Journal of Food and Dairy Sciences

Journal homepage: www.jfds.mans.edu.eg
Available online at: www. jfds.journals.ekb.eg

Maintaining the Viability of Probiotic *Lactobacillus casei* 01 as Affected with different Making Techniques of Ice Milk

Fatma A. M. Ramadan; M. A. Azzam; Ashwak A. Hassan and Nagwa M. Abdl-Hameed*

Dairy Science and Technology Dept. Faculty of Agric. Cairo Univ.

Cross Mark

ABSTRACT

The purpose of this study was to determine the effect of using three making techniques on the survival rate of *Lactobacillus casei* 01 in ice milk containing three different levels of sugar (13, 15 and 17%). To meet this objective, the first technique was done by inoculating of ice milk mix with *L.c* 01culture directly and without fermentation. A culture of *Lactobacillus casei* (*L.c* 01) grown in boiled skim milk at 37°C. once, and at 30°C. again, and until pH of 4.5 and 5.5 reached, respectively. The fermented milk was added to the ice milk mix (50% w/w.). Microbial counts, pH, viscosity of the ice milk mixes and the functional and sensorial properties of the final products were evaluated. When *L.c* 01 was added to ice milk mixes using the second (F_{37}) and third (F_{30}) techniques, the pH value decreased and the viscosity increased in the mixes. No effect on the overrun or melting rate of the final product was observed. The fermentation techniques (F_{37} and F_{30}) could significantly increase the viability of *L.c* 01 in ice milk by extending the storage period. F_{30}–ice milks had the highest sensory attributes.

Keywords: *Lactobacillus casei* 01, ice milk, probiotic, fermentation, viability

INTRODUCTION

The market for probiotic food products has grown rapidly in recent years due to nutritional value and health-promoting properties of these products. The main aim of probiotic food products is to introduce beneficial microorganisms "probiotics" into the human through his daily dietary intake. The therapeutic value of this microorganism normally depends on their quantity and viability in the food, addition to their ability to tolerance the acidic conditions in the stomach and bile in the small intestine. (McBrearty *et al.* 2001,Casarotti and Penna 2015; Dello Staffolo *et al.* 2004, Doleyres *et al.*, 2004 and Sultana *et al.* 2000). Probiotic bacteria were also widely used in preparing cheese (McBrearty *et al.* 2001), fermented milk (Sanchez *et al.* 2009), and ice cream (Cruz *et al.* 2009; Di Criscio *et al.* 2010; Mohammadi *et al.* 2011; Arslan *et al.* 2016).

Because of its neutral pH and high total solids level which provides protection for the probiotic bacteria, ice cream is ideal vehicle to transfer probiotics into the human intestinal tract but the harsh conditions of ice cream formulation and manufacturing may reversely alter the probiotic survival (Nousia *et al.* 2011; Mohammadi *et al.* 2011, A kin 2005; Fávaro- Trindade *et al.* 2006). Using probiotic culture in making ice cream mix before freezing process and without fermentation was examined by Alamprese *et al.* 2002; Magariņ̃os *et al.* 2007; Homayouni *et al.* 2008; Abghari *et al.* 2011; Gheisari *et al.* 2016). Addition of fermented milk to ice cream mix was also tested by Christiansen *et al.* 1996; Hagen and Narvhus 1999; Salem *et al.* 2005; Mohammadi *et al.* 2011; Nousia *et al.* 2011; Arslan *et al.* 2016). for maintaining a minimum of probiotic bacterial cells which should be alive at the time of consumption per gram of product (10^7cfu/g) as suggested by international dairy federation (Hekmat and McMahon, 1997).

Therefore, in this study, some of these techniques were used to produce ice milk with high number of *Lactobacillus casei* (*Lc*-01) cells can resist processing conditions. Thus, the survival of *Lc*-01 during mix ice milk aging and freezing and during ice milk storage for 60 days at - 18°C was evaluated. Moreover, the influence of added *Lc*-01 on physical and sensorial characteristics of resulting ice milk was studied.

MATERIALS AND METHODS

Fresh raw buffalo's milk (15.7% TS & 6.7% Fat) was obtained from the herd of the Faculty of Agriculture Cairo University, Egypt. Skim milk (9.74% TS & 0.1% Fat) and cream (45.79% TS & 40% Fat) were obtained by separating raw buffalo's milk using cream separator (Alfa-Laval 102 separator, Alfa-Laval, Stockholm, Sweden). Low heat skimmed milk powder (96.2% TS & 0.8% Fat) made in USA, cane sugar (sucrose), raw vanillin and gelatin panels were purchased from the local market.

