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Production of a Functional Frozen Yoghurt Fortified with Omega-3 and Vitamin E

Hoda Mahrous[1,*], Rehab Abd-El- Salam[2]

[1]Genetic Engineering and Biotechnology Research Institute, Sadat City University, Egypt
[2]Food Technology Research Institute, Agricultural Research Center, El-Sabhia, Alexandria, Egypt
*Corresponding author: hmahrous7@yahoo.com

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Abstract Frozen yoghurt can be regarded as a healthy alternative to plain ice cream. This study investigates the isolation and identification of beneficial bacterial isolates from Laban Rayeb. Three isolates were selected and identified, namely Lactobacillus acidophilus r1, Lactobacillus acidophilus r2 and Latctococcus lactis subsp lactis r3. Also results showed that Laban Rayeb isolates had an inhibition and bactericidal effects on the growth of some pathogenic microorganisms as Staphylococcus aureus; E.coli ATCC 25922 and Bacillus subtilis NCIB3610. Antagonistic effect of S. aureus and E.coli indicated more pronounced inhibitory effect than Bacillus subtilis especially for Lactobacillus acidophilus r2. Then Low-fat ice cream mix was fermented with probiotic supplemented; omega-3 & vitamin E with traditional starter culture and evaluated for culture survival, composition, and sensory characteristics of frozen product. The developed frozen yoghurt formulae were as follows: Formula 1 with no probiotics, Formula 2 containing 3%w/w of Lactobacillus acidophilus r2 which has shown significant promise in all probiotics characteristics, and Formula 3 containing 3% w/w of probiotic strain (Lactobacillus acidophilus r2); with 2% ω-3 with vitamin E. The survival rate of probiotic in Formula 2 and Formula 3 after 4 weeks was higher than 106 CFU/ ml, the number regulated by FDA for the probiotic products. Formula 3 was containing 39.13±1.13 μg/ g alpha tocopherols after 4 weeks under freezing conditions. Sensory evaluation was carried out among 10 panelists, using the 9-point hedonic scale method for food acceptance. Formula 1, 2, and 3 obtained the mean score of 7.45, 7.98 and 8.01, respectively.

Keywords: *functional foods, probiotics, Lactobacillus acidophilus, frozen yoghurt*

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1. Introduction

Frozen yoghurt is a dessert that combines the texture of ice cream with the nutritive and healthy properties of yoghurt (Rezaei, *et al*., 2011). Its process consists in mixing all ingredients to make natural stirred yoghurt with stabilizers/emulsifiers and sugar, then freezing the mix in a conventional ice cream freezer (Tamine and Robinson 2007). Production of the first fermented milks dates back to 7000 BC with origins in the middle and far-east of Asia, making it one of the oldest methods of long term food preservation. A further spreading east of these traditions, by way of Russia and Eastern Europe, by the Tartars, Mongols and Huns occurred during their conquests (Vasiljevic, *et al*., 2008).

Frozen yoghurt popularity has increased and continues to grow; making it one of the most frequently consumed frozen desserts around the world. As the popularity of yoghurt products continues to grow, manufacturers are continuously investigating value-added ingredients to entice health-conscious consumers (Allgeyer *et al*., 2010).

Frozen yoghurt's attractiveness to consumers include providing a low fat replacement for ice cream and the probiotic benefits of the live cultures present in the yoghurt (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*). According to Tamine and Robinson (2007), the official standard of identity for frozen yoghurts has not been specified yet in most countries. However, some references specify that the final product should have a minimum of 0.15% titratable acidity (expressed as lactic acid), >3.25% milk-fat, a pH <5, and a minimum yoghurt content >70% (Westerbeek, 1995; Marshall *et al*., 2003).

According to Tamine (2002), frozen yoghurt can be classified into three categories: soft, hard, or mousse. Soft frozen yoghurt consists of a mix of 80% yoghurt base (cold) with 20% fruit syrup base and stabilizer/emulsifier; hard frozen yoghurt is a mix containing 65% yoghurt base with 35% fruit syrup plus stabilizer/emulsifier; and mousse frozen yoghurt is a mix of the yoghurt with hot mousse base mixture (skim milk, sugar and stabilizer/emulsifier). According to the International Dairy Foods Association (IDFA), a total of 1.481 billion gallons of frozen yoghurt mix were produced in 2010. In addition, frozen yoghurt production increased by 8.1% with 49.7

million gallons produced in 2010 compared with 2009 (NASS, 2012).

Marine lipids have for the last decades received increasing attention because of their beneficial health effects. Since the first evidences regarding the beneficial health effects of eicosapentaenoic acid (**EPA**) and docosahexaenoic acid (**DHA**) on cardiovascular diseases appeared, many studies have demonstrated that these fatty acids have a positive effect especially on myocardial infarction (Schmidt *et al*., 1992) and other diseases. Foods containing fish oil are very susceptible to oxidation due to the high content of PUFAs (Nielsen *et al*. 2007). In recent years, the number of studies describing the health-promoting benefits of omega-3 fatty acids has increased substantially. Some of the reported activities attributed to the omega-3 fatty acids include improving serum cholesterol profiles, stabilizing arrhythmias, reducing inflammation, regulating endothelial cell function, improving insulin sensitivity in patients with Type 2 diabetes, and enhancing the immune response.

Vitamin E is a fat soluble vitamin and recent research suggests that its deficiency is associated with an elevated risk of atherosclerosis and other degenerative diseases. The physiological role of vitamin E centers on its ability to react with free radicals in cell membranes and other lipid environments, thereby preventing PUFAs from being damaged by lipid oxidation (Bramley *et al*., 2000). Temperature, oxygen and UV light parameters during extraction, encapsulation and storage of vitamin E affect its degradation (Sabliov *et al*., 2009). Frozen desserts can be used to carry health-promoting constituent's vitamins and minerals or fiber or nutraceuticals (minor constituents of foods that have been shown to have health-promoting effects).

