extract, T_2: Aqueous phase substituted with 50% tiger-nut extract, T_3: Aqueous phase substituted with 75% tiger-nut extract and T_4: Aqueous phase substituted with 25% tiger-nut extract

Table 2: Whipping ability of the ice cream mixes made with or without different levels of tiger-nut extract

	Whipping ability of ice cream mixes				
Ice cream mixes-base treatments	0.0	5 min	10 min	15 min	20 min
Cont	150	227^{Ab}	242^{Aab}	247^{Aab}	250^{Aa}
T_1	150	222^{Ab}	232^{Aab}	232^{Aab}	245^{Aa}
T_2	150	230^{Ab}	237^{Ab}	240^{Aab}	250^{Aa}
T_3	150	227^{Aa}	237^{Aa}	237^{Aa}	242^{Aa}
T_4	150	235^{Aa}	232^{Aa}	247^{Aa}	247^{Aa}

Means (n = 3) with the same capital letters in the same column or the same small letters in the same row are not significantly different at $p\leq 0.05$, Cont: Ice cream mix made without tiger-nut extract, T_1: Aqueous phase substituted with 25% tiger-nut extract, T_2: Aqueous phase substituted with 50% tiger-nut extract, T_3: Aqueous phase substituted with 75% tiger-nut extract and T_4: Aqueous phase substituted with 25% tiger-nut extract

Table 3: Levels of total bacterial counts ($\log_{10}$ CFU g^{-1}) of the ice cream made with or without different levels of tiger-nut extract during the storage period

	Total bacterial counts ($\log_{10}$ CFU g^{-1})				
Storage period (days)	Cont	T_1	T_2	T_3	T_4
After freezing	8.50^{Abc}	8.78^{Abc}	9.15^{Aab}	9.48^{Aa}	9.67^{Aa}
15	8.92^{Aa}	8.95^{Aa}	8.97^{Aa}	9.00^{ABa}	9.34^{ABa}
30	8.76^{ABa}	8.81^{Aa}	8.85^{Aa}	8.92^{ABa}	8.96^{BCa}
60	8.40^{Aa}	8.56^{Aa}	8.62^{Aa}	8.71^{BCa}	8.82^{BCa}
90	6.94^{Bb}	7.90^{Ba}	8.04^{Ca}	8.36^{Ca}	8.40^{Ca}

Means (n = 3) with the same capital letters in the same column or the same small letters in the same row are not significantly different at $p\leq 0.05$, Cont: Ice cream mix made without tiger-nut extract, T_1: Aqueous phase substituted with 25% tiger-nut extract, T_2: Aqueous phase substituted with 50% tiger-nut extract, T_3: Aqueous phase substituted with 75% tiger-nut extract and T_4: Aqueous phase substituted with 25% tiger-nut extract

Whipping ability is based on tensile strength and strength of the air cells lamellae. High whipping rate describes the ability to whip rapidly to gain a high overrun[24]. The whipping ability of ice cream mixes made with or without TNE is presented in Table 2. There was no significant difference ($p>0.05$) in the whipping ability of ice cream mixes made with different levels of TNE compared with the control mix. However, the whipping ability of T_2 was the nearest to that of control mix during whipping time (min). Also, the increasing rate with the time was similar between control mix, T_1 and T_2, however, the increasing rate being significant only at 20 min ($p<0.05$).

Properties of ice cream

Microbiological properties: The changes in total bacterial counts ($\log_{10}$ CFU g^{-1}) of resultant ice cream made with or without different levels of TNE during storage at -20°C for 90 days are shown in Table 3. In general, the total bacterial counts were lower in control ice cream than those in ice cream made with different levels of TNE, the difference was significant only when compared with T_2, T_3 and T_4 ($p<0.05$). Over storage, the total bacterial counts in all resultant ice cream treatments slightly decreased as the time of storage increased ($p>0.05$). These results are agreement with Ibrahim *et al.*[37] who mentioned that the total bacterial counts in two types of flavored ice cream like product decreased gradually until the end of storage period. This decrease could be evidentially attributed to the effect of hardening freezing storage period and presence of oxygen which control rate of bacterial growth[38].