Freeze-dried commercial culture (DVS) of probiotic *Lactobacillus casei* (*Lc*-01) (Christian Hansen, Hoersholm, Denmark), was employed (2.5%) to maintain a minimum of 10^7 cfu/g milk. in making ice milk

Making of ice milk

According to the Egyptian standards of ice milk (2005), twelve ice milk mixes (4 kg each) were standardized to contain 4% fat, 11% MSNF, 13, 15 or 17% sugar and 0.7% gelatin (Table 1). All mixes were heated at 80°C for 5 min and then cooled to 4°C. These mixes were divided into four groups.

* **Corresponding author.**
E-mail address: nagwa2011@yahoo.com
DOI: 10.21608/jfds.2020.80839

Table 1. formulations of different ice milk mixes

Ingredients (kg)	Mixes[1]		
	M_{13}	M_{15}	M_{17}
Fresh skim milk	2.919	2.830	2.742
Fresh cream	0.390	0.390	0.390
Skim milk powder	0.143	0.152	0.160
Sugar	0.520	0.600	0.680
Gelatin	0.028	0.028	0.028
Total weight (kg)	4.000	4.000	4.000

[1] M_{13}: ice milk mix with13%sugar,M_{15}: ice milk mix with 15%sugar and M_{17}:ice milk mix with 17% sugar

Each group consists of three mixes containing 13, 15 and 17% sugar respectively. The first group mixes ($NF_{13,\ 15,\ 17}$) were directly inoculated with *Lactobacillus casei* (*Lc*-01) prior to aging and without fermentation. Half the amount of skim milk used in the preparation of the second and third groups was fermented with *Lactobacillus casei* (*Lc*-01) at 37 °C for the second group ($F_{37/13,\ 37/15,\ 37/17}$) and at 30°C for the third group ($F_{30/13,\ 30/15,\ 30/17}$) until a pH of 4.5 and 5.5 was reached, respectively. The coagulated milk was then cooled to 4°C, and added to the remaining ice milk mixes prior to aging. Mixes of the fourth group ($CO_{13,\ 15,\ 17}$) were prepared without addition of *Lactobacillus casei* (*Lc*-01) and applied as a control. After ageing for 18 h at 4°C, all mixes were flavored with 0.03% vanillin, frozen and whipped in a vertical batch freezer (STARMATIC V 500, Italy) at -2.5°C for 15 min. Afterwards, the ice milks were collected, packaged in 50 ml high density polyethylene cups and stored at – 18°C for 60 days (Fig. 1). All experiments were conducted in triplicate.