Polyunsaturated fatty acids (PUFAs) lower the risk of heart disease and vitamin E is an important lipophilic antioxidant that protects cell membranes from oxidation. Our goal in the current research was to isolate and identify of beneficial bacterial strains with properties of probiotic bacteria from Laban Rayeb and develop a successful strategy obtains fermented milks with probiotics that has a satisfactory quality for consumers and also to delivery of ω-3 and to evaluate their effect on sensory characteristics of frozen yoghurt during storage.

2. Materials and Methods

2.1. Source of Bacteria and Antimicrobial Activity

Probiotic strains candidate: *Lactobacillus acidophilus* r1, *Lactobacillus acidophilus* r2 and *Latctococcus lactis* subsp *lactis* r3 were isolated from traditional Egyptian dairy product Laban Rayeb and used after the selection had been done according to Bergey's Manual of Determinative Bacteriology, 9th edition (Holt *et al.*, 1994) with confirm the identification by API 50CH system (Biomerieux, Marcy l'Etoile France). The strains were tested for their probiotic characteristic i.e. gastric acid resistance, bile salt tolerance, antibacterial activity, adhesion to human mucus according to Mahrous et al., (2010). The antimicrobial activity was determined by measuring the diameter of the inhibition zone around the wells. The bacterial isolates showing the widest zone of inhibition against *Staphylococcus aureus; E.coli* ATCC 25922 and *Bacillus subtilis* NCIB3610 were selected for further studies.

Lactobacillus strains were cultivated in MRS (de Man Rogosa Sharpe) broth (Lab M, IDG, UK) and incubated at 37°C in BBL anaerobic jar (Becton Dickinson Microbiology Systems, Sparks, MD) provided with disposable BBL gas generating pack (CO_2 system envelopes, Oxoid, Ltd., West Heidelberg, Victoria, Canada). Other strains were cultivated in Nutrient broth and incubated at 37°C. Isolates were stored at –20°C in MRS broth supplemented with 25% (v/v) glycerol. For routine analysis, the strains were subculture twice in MRS broth at 37°C for 24 h.

2.2. Frozen Yoghurt Manufacture

2.2.1. Preparation of Yoghurt

Experimental plain yoghurt was prepared by heating pasteurized whole milk at 72°C for 10 minutes and subsequently cooled to 43°C. It was then divided into three separated containers. Formula 1 inoculated with 2%w/w starter culture yoghurt with no probiotics, Formula 2 inoculated with 2%w/w starter culture yoghurt with 3%w/w of *Lactobacillus acidophilus* r2, and Formula 3 inoculated with 2%w/w starter culture yoghurt with 3%w/w of *Lactobacillus acidophilus* r2; with 2% ω-3 with vitamin E (after separated 2% of milk fat). The inoculated mixes were incubated at 43°C until the acidity of 0.80-0.88 was obtained.

2.2.2. Probiotic Frozen Yoghurt Preparation

The frozen yoghurt mixes were prepared by combining experimental plain yoghurts (three formulas), sucrose, 11%w/w, guar gum, 0.3%w/w and the mix was standardized to a total solid content of up to 12% by addition of skim milk powder. Weighed guar gum was dissolved into hot water (60°C). Other ingredients (without plain yoghurts) were weighed and mixed in the containers. Hydrated guar gum suspension was poured into the mixes and pasteurized at 85°C for 25 seconds. The mix was divided into three separated containers and mix with the tree formulas of plain yoghurts. The final formula as Formula 1 with no probiotics, Formula 2 containing 3%w/w of *Lactobacillus acidophilus* r2, and Formula 3 containing 3%w/w of probiotic strain (*Lactobacillus acidophilus* r2); with 2% ω-3 with vitamin E (50 μg/g). All three yoghurt mixes were each transferred into the ice-cream maker. The finished products were stored at -18°C for further tests.

2.3. Chemical Analysis

2.3.1. Approximate Analysis

The frozen yoghurt samples were analyzed for total solids, fat, lactose, protein, titratable acidity (%), pH and acetaldehyde (Robinson *et al*., 1977).

2.3.2. Determination of Alpha Tocopherol Content using HPLC

The α-tocopherol concentration was determined using the method described by Jang and Xu (2009). Oils were

extracted from frozen yoghurts using the modified Folch extraction method (AOCS, 1985). All solvents used were HPLC-grade (Fisher Scientific). A sample (0.2 g) of oil was dissolved in 2 mL of hexane in a glass test tube and vortexed. The mixture was transferred to HPLC vials and 25 µL were injected into the HPLC system for analysis (1260 HPLC Liquid Chromatography and Waters), Bondapak C18 3.9×300 mm column. The mobile phase consisted of 0.5% ethyl acetate and 0.5% acetic acid in hexane at a flow rate of 1.5 mL/ min. The fluorescence detector was set at 290 nm excitation and 330 emissions to monitor α-tocopherol. The α-tocopherol concentration was determined and expressed as µg/ g frozen yoghurt.

2.4. Microbiological Analysis

2.4.1. Lactic acid Bacteria Counts

Str. thermophilus and *Lb. bulgaricus* counts and probiotic bacteria counts were enumerated in each fermented milk samples. Each sample was serially diluted to 10^{-6} with Ringers' solution. Appropriate dilutions were prepared using the following media: MRS Agar (Merck, Darmstadt/Germany) for the enumeration of *Lb. bulgaricus*; was incubated anaerobically at 42±2°C for 3 days (Dave and Shah, 1997); M-17 medium agar for the enumeration of *Str. thermophilus* was made of the following (g/L of dH_2O): phytone peptone, 5; polypeptone, 5; lactose, 5; beef extract, 5; yeast extract, 2.5; L-ascorbic acid, 0.5; sodium glycerophosphate, 19; $MgSO4*7H_2O$ 1M solution, 1 ml; and agar, 12. Medium was adjusted to pH 5.4, dispensed into 150-ml screw-cap bottles, sterilized (20 psi, 20 min) and stored in the dark until used; was anaerobically incubated at 37±2°C for 3 days and MRS agar containing D-sorbitol for the enumeration of *Lb. acidophilus*; was incubated at 37°C for 72 hours anaerobically (Dave and Shah, 1997).