Table 4 shows the changes in viability of *B. bifidium* and *L. acidophilus* ($\log_{10}$ CFU g^{-1}) in mixes and ice cream made with or TNE during storage at -20°C for 90 days. Initial freezing in batch freezer caused a slight decrease of both *B. bifidium* and *L. acidophilus* in all resultant ice cream treatments compared with ice cream mix treatments. The mechanical stresses of the mixing and freezing process and also the incorporation of oxygen into the mix might have resulted in a further decrease in bacteria count[39]. However, the decrease was more pronounced in control ice cream (0.5-0.6 $\log_{10}$ CFU g^{-1}) compared with that containing different levels of tiger-nut extract (0.2-0.3 $\log_{10}$ CFU g^{-1}), the reduction was less than one log cycle in total colony count of probiotic strains. A similar observation was found by Alamprese *et al.*[40] during freezing of ice cream. Salem *et al.*[15] showed that the initial freezing of ice cream mix followed by hardening caused a reduction of less than one log cycle in viable counts of probiotic. The viable counts decreased by 2.23, 1.68, 1.54, 1.23 and 1.77 $\log_{10}$ CFU g^{-1} for *L. acidophilus*, *B. bifidium*, *L. reuteria*, *L. gasseri* and *L. rhamnosus*, respectively during 12 weeks of frozen storage. However, the reduction in both *B. bifidium* and *L. acidophilus* after freezing was less marked than that (0.7-0.8 $\log_{10}$ CFU g^{-1}) reported by Hekmat and McMahon[22], Hagen and Narvhus[39] and Salem *et al.*[15].

Table 4: Changes in viable counts ($\log_{10}$ CFU g^{-1}) of *Bifidobacterium bifidium* and *Lactobacillus acidophilus* in mix and resultant ice cream made with and without tiger nut extract during storage at -20°C for 90 days

Strains	Storage period (days)	Treatments Cont	T_1	T_2	T_3	T_4
Bifidobacterium bifidium ($\log_{10}$ CFU g^{-1})	Before freezing	9.62^{Aa}	9.66^{Aa}	9.74^{Aa}	9.60^{Aa}	9.67^{Aa}
	After freezing	9.08^{Ba}	9.36^{ABa}	9.48^{ABa}	9.40^{Aa}	9.38^{Aa}
	15	8.97^{Ba}	9.28^{ABa}	9.45^{ABa}	9.32^{ABa}	9.27^{ABa}
	30	8.81^{BCa}	8.95^{Ba}	9.11^{Ba}	8.89^{Ba}	8.81^{Ba}
	60	8.40^{Cb}	8.92^{Ba}	8.96^{Ba}	8.85^{Bab}	8.48^{Bab}
	90	6.54^{Db}	7.44^{Cab}	7.85^{Ca}	7.80^{Cab}	7.34^{Cb}
Lactobacillus acidophilus ($\log_{10}$ CFU g^{-1})	Before freezing	9.49^{Aa}	9.70^{Aa}	9.51^{Aa}	9.64^{Aa}	9.56^{Aa}
	After freezing	8.90^{Bb}	9.40^{ABa}	9.34^{ABa}	9.38^{ABa}	9.26^{ABab}
	15	8.87^{BCa}	9.18^{BCa}	8.89^{BCa}	9.04^{BCa}	8.86^{BCa}
	30	8.78^{BCa}	8.95^{BCa}	8.84^{BCa}	8.93^{BCa}	8.65^{Ca}
	60	8.49^{Ca}	8.80^{Ca}	8.72^{Cab}	8.76^{Ca}	8.30^{Cb}
	90	6.96^{Db}	7.94^{Da}	7.97^{Da}	7.80^{Da}	7.40^{Da}

Means (n = 3) with the same capital letters in the same column or the same small letters in the same row are not significantly different at $p \leq 0.05$, Cont: Ice cream mix made without tiger-nut extract, T_1: Aqueous phase substituted with 25% tiger-nut extract, T_2: Aqueous phase substituted with 50% tiger-nut extract, T_3: Aqueous phase substituted with 75% tiger-nut extract and T_4: Aqueous phase substituted with 25% tiger-nut extract

Table 5: Survival percentage of *Bifidobacterium bifidium* and *Lactobacillus acidophilus* in resultant ice cream made with and without tiger-nut extract after storage at -20°C for 90 days