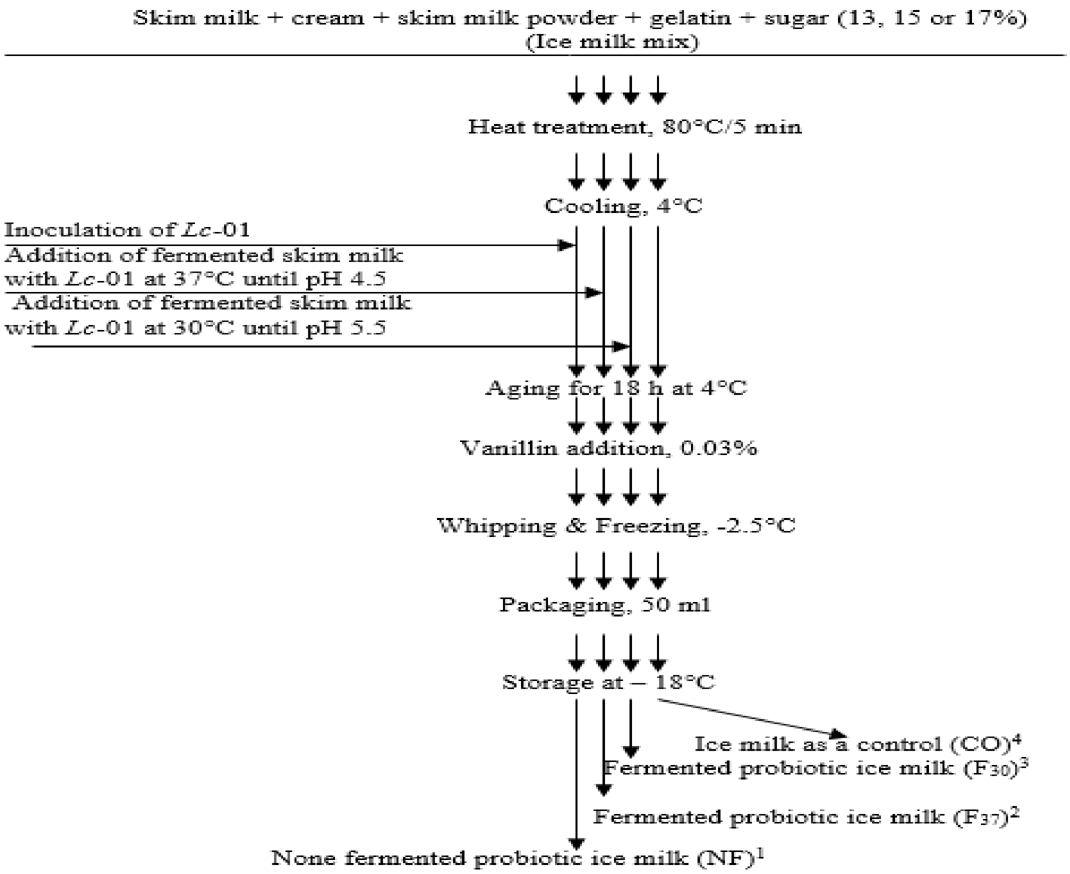

Fig.1. Three different techniques for the production of probiotic ice milks. [1] technique 1: ice milk mixes were directly inoculated with *Lc*-01 prior to aging and without fermentation (NF), [2] technique 2: 50% of total skim milk used in the preparation of ice milk mixes was fermented with *Lc*-01at 37 °C until a pH of 4.5 was reached; the coagulated milk was cooled to 4°C, and then added to the remaining ice milk mixes prior to aging (F_{37}). [3] technique 3: 50% of total skim milk used in the preparation of ice milk mixes was fermented with *Lc*-01at 30 °C until a pH of 5.5 was reached; the coagulated milk was cooled to 4°C, and then added to the remaining ice milk mixes prior to aging (F_{30}). [4] CO: ice milk mixes were prepared without addition of Lc-01 and applied as a control.

The dry matter content (%TS) and fat content (%)of raw buffalo's milk, skim milk and cream used in preparing the examined ice milks were determined using gravimetric and Gerber methods respectively, according to the AOAC (1990) Standard Procedures.

pH values of the coagulated milks and Ice milk mixes were measured using a pH meter (Jenway model, USA). The specific gravity and weight per gallon (Lb) of ice milk mixes and ice milk samples were determined as described by Winton (1958) and Burke (1947), respectively. After ageing, all mixes were analyzed for viscosity using a Brookfield viscometer (Brookfield DV-III, Brookfield Engineering Laboratories, Inc., USA). The viscometer was operated at 40 rpm (spindle number 2).

Each result in triplicate was recorded in cP (centi poise) after 1 min rotationIce milk overrun was determined using the following equation (Marshall *et al.*, 2003):

Overrun % = 100 x (weight per gallon of ice milk mix – weight per gallon of ice milk) x (weight per gallon of ice milk)$^{-1}$

Melting rate of ice milk samples was measured according to Güven and Karaca (2002). 35 g of ice milk was placed on the screen of stainless steel (mesh size 2 mm) under which a conical flask was placed to collect ice milk melted at room temperature, 25 ± 1°C. The timing of the melting rate began when the first drop of the melt touched the bottom of the flask. The weight of the ice milk melted was recorded every 15 min for 45 min. All measurements were done in triplicates. The viability of *Lactobacillus casei* (*Lc*-01) in ice milk mixes was estimated after 0, 4 and 18 h of aging, when fresh at zero time and after 5, 10 and 15 mins of whipping and freezing at -2.5°C. Ice milk samples were also tested after 0, 15, 30 and 60 days of freezing storage at -18°C. Ten grams of sample were diluted in 100 ml of sterile peptone water and 1 ml aliquot dilutions were poured onto plates of Deman, Rogosa, Sharpe agar medium containing 2 g/l Lithium chloride and 3 g/l sodium propionate (LP-MRS agar) in triplicate. Enumeration of *Lc*-01 was done as described by Lima *et al.*, (2009). All cultured plates of *Lc*-01 were incubated aerobically at 37°C for 72 h. The results were expressed as colony-forming units per gram of sample (cfu/g). The ice milk samples were organoleptically assessed by 12 staff from Dairy Department in Faculty of Agriculture at Cairo University in Egypt, after 1and 60 days of freezing storage at -18°C using a sensory rating scale of 1-10 for flavor & taste, 1-5 for body & texture and 1-5 for color & appearance, as described by Homayouni *et al.*, (2006). The properties evaluated included: (a) eight attributes for flavor and taste (no criticism: 10, cooked flavor: 9-7, lack of sweetness and too sweet: 9-7, lack of flavor: 9-6, yogurt/probiotic flavor: 8-6, acidic/sour: 8-6, rancid and oxidized: 6-1, and other: 5-1), (b) seven characteristics of body and texture (no criticism: 5, crumbly: 4-2, coarse: 4-1, weak: 4-1, gummy: 4-1, fluffy: 3-1, sandy: 2-1) and (c) four terms describing color and appearance (no criticism: 5, pale color: 4-1, non-uniform color: 4-1, unnatural color: 3-1). Total score of flavor & taste, body & texture, and color & appearance was defined as total acceptability. The samples stored at -18°C were tempered at room temperature for 5 min prior to sensory evaluation. Physical, bacteriological and sensory analyses were carried out one day after production. The data were statistically analyzed using ANOVA and treatment means were compared by using Duncan's Multiple Range test to determine the effects of treatments when the F-test was statistically significant at $P<0.05$ (Steel *et al.*, 1997). Skim milk + cream + skim milk powder + gelatin + sugar (13, 15 or 17%) (Ice milk mix)