2.4.2. Other Microbial Counts

Selective media for total count bacteria Plate count agar (PCA), Coliforms Violet Red Bile Glucose Agar (V. R), *Enterobacter* spp. Violet Red Bile Glucose Agar (V.R), *Entercoccus* spp. *Streptococcus faecalis* Medium (S.F) and yeasts and molds Potato Dextrose Agar (PDA) contained chloramphenichole, the plates incubated at 37°C for 72 hours.

2.5. Sensory Evaluation

Ten panelists participated in a sensory evaluation of the yoghurt on the basis of appearance, flavour, texture, and overall acceptability. The panelists rated these characteristics according to the hedonic scale where 1 corresponds with "dislike extremely" and 9 corresponds with "like extremely". Panelists were asked to assess the sensory characteristics of the three different types of frozen yoghurt: frozen yoghurt 1 = plain (non-probiotic) yoghurt, frozen yoghurt 2 = probiotic yoghurt and frozen yoghurt 3=probiotic yoghurt with 2% ω-3 with vitamin E. All participants received a letter of information and consent document, and gave signed consent before participating in the study. Panelists were then given three samples at a time at storage temperature (4°C), a pencil, and a glass of cold water to rinse their mouths between samples.

2.6. Statistical Analysis

Data are presented as the mean ± standard deviation, and n represents the number of samples from the frozen yoghurt and the control.

3. Results and Discussion

Table 1. Biochemical identification of isolates bacteria

No.	Biochemical tests					API
	Gram	Catalase	Groth at $10^{o}c$	growth at $45^{o}c$	production of CO_2	
r1	+	-	-	+	-	99.9% *Lactobacillus acidophilus* r1
r2	+	-	-	+	-	99.9% *Lactobacillus acidophilus* r2
r3	+	-	-	+	-	99.9% *Latctococcus lactis* subsp *lactis* r3

3.1. Identification of Isolates and Probiotic Properties

Three isolates were selected from Laban Rayeb were identified by morphological & biochemical tests (Gram staining and catalase production, growth at 10, 45°C for 48 h, and CO_2 production from glucose) and API 50CH system (Biomerieux, Marcy l'Etoile France). Gram positive, catalase-negative rods, which grew at 45°C and not grow at 10°C, were considered as thermophilic lactobacilli, while those that didn't grow at 45°C and grew at 10°C were considered as mesophilic lactobacilli. The identified *Lactobacillus* strains were *Lactobacillus acidophilus* r1, *Lactobacillus acidophilus* r2 and *Latctococcus lactis* subsp *lactis* r3 as shown in Table 1.

A potentially successful probiotic strain is expected to have several desirable properties in order to be able to exert its beneficial effects. The selection criteria that have been considered to be relevant for any potential probiotic microorganism are: acid and bile stability; Adhesion to mucosal surfaces; antimicrobial activity and good technological properties (Ouwehand *et al*., 1999).

The LAB survival in low pH is very important for bearing initial stress in the stomach (Gilliland and Walker, 1989). At the application level, when LAB enter into human body, first constraint is gastric acid with very low pH level around 2- 3 (Minellia *et al*., 2004). Resistance of isolates for Gastric Juice (*In Vitro*) was determined according to the method of Pennacchia (2004). All isolates were tested for their survival in low pH level around 2- 3 and *Lactobacillus acidophilus* r1 was the best isolates for survival in these levels. The acid resistant LAB isolates were moreover tested for bile tolerance according to their survival in MRS broth containing 0.1, 0.2 and 0.3% bile salt and MRS broth without bile salt as a control. Therefore, all isolates were able to grow and survive at bile salt condition after six hours especially *Lactobacillus acidophilus* r1. The survival at bile salt condition is one of the main criteria for *in vitro* selection of potentially probiotic bacteria and critical points for the

microorganisms. Because some of LAB are able to survive at bile salt condition.

The ability of the probiotics present in dairy products that inhibit the growth of pathogens confirms the health benefits. Our study suggests that probiotics are helpful in the protection and improvement of some pathogenic bacteria. Table 2 showed the antibacterial effect of the three isolates against some pathogenic microorganisms as *Staphylococcus aureus; E. coli* ATCC 25922 and *Bacillus subtilis* NCIB3610. The results showed that all isolates had ability to inhibit the pathogenic bacteria and *Lactobacillus acidophilus* r2 was the highest ability in inhibition the growth of *Staphylococcus aureus; E. coli* ATCC 25922 and *Bacillus subtilis* NCIB3610 so it was used in the production of our product. Consuming these dairy products can help human healthcare and can also protect against occurrences of diarrhea and food contaminations (Walencka, 2008).

Table 2. Antimicrobial effect of isolates bacteria

No.	*Staphylococcus aureus* mm.	*E.coli* ATCC 25922mm.	*Bacillus subtilis* NCIB3610 mm.
Lactobacillus acidophilus r1	16±2.00	17±1.01	12±3.02
Lactobacillus acidophilus r2	17±0.01	18±0.03	13±1.03
Latctococcus lactis subsp *lactis* r3	10±0.03	12±1.10	9±0.05

Data are presented as mean ± standard deviation of triplicate values.