Ice cream treatments	Strains *Bifidobacterium bifidium* (survival%)	*Lactobacillus acidophilus* (survival %)
Cont	72.02^{B}	78.20^{C}
T_1	79.48^{A}	84.46^{AB}
T_2	82.80^{A}	85.69^{A}
T_3	80.85^{A}	83.2^{ABC}
T_4	78.25^{AB}	79.91^{BC}

Means (n = 3) with the same capital letters in the same column or the same small letters in the same row are not significantly different at $p \leq 0.05$, Cont: Ice cream mix made without tiger-nut extract, T_1: Aqueous phase substituted with 25% tiger-nut extract, T_2: Aqueous phase substituted with 50% tiger-nut extract, T_3: Aqueous phase substituted with 75% tiger-nut extract and T_4: Aqueous phase substituted with 25% tiger-nut extract

Table 6: Changes in sporeform ($\log_{10}$ CFU g^{-1}) in resultant ice cream made with and without tiger-nut extract during storage at -20°C for 90 days

Strains	Storage period (days)	Treatments Cont	T_1	T_2	T_3	T_4
Sporeform ($\log_{10}$ CFU g^{-1})	After freezing	1.30^{Ab}	1.74^{Aa}	1.86^{Aa}	1.90^{Aa}	1.97^{Aa}
	15	1.08^{ABb}	$1.56A^{Bab}$	1.71^{ABa}	1.82^{ABa}	1.88^{Aa}
	30	0.90^{Bb}	1.34^{Ba}	1.46^{Ba}	1.45^{Ba}	1.59^{ABa}
	60	-	0.95^{Ba}	1.08^{Ba}	1.11^{Ba}	1.23^{Ba}
	90	-	-	-	-	-

Means (n = 3) with the same capital letters in the same column or the same small letters in the same row are not significantly different at $p \leq 0.05$, Cont: Ice cream mix made without tiger-nut extract, T_1: Aqueous phase substituted with 25% tiger-nut extract, T_2: Aqueous phase substituted with 50% tiger-nut extract, T_3: Aqueous phase substituted with 75% tiger-nut extract and T_4: Aqueous phase substituted with 25% tiger-nut extract

After 90 days of frozen storage, the viability of *B. bifidium* and *L. acidophilus* decreased in all ice cream treatments, however, the reduction was more pronounced in control ice cream compared with that made with TNE. In particular, the reduction were 2.58, 1.92, 1.63, 1.60 and 2.04 $\log_{10}$ CFU g^{-1} for *B. bididium* and 1.94, 1.46, 1.33, 1.58 and 1.86 $\log_{10}$ CFU g^{-1} for *L. acidophilus* in control, T_1, T_2, T_3 and T_4, respectively. In addition, the survival percentage of *B. bididium* and *L. acidophilus* in control ice cream was lower (p<0.05) than that in ice cream made with TNE (Table 5). The T_2 had the highest survival percentage of *B. bididium* and *L. acidophilus* compared with other treatments. However, all ice cream treatments still has higher numbers of viable organism throughout 90 days of storage at -20°C and all were above the recommended minimum limit of 10^6 CFU g^{-1} which is the level suggested by some authors to have a health promoting effect[41,42].

In Table 6, the sporeform bacterial ($\log_{10}$ CFU g^{-1}) was found in all ice cream treatments after freezing. However, the counts of sporeform were lower in control ice cream (1.30 $\log_{10}$ CFU g^{-1}) than that made with TNE (p<0.05). The presence of the spore forming may due to these bacteria are

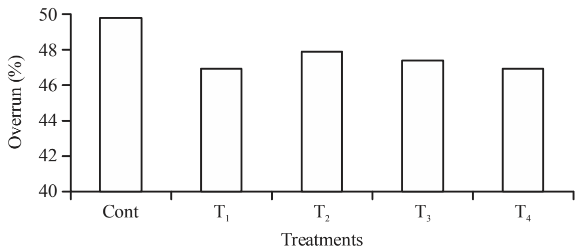

Fig. 2: Overrun of the ice cream made with and without tiger nut extract, Cont: Ice cream mix made without-tiger nut extract, T_1: Aqueous phase substituted with 25% tiger-nut extract, T_2: Aqueous phase substituted with 50% tiger-nut extract, T_3: Aqueous phase substituted with 75% tiger-nut extract and T_4: Aqueous phase substituted with 25% tiger-nut extract