RESULTS AND DISCUSSION

Physical properties of ice milk mixes:

pH values, specific gravity, weight per gallon and viscosity values of both probiotic ice milk mixes (NF, F_{37} and F_{30} groups) and ice milk mix as control (CO group) are given in Table 2. The pH, weight per gallon and viscosity values of all ice milk mixes ranged between 6.3 and 5.2, 10.25 and 11.24 Lb and between 104.7 and 139.9 cP, respectively.

The freeze-dried *Lactobacillus casei* (*Lc*-01) culture being added directly to ice milk mixes without fermentation was of no significant effect on the pH, weight per gallon and viscosity values of NF group, compared with CO group.

The low pH values of F_{37} and F_{30} groups of 5.2 and 5.9, respectively, are due to the metabolic activity of *Lc*-01 during the fermentation step. Addition of sugar to ice milk mix increased the weight per gallon of this mix significantly, which might be attributed to the increase in the dry matter content of the mix. Also, the added concentration of to ice milk mix and the fermentation technique increased the viscosity of the mix. Therefore, the highest viscosity was related to $F_{37/17}$ ice milk mix, followed by the $F_{30/17}$ mix (139.9 and 135.3 cP, respectively), while, NF_{17} and CO_{17} mixes achieved viscosity ranged between 127.8 and 126.7 cP, respectively (Table 2). This high viscosity might be due to the microbial production of exo-polysaccharides during fermentation (Patel *et al.* 2010). Also, during fermentation casein micelles begin to aggregate and form a complex with calcium that contributes to the increase in viscosity (Ordonez *et al.* 2000).

Physical properties of probiotic ice milk samples:

Data illustrated in Table 3 revealed that the highest weight per gallon was related to all ice milk samples made with 17 % sugar. When sugar and fermented skim milk with *Lactobacillus casei* (*Lc*-01) were added in the formulation, no significant difference was verified for overrun of F_{37} and F_{30} groups, compared with NF and CO groups. Overrun ranged between 28.65 and 31.99 % (Table 3). Similar results were found in probiotic ice cream made with *Lactobacillus rhamnosus* GG and 15 or 22 % sugar by Alamprese *et al.* (2005).

Table 2. physical properties of aged probiotic ice milk mixes[1] made with different sugar concentrations

Aged mixes[1]	pH	Specific gravity	Weight per gallon (Lb)	Viscosity (cP)
CO group				
CO_{13}	6.3^{a}	1.2293	10.26^{c}	105.33^{g}
CO_{15}	6.3^{a}	1.2798	10.68^{b}	113.00^{ef}
CO_{17}	6.3^{a}	1.3356	11.15^{a}	126.67^{c}
NF group				
NF_{13}	6.3^{a}	1.2294	10.26^{c}	104.67^{g}
NF_{15}	6.3^{a}	1.2737	10.63^{b}	113.87^{e}
NF_{17}	6.3^{a}	1.3375	11.16^{a}	127.80^{c}
F_{37} group				
$F_{37/13}$	5.2^{c}	1.2289	10.25^{c}	113.19^{ef}
$F_{37/15}$	5.2^{c}	1.2718	10.61^{b}	122.78^{d}
$F_{37/17}$	5.2^{c}	1.3365	11.15^{a}	139.88^{a}
F_{30} group				
$F_{30/13}$	5.9^{b}	1.2330	10.29^{c}	110.18^{f}
$F_{30/15}$	5.9^{b}	1.2635	10.54^{b}	120.32^{d}
$F_{30/17}$	5.9^{b}	1.3463	11.24^{a}	135.30^{b}
LSD	0.1444		0.1633	3.671

[1] See Table 1 and Fig. 1

[a,b,c,…g] Means in the same column without a common subscript are significantly different (P < 0.05)

Melting behavior of ice milk samples is not changed by the addition of *Lc*-01 strain, compared to the control. Sugar content of ice milk sample negatively affected its melting rate. All ice milk samples made with

17 % sugar characterized with faster melting than the other samples, which made with 15 or 13 % sugar (Table 3). These results matched those reported by Akin *et al.* (2007).