Table 3. Adhesion of *Lactobacillus acidophilus* r1, *Lactobacillus acidophilus* r2 and *Latctococcus lactis* subsp *lactis* r3 to intestinal mucous of isolates bacteria

No.	Adhesion (O. D at 630 nm)
Lactobacillus acidophilus r1	1.2 ±0.001
Lactobacillus acidophilus r2	2.4 ±0.01
Latctococcus lactis subsp *lactis* r3	1.4±0.03
Bifidobacterium bifidum (B)	2.5±0.015

Data are presented as mean ±SD.Intestinal mucous isolated from faecal samples of 30 days old infant. Adhesion is expressed as the turbidity caused by crystal violet stain bound to the adheri bacteria as released by 20 mmole^{-1}citrate buffer. Bars represent the mean ± standard deviation of triplicate O.D at 630nm values recorded for each strain, Bifidobacterium bifidum B reference strain used.

Many different intestinal mucosal models have been used to assess the adhesive ability of probiotics. In this study we had focused on the adhesion to intestinal mucous which is overlying the enterocytes. For the adherence assay, the crystal violet method was selected (Vesterlund *et al*., 2005). Table 3 indicates that all isolates strains had the ability to adhere on intestinal mucous to various extents. The strongest *in vitro* adhesion was observed for strains of *Lactobacillus acidophilus* r2 compared to the reference probiotic strain *Bifidobacterium bifidum* (B). From the obtained results it is clear that there are many differences in the adhesive characteristics among strains and species in the genus *Lactobacillus* these results agreed with several literatures (Jonsson *et al*., 2004; MacKenzie *et al*., 2009).

From the obtained results frozen yoghurt were made by using *Lactobacillus acidophilus* r2 which has shown significant promise in all probiotics characteristics. The formulas of prepared frozen yoghurt were: Formula 1 inoculated with 2%w/w starter culture yoghurt with no probiotics, Formula 2 inoculated with 2%w/w starter culture yoghurt with 3%w/w of *Lactobacillus acidophilus* r2, and Formula 3 inoculated with 2%w/w starter culture yoghurt with 3%w/w of *Lactobacillus acidophilus* r2; with 2% ω-3 with vitamin E (after separated 2% of milk fat). The following analyses were tested in these formulas.

3.2. Approximate Analysis

Table 4 showed that the best features of this product were the high levels of protein (8.2±0.02; 8.5±0.15 & 8.3±0.03%) in the three formulas 1; 2&3, respectively. Titratable acidity in zero time of formula 1, 2& 3 were observed to be 0.81, 0.85& 0.89%, respectively. According to ISI (1974), the maximum lactic acid acidity should be 0.8 percent. After 4 weeks there were increasing in titratable acidity as 0.89; 0.96& 0.99 in the three formulas 1; 2&3, respectively. The difference in acidity was due to addition of probiotic *Lb. acidophilus* r1. Titratable acidity found increased and pH decreased with time after 4 weeks mainly due to sugar fermentation and conversion of lactose to lactic acid. Similar findings were reported Tamine and Robinson, 1985; Shin et al. 1991 and Salwa et al. 2001 for yoghurt.

Table 4. Chemical analysis of frozen Yogurts

Trial	Parameter (%)						
	Total solid	Fat	Lactose	Protein	Acidit	pH	Acetaldhyde (µg/mL)
0 time							
Formula1	29.5±0.01	3.6±0.03	4.22±0.15	8.2±0.02	0.81±0.01	5.9	6.47±0.02
Formula2	29.9±0.1	3.7±0.02	4.20±0.52	8.5±0.15	0.85±0.12	5.5	6.89±0.01
Formula3	30.1±0.014	3.9±0.01	4.21±0.12	8.3±0.03	0.89±0.02	5.45	6.99±1.01
After 4 weeks							
Formula1	28.1±0. 2	3.0±0.12	3.95±0.3	7.3±0.02	0.89±0.1	5.5	7.33±0.03
Formula2	28.5±0.013	3.0±0.02	3.75±0.12	7.0±0.03	0.96±0.02	5.0	8.85±0.02
Formula3	28.9±0.02	3.2±0.15	3.72±0.1	6.9±0.02	0.99±0.12	5.0	8.95±0.05

Data are presented as mean ± SD. Formula 1 with no probiotics, Formula 2 containing 3%w/w of *Lactobacillus acidophilus* r2, Formula 3 containing 3%w/w of probiotic strain (*Lactobacillus acidophilus* r2); with 2% ω-3 with vitamin E.

Lactose concentrations were detected in all formulas to determine if additional organisms (*Lactobacillus acidophilus* r1) increased lactose hydrolysis or not. Lactose concentration was decreased after 4 weeks especially in formula 2 &3. The differences were observed in lactose concentration due to the difference in levels of acidity. Thompson and Mistry (1994) observed no significant changes in lactose concentrations in frozen yoghurt mix when stored frozen for 1 and 3 months. Gilliland and Kim found that lactose decreased from 6.26% in uninoculated yoghurt mix to 4.23% in inoculated.

Our results generally agree reports Hekmat and McMahon (1992).

Acetaldehyde concentrations were increased in all formulas (1; 2&3) from zero to 4 weeks from 6.47±0.02; 6.89±0.01 &6.99±1.01 (µg/mL), respectively at zero time to 7.33±0.03; 8.85±0.02 & 8.95±0.05 (µg/mL), respectively after 4 weeks. The higher levels of acetaldehyde in formula 2 and 3 were thought to be due to the fast metabolic activity of starter bacteria with probiotic bacteria compared with formula 1. Recently, Martin et al. (2011) reported on the influence of the oxidoreduction potential value on the production of aroma compounds, as it can modify the metabolic pathways of the yogurt bacteria. Oxidative conditions contribute to the stability of the acetaldehyde during the yogurt storage, whereas reducing conditions provide the opposite effect.