Table 7: Meltdown of the resultant ice cream made with different levels of tiger-nut extract after hardening for 24 h

	Meltdown properties of ice cream		
Ice cream mixes-base treatments	10 min	20 min	30 min
	(g/100 g ice cream)		
Cont	29.6^{Ac}	65.7^{Ab}	85.0^{Aa}
T_1	34.3^{Ac}	66.5^{Ab}	84.2^{Aa}
T_2	36.0^{Ac}	66.6^{Ab}	85.5^{Aa}
T_3	33.9^{Ac}	65.9^{Ab}	83.1^{Aa}
T_4	34.0^{Ac}	70.7^{Ab}	83.8^{Aa}

Means (n = 3) with the same capital letters in the same column or the same small letters in the same row are not significantly different at $p\leq 0.05$, Cont: Ice cream mix made without tiger-nut extract, T_1: Aqueous phase substituted with 25% tiger-nut extract, T_2: Aqueous phase substituted with 50% tiger-nut extract, T_3: Aqueous phase substituted with 75% tiger-nut extract and T_4: Aqueous phase substituted with 25% tiger-nut extract

Table 8: Sensory properties of the resultant ice cream made with different levels of tiger-nut extract after hardening for 24 h

	Sensory properties of resultant ice cream		
Ice cream mixes-base treatments	Flavour (50)	Body and texture (40)	Melting quality (10)
Cont	44.11^{A}	38.22^{A}	8.44^{A}
T_1	43.44^{A}	37.11^{A}	8.22^{A}
T_2	44.11^{A}	37.78^{A}	8.00^{A}
T_3	43.33^{A}	36.78^{A}	8.00^{A}
T_4	41.56^{A}	36.67^{A}	7.67^{A}

Means (n = 3) with the same capital letters in the same column or the same small letters in the same row are not significantly different at $p\leq 0.05$, Cont: Ice cream mix made without tiger-nut extract, T_1: Aqueous phase substituted with 25% tiger-nut extract, T_2: Aqueous phase substituted with 50% tiger-nut extract, T_3: Aqueous phase substituted with 75% tiger-nut extract and T_4: Aqueous phase substituted with 25% tiger-nut extract

ubiquitous organisms present in virtually all environments due to sporeforming ability and can also survive pasteurization which provides resistance to harsh environmental conditions or processing treatment[43].

Over storage period of 90 days, all ice cream treatments showed a gradual decrease in the sporeform bacterial, the decrease being significant only at day 30 (p>0.05). The sporeform bacterial was not detected at day 60 in control ice cream and at day 90 in that made with TNE.

Physical properties: Figure 2 shows that control ice cream (made without TNE) exhibited higher overrun than that made with different levels TNE, however, there was no significant difference in the percentage of overrun among all treatments (p>0.05). In the same time, slight decrease was observed in overrun of ice cream as the percentage of TNE increased (p>0.05). The decrease in overrun of ice cream containing TNE may be correlated with the increase in the viscosity of ice cream mix which in turn resulted in lower overrun or TNE may contain some components decrease tensile strength or strength of the air cells lamellae. Adapa *et al.*[26] reported that increase of the mix viscosity could have been the primary reason for the decrease of whipping abilities. Meltdown behavior of ice cream made with or without TNE is presented in Table 7. At 10 min, although the melting rate of control ice cream was lower compared with that made with different levels of TNE, the difference was not significant (p>0.05). After that the melting rate was similar in all ice cream treatments, which reflected that TNE had no significant effect on melting properties of resultant ice cream (p>0.05).

Sensory properties: The sensory properties of resultant ice cream are shown in Table 8. The results revealed that there was no significant difference in the score of flavor, body and texture and melting properties between the control ice cream and that made with TNE (p>0.05). However, T_2 was the nearest to the control in flavor and body and texture.

CONCLUSION

It could be concluded that substitution of the aqueous phase of the ice cream mix with 50% tiger-nuts extract together with incorporation of probiotic bacteria (*Lactobacillus acidophilus* and *Bifidobacterium bifidum*) can be done for production of healthful new probiotic ice cream.

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