Table 3. physical properties of probiotic ice milk samples[1]

Ice milk samples[1]	Specific gravity	Weight per gallon (Lb)	Overrun (%)	Melted ice milk (%)[2]
CO group				
CO_{13}	0.9571	7.99^{d}	28.65^{a}	87.97^{b}
CO_{15}	0.9776	8.16^{bcd}	30.93^{a}	91.37^{a}
CO_{17}	1.0165	8.48^{ab}	31.43^{a}	91.77^{a}
NF group				
NF_{13}	0.9523	7.95^{d}	29.20^{a}	88.88^{b}
NF_{15}	0.9765	8.15^{cd}	30.44^{a}	91.44^{a}
NF_{17}	1.0188	8.50^{a}	31.30^{a}	91.50^{a}
F_{37} group				
$F_{37/13}$	0.9401	7.85^{d}	30.75^{a}	88.11^{b}
$F_{37/15}$	0.9696	8.09^{d}	31.19^{a}	88.57^{b}
$F_{37/17}$	1.0141	8.46^{abc}	31.84^{a}	91.12^{a}
F_{30} group				
$F_{30/13}$	0.9424	7.86^{d}	30.87^{a}	87.95^{b}
$F_{30/15}$	0.9640	8.05^{d}	31.14^{a}	88.72^{b}
$F_{30/17}$	1.0206	8.52^{a}	31.99^{a}	90.89^{a}
LSD		0.3285	4.7486	1.8771

[1] See Table 1 and Fig. 1 [2] Melted ice milk after 45 min

[a,b,c,d] Means in the same column without a common subscript are significantly different ($P < 0.05$)

The effect of making stages on the viability of *Lactobacillus casei* 01 in ice milk:

The viable counts of *Lactobacillus casei* (*Lc*-01) during aging period are shown in Table 4. Significant decrease in the viable counts was detected after 18 h aging at 4°C in all of the experimented groups. The lowest of viable counts of *Lc*-01 of 1.06×10^{7} cfu/g, were observed in NF group, compared with $24.5\times 10^{7}$and 19.0×10^{7} cfu/g, in F_{37} and F_{30} groups, and $24.5\times 10^{7}$and 19.0×10^{7} cfu/g, respectively, during aging period. This might be due to the resistance of Lactobacilli cells towards the concentration of sugar and the cooling temperature provided by the fermentation technique that may provide resistance to Lactobacilli cells against the concentration of sugar (Streit *et al.* 2008; Mohammadi *et al.* 2011; Arslan *et al.* 2016). The fermentation technique also resulted in an increase in an average of initial number of 25.0×10^{7} cfu/g and 19.1×10^{7} cfu/g, respectively, in *Lc*-01 in both F_{37} and F_{30} group, compared to NF group (1.75×10^{7} cfu/g). However, the initial number of *Lc*-01 in F_{37} group was higher than that in F_{30} group. This could be attributed to the incubation temperature. Generally, the highest viable counts of *Lc*-01 were related to $F_{37/15}$ and $F_{37/17}$ ice milk mixes followed by the $F_{37/13}$ then $F_{30/15}$ mix (Table 4).

Table 4. viable counts of *Lactobacillus casei* 01($\times 10^{7}$ cfu/g) in ice milk mixes[1] made with different sugar concentrations during 18 h aging at 4°C

Aging period (h)	Ice milk mixes (3 groups)[1]									
	NF (group 1)			F_{37} (group 2)			F_{30} (group 3)			
	ϒ NF_{13}	NF_{15}	NF_{17}	$F_{37/13}$	$F_{37/15}$	$F_{37/17}$	$F_{30/13}$	$F_{30/15}$	$F_{30/17}$ ϒ	Mean[2]
0	1.77^{j}	1.69^{jk}	1.80^{j}	23.9^{c}	25.4^{b}	25.7^{ab}	17.7^{i}	20.4^{fg}	19.2^{gh}	15.3^{a}
4	1.10^{jkl}	0.88^{jkl}	0.57^{jkl}	25.5^{ab}	26.7^{a}	26.4^{ab}	17.7^{i}	20.4^{fg}	18.3^{hi}	15.3^{a}
18	0.87^{jkl}	0.51^{kl}	0.33^{l}	21.8^{de}	22.6^{d}	22.1^{d}	18.8^{hi}	20.6^{ef}	18.1^{hi}	14.0^{b}
Mean[3]	1.26^{E}	1.02^{E}	0.90^{E}	23.8^{B}	24.9^{A}	24.7^{A}	18.1^{D}	20.5^{C}	18.5^{D}	
Mean[4]		1.06^{C}			24.5^{A}			19.0^{B}		