3.2.1. Alpha Tocopherol Content using HPLC

Means and standard deviations of α-tocopherol content during 4 weeks of storage of frozen yoghurt are illustrated in Table 5. Initial α-tocopherol contents in the three formulas 1, 2 &3 were 18.21 ± 1.15, 20.15 ± 0.05 and 42.13 ± 1.13 µg/ g frozen yoghurt, respectively. After 4 weeks of storage, α-tocopherol contents in the three formulas1, 2 &3 were reduced to 15.40±1.15; 16.25±0.05 and 39.13±1.13 µg/ g frozen yoghurt, respectively; however α-tocopherol content in formula 3 was still greater than the other formulas. A number of factors such as oxygen, light, heat, alkali, trace minerals, and hydroperoxides can cause decomposition of vitamin E vitamers (Bramley *et al*., 2000). The decrease in α-tocopherol content during refrigerated storage may be primarily caused by oxygen dissolved in the yoghurt matrix, and reaction with hydroperoxides produced by initial lipid oxidation reactions. α-Tocopherol is expected to have chain-breaking antioxidant activity in yogurts as well as in human body tissues. Burton et al. (1985) showed that α-tocopherol donates its phenolic hydrogen atom to peroxyl radicals arising and in the process becomes an α- tocopheroxyl radical.

Table 5. Alpha tocopherol content of frozen Yogurts

Trial	α-Tocopherol (µg/ g frozen yogurt)				
	Initial	1week	2week	3 week	4 week
Formula 1	18.21±1.15	18.0±1.15	17.12±1.15	16.10±1.15	15.40±1.15
Formula 2	20.15±0.05	19.12±0.05	18.11±0.05	17.15±0.05	16.25±0.05
Formula 3	42.13±1.13	41.11±1.13	40.45±1.13	40.21±1.13	39.13±1.13

Data are presented as mean ± standard deviation of triplicate values.

Table 6. Total plate count for lactic acid bacteria of frozen yoghurt samples

Time (day) / Trial	Total count (cfu/ml)				
	0	7	14	21	28
Formula 1					
S.thermophilus	$4.5\times10^6\pm1.0$	$4.3\times10^6\pm2.0$	$4.2\times10^6\pm3.0$	$4.0\times10^6\pm0.1$	$3.8\times10^6\pm0.1$
Lb.bulgaricus	$5.6\times10^6\pm2.1$	$5.5\times10^6\pm3.0$	$5.0\times10^6\pm2.0$	$4.4\times10^6\pm3.0$	$4.0\times10^6\pm3.0$
Formula2					
L.acidophilus r2	$1.6\times10^8\pm3.0$	$1.0\times10^8\pm3.0$	$7.9\times10^7\pm3.0$	$6.5\times10^7\pm3.0$	$2.6\times10^7\pm3.0$
S.thermophilus	$5.5\times10^6\pm3.0$	$5.4\times10^6\pm3.0$	$5.2\times10^6\pm3.0$	$5.0\times10^6\pm3.0$	$4.6\times10^6\pm3.0$
Lb.bulgaricus	$5.6\times10^6\pm3.0$	$5.6\times10^6\pm3.0$	$5.4\times10^6\pm3.0$	$5.2\times10^6\pm3.0$	$4.6\times10^6\pm3.0$
Formula 3					
L.acidophilus r2	$1.8\times10^8\pm2.0$	$1.3\times10^7\pm1.0$	$8.8\times10^7\pm0.1$	$6.9\times10^7\pm1.0$	$3.2\times10^7\pm1.0$
S.thermophilus	$5.5\times10^6\pm1.0$	$5.5\times10^6\pm2.0$	$5.4\times10^6\pm3.0$	$5.3\times10^6\pm0.1$	$5.1\times10^6\pm0.1$
Lb.bulgaricus	$5.6\times10^6\pm2.1$	$5.6\times10^6\pm3.0$	$5.4\times10^6\pm2.0$	$5.2\times10^6\pm3.0$	$5.0\times10^6\pm3.0$

Data are presented as mean ± SD. Formula 1 with no probiotics, Formula 2 containing 3%w/w of *Lactobacillus acidophilus* r2, Formula 3 containing 3%w/w of probiotic strain (*Lactobacillus acidophilus* r2); with 2% ω-3 with vitamin E.

3.3. Microbiological Counts

3.3.1. Lactic Acid Bacteria Counts

The dairy products, especially frozen yoghurt are good vehicle to transfer probiotics to the human intestinal tract. Consumption of probiotic bacteria via dairy food product is an ideal way to re-establish the intestinal micro-flora balance. Survival of cultures in frozen yoghurt has great importance for the healthy properties of the product (Tamine and Robinson, 1999). Table 6 showed lactic acid bacteria counts in the three trials of the frozen yoghurt. The initial count of probiotic in Formula 2 was $1.6\text{x}10^8$ cfu/ ml and that of Formula 3 was $1.8\text{x}10^8$ cfu/ml. The counts of probiotic in the two formulae decreased gradually during storage. After four weeks, the counts of probiotic in Formula 2 and Formula 3 were $2.6\text{x}10^7$ and $3.2\text{x}10^7$cfu/ml respectively. However the bacterial contents of the two formulae were still higher than $1.0\text{x}10^6$ cfu/ml, the number regulated by Thai FDA (2008) for the probiotic products. Lopez et al. (1998) observed only a slight decline in lactic acid bacteria in three batches (pH = 4.32, 5.09, and 5.53) of commercial frozen yogurt stored at –23°C for 1 yr. Modler and Villa- Garcia (1993) reported a 2 $\log^{10}$ cfu/ml loss in B. longum concentrations from acidification of frozen yogurt caused by fermentation and refreezing. Holcomb et al. (1991) observed no decrease in B. longum in frozen yogurt that was frozen at –5°C for 6 h.

The overall rate of reduction for *Lactobacillus bulgaricus* and *Streptococcus thermophilus* in the frozen yoghurt was found during the storage time but this decrease was reduced in formula 2 & 3. This result was in agreement with Gilliland and Kim (1984) & Chandan and Shahani, 1992 that due to the frozen yoghurt environment is not optimum for survival of bacteria. The freezing process of the mix may cause a loss of ½ to 1 log cycle in viable counts. The obtained data suggested that incorporation of probiotic strain and ω-3 caused protection of LAB against freezing process. The decline in bacterial counts, as a result of freezing, is likely due to the freeze injury of cells, leading eventually to the death of cells.