[1] See Table 1 and Fig. 1 [2] Time: LSD ($P < 0.05$) = 0.43 [3] Group × Sample: LSD ($P < 0.05$) = 0.744

[4] Group: LSD ($P < 0.05$) = 0.43 ▫ Group × Sample × Time: LSD ($P < 0.05$) = 1.288

[A,B,C,D,E] Means on the same line without a common subscript are significantly different ($P < 0.05$)

[a,b,c ,…..l] Means in the same column without a common subscript are significantly different ($P < 0.05$)

Effect of freezing

The viable counts of *Lactobacillus casei* (*Lc*-01) in 3 groups of ice milk mixes during different freezing periods are shown in Table 5. Comparison of the viable counts after 5, 10 and 15 mins revealed that the freezing period was of slightly effect on the viability of Lactobacilli cells in the examined groups. The viable counts of *L.c* 01 in NF, F_{37} and F_{30} groups after 15 min freezing at -2.5°C reduced by 45.6, 2.12 and 2.3%, respectively. The lowest decrease in the viable counts of *Lc*-01 in F_{37} and F_{30} groups due to pre-adaptation of Lactobacilli cells during fermentation against the freezing temperature, in addition to sugar in ice milk mixes increased a probiotic's viability through acting as cryoprotectant (Champagne and Rastall 2009). Similar results were obtained by Alamprese *et al.* (2005), who indicated that the ice cream freezing process (with a mix containing 10% fat and without fermentation) caused a reduction by 33% of the initial count of *Lactobacillus rhamnosus* GG. In general, this decrease is due to the mechanical stresses of whipping and freezing processes caused by the formation of ice crystals and by scraping of the cylinder wall by blade of freezer(Gill 2006; Akin *et al.* 2007; Akalin and Erişir 2008; Homayouni *et al.* 2008; Mohammadi *et al.* 2011). With regard to the individual samples during freezing period, it was observed that the highest viable counts of *Lc*-01 were obtained in $F_{37/15}$ and $F_{37/17,}$ followed by the $F_{37/13}$ then $F_{30/15}$(Table 5).

Table 5. viable count of *Lactobacillus casei* 01 ($\times 10^{7}$ cfu/g) in ice milk mixes[1] made with different sugar concentrations during 15 min freezing at – 2.5°C

Freezing period (min)	Ice milk mixes (3 groups)[1]									
	NF (group 1)			F_{37} (group 2)			F_{30} (group 3)			
	NF_{13}	NF_{15}	NF_{17}	$F_{37/13}$	$F_{37/15}$	$F_{37/17}$	$F_{30/13}$	$F_{30/15}$	$F_{30/17}$	Mean[2]
0	0.87^{h}	0.51^{h}	0.33^{h}	21.8^{ab}	22.6^{a}	22.1^{ab}	18.8^{g}	20.6^{cdef}	18.1^{g}	14.0^{a}
5	0.70^{h}	0.40^{h}	0.24^{h}	21.4^{bcd}	22.3^{ab}	21.9^{ab}	18.5^{g}	20.3^{def}	17.9^{g}	13.7^{a}
10	0.64^{h}	0.35^{h}	0.21^{h}	21.3^{bcde}	22.2^{ab}	21.8^{ab}	18.4^{g}	20.2^{ef}	17.8^{g}	13.6^{ab}
15	0.51^{h}	0.26^{h}	0.16^{h}	21.3^{bcde}	22.1^{ab}	21.7^{abc}	18.3^{g}	20.1^{f}	17.8^{g}	13.5^{b}
Mean[3]	0.68^{F}	0.38^{F}	0.23^{F}	21.5^{B}	22.3^{A}	21.9^{AB}	18.5^{D}	20.3^{C}	17.9^{E}	
Mean[4]		0.43^{C}			21.9^{A}			18.9^{B}		

[1] See Table 1 and Fig. 1 [2] Time: LSD ($P < 0.05$) = 0.374 [3] Group × Sample: LSD ($P < 0.05$) = 0.5602 [4] Group: LSD ($P < 0.05$) = 0.324

▫ Group × Sample × Time: LSD ($P < 0.05$) = 1.121

[A,B,C,D,E,F] Means on the same line without a common subscript are significantly different ($P < 0.05$)

[a,b,c ,…..h] Means in the same column without a common subscript are significantly different ($P < 0.05$)

Effect of storage:

Results in Table 6 revealed a significant decrease in the obtained viable counts of *Lactobacillus casei* (*Lc*-01) in the three groups (NF, F_{37} and F_{30}) during 60 days of freezing storage at - 18°C., which might be due to the continuous effect of the freezing temperature on the viability of *Lc*-01. However, the viable counts of *Lc*-01 in NF group were the lowest (0.29×10^7 cfu/g), compared with F_{37} and F_{30} groups (20.6×10^7 and 18.3×10^7 cfu/g, respectively) during storage. It was also observed that $F_{37/15}$ and $F_{37/17}$ samples contained the highest numbers of viable cells of 21.0×10^7 and 20.9×10^7 cfu/g, respectively, while $NF_{17,15,13}$ samples were of the lowest numbers of 0.15, 0.25 and 0.47×10^7 cfu/g, respectively. Viable counts of *Lc*-01 in all samples were higher than that of the recommended minimum limit of 7 log cfu/g to have the beneficial effects on the consumer's health (Hekmat and McMahon, 1997). Similar results were also obtained by Alamprese *et al.* 2005, Akin *et al.* 2007 and Gheisari *et al.* 2016). Gheisari *et al.* (2016), who reported that the viable counts of *Lactobacillus casei* in stored ice cream sample (8% fat and 15% sugar) at -18°C for 60 days were 16.9×10^7 cfu/g. Survival rate (%) of *Lactobacillus casei* 01 In ice milk samples.

Results in Table 7 reveal the survival rate of the examined *Lactobacillus casei* (*Lc*-01)' during aging, freezing and storage for 60 days at -18°C. Survival rate of *Lc*-01 in F_{30} –ice milks was the highest, followed by F_{37} – ice milks.

This result was consistent with that reported by Arslan *et al.* (2016), who observed that the inoculation of a 0.9% fat ice cream mixture with 10% fermented milk with *Lactobacillus acidophilus* ATCC 4356.

Table 6. viable count of *Lactobacillus casei* 01 ($\times 10^7$ cfu/g) in ice milk samples during 60 days storage at – 18.0°C

Storage period (day)	Ice milk sampes (3 groups)[1] NF (group 1)			F_{37} (group 2)			F_{30} (group 3)			
	NF_{13}	NF_{15}	NF_{17}	$F_{37/13}$	$F_{37/15}$	$F_{37/17}$	$F_{30/13}$	$F_{30/15}$	$F_{30/17}$	**Mean[2]**
0	0.51^{k}	0.27^{k}	0.16^{k}	21.3^{abc}	22.1^{a}	21.7^{ab}	18.3^{hi}	20.2^{def}	17.8^{ij}	13.6^{a}
15	0.48^{k}	0.25^{k}	0.15^{k}	19.6^{efg}	20.7^{bcd}	21.0^{bcd}	18.3^{hi}	19.2^{fgh}	17.8^{ij}	13.1^{b}
30	0.45^{k}	0.24^{k}	0.15^{k}	19.6^{efg}	20.6^{cde}	20.6^{cde}	18.8^{ghi}	18.8^{ghi}	17.8^{ij}	13.0^{bc}
60	0.42^{k}	0.23^{k}	0.15^{k}	19.2^{fgh}	20.6^{cde}	20.5^{cde}	17.1^{j}	18.3^{hi}	17.0^{j}	12.7^{c}
Mean[3]	0.47^{E}	0.25^{E}	0.15^{E}	19.9^{B}	21.0^{A}	20.9^{A}	18.1^{D}	19.1^{C}	17.6^{D}	
Mean[4]		0.29^{C}			20.6^{A}			18.3^{B}		

[1] See Table 1 and Fig. 1 [2] Time: LSD ($P < 0.05$) = 0.36 [3] Group × Sample: LSD ($P < 0.05$) = 0.54 [4] Group: LSD ($P < 0.05$) = 0.31
□ Group × Sample × Time: LSD ($P < 0.05$) = 1.08
[A,B,C,D,E] Means on the same line without a common subscript are significantly different ($P < 0.05$)
[a,b,c,…..k] Means in the same column without a common subscript are significantly different ($P < 0.05$)

Table 7 survival rate (%) of *Lactobacillus casei* 01 in ice milk samples after 60 days storage at -18°C

Ice milk samples[1]	The initial number of *Lc*-01 (cfu/g)[2]	The final number of *Lc*-01 (cfu/g)[3]	Survival rate (%)
NF group			
NF_{13}		0.42×10^7	24.0
NF_{15}	1.75×10^7	0.23×10^7	13.1
NF_{17}		0.15×10^7	8.6
Average		0.27×10^7	15.2
F_{37} group			
$F_{37/13}$		19.2×10^7	76.8
$F_{37/15}$	25.0×10^7	20.6×10^7	82.4
$F_{37/17}$		20.5×10^7	82.0
Average		20.1×10^7	80.4
F_{30} group			
$F_{30/13}$		17.1×10^7	89.5
$F_{30/15}$	19.1×10^7	18.3×10^7	95.8
$F_{30/17}$		17.0×10^7	89.0
Average		17.5×10^7	91.6