3.3.2. Other microbial Counts

The rate of decrease of count bacteria on Plate count agar (PCA) in the frozen yoghurt was also observed Table 7 especially in the occurrence of probiotic strain (formula 2,3) compared with the control (formula 1); the same observation was found in the three trials for occurrence of Coliforms on Violet Red Bile Glucose Agar (V. R); *Enterobacter* spp. on Violet Red Bile Glucose Agar (V.R); *Entercoccus* spp. *Streptococcus faecalis* Medium (S.F) and yeasts and molds Potato Dextrose Agar (PDA) contained chloramphenichole. According to PFA (2004) the acceptable value of coliform count in yoghurt should not be more than 10/ml. These organisms are killed during pasteurization and if they are present in the product they are the result of post pasteurization contamination. The low coliform organism indicates that there was no post processing contamination and proper care was taken during processing and this probiotic product could be making without having contamination and can be stored for extra period of time.

Table 7. Other microbial counts of frozen yoghurt samples

Time (day) / Trial	Total count (cfu/ml)				
	0	7	14	21	28
Total count					
Formula1	$5.5\times10^4\pm2.0$	$5.1\times10^4\pm0.02$	$4.3\times10^4\pm0.01$	$4.0\times10^4\pm1.0$	$3.3\times10^4\pm1.0$
Formula2	$3.9\times10^4\pm1.0$	$3.3\times10^4\pm2.0$	$2.5\times10^4\pm0.04$	$1.8\times10^4\pm2.0$	$1.5\times10^4\pm1.01$
Formula3	$4.2\times10^4\pm0.01$	$3.1\times10^4\pm1.01$	$2.2\times10^4\pm2.0$	$1.5\times10^4\pm2.0$	$1.2\times10^4\pm1.00$
Coliform					
Formula1	$7.5\times10^2\pm1.0$	$5.8\times10^2\pm2.0$	$3.9\times10^2\pm0.16$	$7.0\times10^2\pm1.0$	$7.0\times10^2\pm1.0$
Formula2	$3.9\times10^2\pm0.15$	$3.7\times10\pm1.0$	$2.9\times10\pm2.0$	$1.5\times10\pm0.13$	85 ± 2.0
Formula3	$3.7\times10^2\pm2.0$	$3.5\times10\pm0.12$	$1.9\times10\pm0.01$	$1.0\times10\pm2.0$	75 ± 0.15
Enterobacter spp.					
Formula1	$7.5\times10^3\pm1.0$	$5.8\times10^2\pm0.15$	$3.9\times10^2\pm2.0$	$7.0\times10\pm1.0$	$7.0\times10\pm1.0$
Formula2	$3.9\times10^3\pm0.23$	$3.9\times10^2\pm2.0$	$3.9\times10\pm0.03$	$3.9\times10\pm2.0$	$3.9\times10\pm0.02$
Formula3	$3.9\times10^3\pm2.0$	$3.9\times10^2\pm0.01$	$3.9\times10\pm2.0$	$3.9\times10\pm1.0$	$3.9\times10\pm0.05$
Enterococcus spp.					
Formula1	$7.5\times10^3\pm2.0$	$5.8\times10^3\pm0.01$	$3.9\times10^2\pm0.05$	$7.0\times10\pm1.0$	$7.0\times10\pm1.0$
Formula2	$3.9\times10^3\pm1.0$	$3.9\times10^2\pm0.03$	$3.9\times10\pm0.02$	$3.9\times10\pm0.02$	$3.9\times10\pm0.03$
Formula3	$3.9\times10^3\pm0.13$	$3.9\times10^2\pm0.02$	$3.9\times10\pm0.01$	$3.9\times10\pm2.0$	$3.9\times10\pm2.0$
Yeasts and Molds					
Formula1	$2.5\times10^2\pm2.0$	$1.8\times10^2\pm2.0$	$1.0\times10^1\pm3.0$	$1.0\times10^1\pm1.0$	95 ± 1.0
Formula2	$2.9\times10^2\pm1.0$	$2.3\times10^2\pm1.0$	$1.9\times10^1\pm0.01$	$0.8\times10^1\pm1.0$	55 ± 2.01
Formula3	$1.9\times10^2\pm0.03$	$1.5\times10^2\pm0.05$	$1.1\times10^1\pm0.04$	$0.5\times10^1\pm2.0$	40 ± 1.0

Data are presented as mean ± SD Formula 1 with no probiotics, Formula 2 containing 3%w/w of *Lactobacillus acidophilus* r2, Formula 3 containing 3%w/w of probiotic strain (*Lactobacillus acidophilus* r2); with 2% ω-3 with vitamin E.

Table 8. Sensory evaluation of probiotic frozen yoghurts

Properties	Mean scores		
	Formula1	Formula 2	Formula 3
Aroma	7.85±0.2	7.95±1.2	8.02±2.02
Color	7.78±0.1	7.93±0.2	7.95±0.1
Texture	7.46±1.02	7.75±1.2	7.95±0.2
Sourness	7.43±0.04	7.96±0.02	8.02±0.3
Sweetness	7.83±0.2	7.78±0.01	7.77±0.04
Meltability	7.87±1.02	7.76±0.2	7.90±1.02
Overall acceptability	7.45±0.2	7.98±1.05	8.01±1.2

Data are presented as mean ± SD Formula 1 with no probiotics, Formula 2 containing 3%w/w of *Lactobacillus acidophilus* r2, Formula 3 containing 3%w/w of probiotic strain (*Lactobacillus acidophilus* r2); with 2% ω-3 with vitamin E.

3.4. Sensory Evaluation

Activity of yoghurt culture and probiotic bacteria causes specific changes in the chemistry of the product that affect sensory characteristics of the product. Carbonyl compounds, such as lactic and acetic acids, acetaldehyde, acetone, and diacetyl result from fermentation of lactose and proteins and contributes to sensory attributes in frozen yoghurt (Chandan *et al*., 2006). Consumers have shown a great interest in frozen yoghurt because of its potential as a low fat replacement for ice cream and the perceived probiotic effects of the lactic acid bacteria used in its manufacture (Hughes and Hoover, 1991). Table 8 shows mean sensory scores of overall sensory evaluation of the 3 formulae of frozen yoghurts was carried out among 10 panelists, using 9-point Hedonic Scale Method. According to acceptability of each group, there was low difference in the mean scores of aroma, texture, sourness, sweetness, melting, and the overall acceptability among the 3 formulae. The overall aroma of all three mixes was acceptable. However, the aroma of mix 1 was rated 7.85±0.2 and for formula 2 & 3 were 7.95±1.2 &8.02±2.02, respectively. On the other hand, there was difference in the texture of the three formulas (7.46±1.02; 7.75±1.2 and 7.95±0.2, respectively). Overall scores for body and texture suggested that addition of omega 3 were better in textural quality. Overall scores for mixes 2 and 3 ranged from 7.98±1.05 to 8.01±1.2, respectively, which were equivalent to “very good” on the sensory scale. No serious body and texture defects were detected by the sensory panel in all formulas but formula 3 had higher scores. The colour of the frozen yoghurt was as natural milk colour and fresh appearance it is the same observation was detected by (Ranganadham and Gupta 1987).

In conclusion, addition of probiotic bacteria with omega-3 and vitamin E did not overlap with the overall acceptance of the frozen yoghurt.

4. Conclusions

There is growing consumer interest in food products that can provide health benefits. The frozen dairy desserts might be a good source for probiotic cultures, due to their composition and low storage temperatures. Frozen storage of the product has little or no effect on culture survival, and bacterial cultures remained at levels sufficient to offer the suggested therapeutic effects. Supplementation with probiotic bacteria has little effect on flavor or compositional characteristics of frozen yogurt. However, fermentation to a lower pH (5.6) does significantly increase the acid flavor of the product.

Our study showed the potential in the development of probiotic frozen yoghurt products which provide the advantage of viable probiotic in the products with omega-3 and vitamin E. The probiotic *L. acidophilus* r2 in addition to the traditional yoghurt cultures grew well in the frozen yoghurt and were stable to the freezing process, and survival remained high during 4weeks of storage at -18°C. The finished products contained high levels of protein and had good flavor and body characteristics. These additions can be adapted to larger production scales.

References

[1] Allgeyer L, Miller M, Lee S. (2010). Sensory and microbiological quality of yogurt drinks with prebiotics and probiotics. J. Dairy Sci. 93: 4471-4479.

[2] AOCS, (1985). Annual Meeting Technical Sessions on Lipids and Related Topics. Volume 20, Issue 1, pp 57-67.

[3] Bramley PM, Elmadfa I, Kafatos A, Kelly FJ, Manios Y, Roxborough HE, Schuch W, Sheehy PJA, Wagner KH. (2000). Review vitamin E. Journal of the Science of Food and Agriculture 80: 913-938.

[4] Burton GW, Foster DO, Perly B, Slater TF, Smith ICP, Ingold KU. (1985). Biological antioxidants. Phil Trans Royal Society (London) B 311: 565-578.

[5] Chandan RC, White CH, Kilara A and Hui YH. (2006). Manufacturing Yogurt and Fermented Milks. Blackwell Publishing, Ames, IA.

[6] Chandan, R. C., and K. M. Shahani. (1992). Pages 1-56 in Yogurt. Y. H. Hui, ed. Dairy Science and Technology Handbook. 2. Product Manufacturing. VCH Publishers Inc., New York.

[7] Dave, R., Shah, N. (1996). Viability of Yoghurt and Probiotic Bacteria in Yoghurts Made from Commercial Starter Cultures. Int. Dairy Journal. 7: 3141. Bramley A.J, Dodd F.H. (1984). Reviews of the progress of dairy science: mastitis control—progress and prospects. J Dairy Res 51: 481-512.

[8] FDA. (2008). Food Labeling Guide: Appendix B: Additional Requirements for Nutrient Content Claims. http://www.cfsan.fda.gov/~dms/2lg-xb.html Accessed Mar., 2011.

[9] Gilliland, S. E., and H. S. Kim. (1984). Effect of viable starter culture bacteria in yogurt on lactose utilization in humans. J. Dairy Sci. 67: 1-6.

[10] Gilliland, S.E., and Walker, D.K. (1989). Jour. Dairy Sci., Vol. 73, PP. 905-911.

[11] Hekmat, S. and Mcmahon, D.J. (1992). Survival of *Lactobacillus acidophilus* and *Bifidobacterium bifldum* in ice cream for use as a probiotic food. I. Dairy Sci. 75, 1415-1421.

[12] Holcomb, J. E., J. F. Frank, and J. U. McGregor. (1991). Viability of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in softserve frozen yogurt. Cult. Dairy Prod. J. 26: 4-5.

[13] Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley, J.T. and Williams, S.T. (1994). Bergey, s manual determinative of bacteriology (9th Ed.). Baltimore: Williams and Wilkins.

[14] Hughes, D.B. and Hoover, D.G. (1991). Bifidobacteria: Their potential use in American dairy products. Food Technol. 45 (4), 74-83.

[15] ISI: 2311 (1974) Determination of SNF in milk, Bureau of Indian Standard Institute, New Delhi. Pp 135-137.

[16] Jang S, Xu Z. (2009). Lipophilic and hydrophilic antioxidants and their antioxidant activities in purple rice bran. J. Agric. Food Chem. 57: 858-862.

[17] Jonsson H, Ström E and Roos S. (2004). Addition of mucin to the growth medium triggers mucus-binding activity in different strains of *Lactobacillus reuteri* in vitro. FEMS Microbiol. Lett., 204, 19-22.

[18] Lopez, M. C., L. M. Medina, and R. Jordano. (1998). Survival of lactic acid bacteria in commercial frozen yogurt. J. Food Sci. 63: 706-708.

[19] MacKenzie DA, Tailford LE, Hemmings AM and Juge N. (2009). Crystal structure of a mucus-binding protein repeat reveals an unexpected functional immunoglobulin binding activity. J. Biol. Chem., 284, 32444-32453.

[20] Mahrous Hoda, Khalil El-Halafawy, Morsi A E El-Soda. (2010). Functionalities of Lactic Acid Bacteria Isolated from Egyptian Environment. Springer Heidelberg, Germany. VDM Verlag Dr. Müller E.K. ISBN 978-3-639-23871-6, paperback, 192 Pages.

[21] Marshall R T, Goff, H D and Hartel, R W. (2003). Ice Cream. 1st Ed.; Aspen Publishers, New York.

[22] Martin, F., R. Cachon, K. Pernin, J. De Coninck, P. Gevais, E. Guichard and N. Cayott. 2011. Effect of oxidoreduction potential on aroma biosynthesis by lactic bacteria in nonfat yogurt. J. Dairy Sci. 94: 614-622.

[23] Minellia EB, A Beninia, M Marzottob, A Sbarbat ic, ORuzzenented, R Ferrarioe, H Hendriksf and FDellaglio. (2004). International Dairy J. 14: 723-736.

[24] Modler, H.W., & Villa-Garcia, L. 1993. The growth of Bifidobacterium longum in a whey based medium and viability of this organism in frozen yoghurt with low and high levels of developed acidity. Cultured Dairy Products J. 28 (1), 4-8.

[25] NASS. (2012). Deaths: Final data for 2008. National Vital Statistics Report. 59 (10): 1-126.

[26] Nielsen NS, Debnath D, Jacobsen C. (2007). Oxidative stability of fish oil enriched drinking yoghurt. Int. Dairy J. 17 (12): 1478-1485.

[27] Ouwehand, A.C., Kirjavainen, P.V., Shortt, C., and Salminen, S. (1999). Probiotics: Mechanisms and established effects. Int. Dairy J. 9, 43-52.

[28] Pennacchia, C., Ercolini, D., Blaiotta, G., Pepme, O., Mauriello, G., Villani, F. (2004).Jour. Meat Science, Barking. Vol. 67, PP. 309-317.

[29] Ranganadham, M. and Gupta, S.K. (1987). Indian Dairyman, 39 (10): 493.

[30] Rezaei, R., Khomeiri, M., Kashaninejad., Alami, M. (2011). Effects of guar gum and arabic gum on the physicochemical, sensory and flow behavior characteristics of frozen yoghurt. International Journal of Dairy Technology. 64 (4): 563-568.

[31] Robinson RK, Tamime AY, Chubb LW. (1977). Acetaldehyde as an indicator of flavour intensity in yoghurt. Milk Ind., 79: 4-6.

[32] Sabliov C, Fronczek C, Astete E, Khachaturyan M, Khachatryan L, Leonardi C. (2009). Effects of temperature and UV light on degradation of α-tocopherol in free and dissolved form. J. Am. Oil Chem. Soc. 86: 895-902.

[33] Salwa A.A., Galal E.A. and Neimat A.E. (2001). Carrot yoghurt: Sensory, Chemical, Microbiological properties and consumer acceptance. Pakistan J. of Nutri. 3 (6): 322-330.

[34] Schmidt E, Varming K, Pedersen J, Lervang H, Grunnet N, Jersild C. (1992). Long-term supplementation with n-3 fatty acids. 2. Effect on neutrophil and monocyte chemotaxis. Scandinavian Journal of Clinical & Laboratory Investigation 52: 229-236.

[35] Shin J.G., Lee J.J., Kim H.Y. and Beak Y.J. (1991). Studies on the changes in quality and sensory evaluation of stirred yoghurt stored at different temperatures. Korean J. Of Dairy Sci. 13 (12): 148-155.

[36] Tamime AY, Robinson RK. (2007). Background, standards and marketing of frozen yogurt: Yoghurt: Science and Technology (ed. AY Tamime and RK Robinson). 3nd ed. CRC Press, Boca Raton, FL. p. 392-393.

[37] Tamime AY, Robinson RK.(1999). Historical Background In: Yoghurt: Science and Technology (ed. AY Tamime and RK Robinson). 2nd ed. CRC Press, Boca Raton, FL. p. 7-8.

[38] Tamime AY. (2002). Fermented milks: a historical food with modern applications-a review. European Journal of Clinical Nutrition 56: 2-15.

[39] Tamime, A.Y. and Robinson, R.K. (1985). Yogurt Science and Technology. Pergamon Press Ltd., Oxford.

[40] Thompson, L. D., and A. N. Mistry. (1994). Compositional changes in frozen yogurt during fermentation, frozen storage and soft serve freezing. Cult. Dairy Prod. J. 29: 12-16.

[41] Traber MG. 1999. Utilization of Vitamin E. Biofactors, 10: 115-120.

[42] Vasiljevic, T. and Shah, N.P. (2008) Probiotics-From Metchnikoff to bioactives. International Dairy Journal 18: 714-728.

[43] Vesterlund S, Paltta J, Karp M and Ouwehand AC. (2005). Measurement of bacterial adhesion-in vitro evaluation of different methods. J. Int. microbial methods, 60: 225-233.

[44] Walencka E, Roazlska S, Sadowska B, Rozalska B. (2008). Folia microbiologica, 53: 61-66.

[45] Westerbeek, J.M.M. (1995). Fermented ice cream products. A healthy challenge to the ice cream industry. Scandinavian Dairy Information. 4: 29-31.