[1] See Table 1 and Fig. 1 [2] See Table 4 [3] See Table 6

Table 8 shows the sensory scores of probiotic ice milk samples at 1 and 60 days of storage at -18°C. The obtained results showed that the inoculation of ice milk mixtures with fermented skim milk with *Lactobacillus casei* 01 at 37°C had significant effect on ice milk flavor only. Therefore, the probiotic taste was found to be particularly noticeable in all F_{37} – ice milk samples, whether fresh or stored. This could be due to the lower pH value for F_{37} – ice milk mixes (Table 2). For example, $F_{37/17}$– ice milk was the lowest preferred sample and obtained the lowest score especially in flavor attribute. Also, sweetened taste was found to be noticeable in all ice milk samples with 17% sugar. Thus, the high concentration of sugar affected the acceptability of these samples. $F_{30/15}$–ice milk sample had the highest acceptable and gave a good total impression with no marked off-flavor during the storage period despite its very light probiotic taste. None of the ice milks were judged to be crumbly, weak, fluffy or sandy.

Sensory evaluation

Table 8. Organoleptic properties of probiotic ice milk samples[1] at days 1 and 60 of storage at – 18°C

Storage period (day)	Organoleptic properties	Ice milk samples[1] CO			NF			F_{37}			F_{30}		
		CO_{13}	**CO_{15}**	**CO_{17}**	**NF_{13}**	**NF_{15}**	**NF_{17}**	**$F_{37/13}$**	**$F_{37/15}$**	**$F_{37/17}$**	**$F_{30/13}$**	**$F_{30/15}$**	**$F_{30/17}$**
	Color and appearance#	5.00^{a}	5.00^{a}	5.00^{a}	5.00^{a}	5.00^{a}	5.00^{a}	5.00^{a}	5.00^{a}	5.00^{a}	5.00^{a}	5.00^{a}	5.00^{a}
1	Body and texture*	3.83^{a}	3.87^{a}	4.00^{a}	3.67^{a}	3.67^{a}	4.00^{a}	4.00^{a}	4.00^{a}	4.00^{a}	4.00^{a}	4.00^{a}	4.00^{a}
	Flavor and taste**	8.83^{b}	10.0^{a}	7.67^{c}	8.67^{b}	10.0^{a}	7.67^{c}	6.67^{d}	7.67^{c}	6.33^{d}	9.00^{b}	10.0^{a}	8.33^{bc}
	Total acceptability***	17.66^{c}	18.87^{a}	16.67^{d}	17.33^{cd}	18.67^{ab}	16.67^{d}	15.67^{e}	16.67^{d}	15.33^{e}	18.0^{bc}	19.0^{a}	17.33^{cd}
	Color and appearance#	5.00^{a}	5.00^{a}	5.00^{a}	5.00^{a}	5.00^{a}	5.00^{a}	5.00^{a}	5.00^{a}	5.00^{a}	5.00^{a}	5.00^{a}	5.00^{a}
60	Body and texture*	3.83^{a}	3.87^{a}	4.00^{a}	3.67^{a}	3.67^{a}	4.00^{a}	4.00^{a}	4.00^{a}	4.00^{a}	4.00^{a}	4.00^{a}	4.00^{a}
	Flavor and taste****	8.0^{d}	10.0^{a}	8.67^{c}	8.0^{d}	10.0^{a}	8.67^{c}	7.33^{e}	8.0^{d}	7.0^{e}	9.33^{b}	10.0^{a}	8.67^{c}
	Total acceptability*****	16.83^{de}	18.87^{ab}	17.67^{c}	16.67^{de}	18.67^{ab}	17.67^{c}	16.33^{ef}	17.0^{d}	16.0^{f}	18.33^{b}	19.0^{a}	17.67^{c}

[1] See Table 1 and Fig. 1 # NS = not significant * LSD= 0.3854 ** LSD= 0.7357 * LSD= 0.8471 **** LSD= 0.6195 ***** LSD= 0.6537**
[a,b,c,d,e,f] Means on the same line without a common subscript are significantly different ($P < 0.05$)

CONCLUSION

Results obtained in the present study indicated that the addition of fermented skim milk with *Lactobacillus casei* 01 at 37 or 30°C to a 4% fat ice milk mixtures (F_{37} and F_{30}) allowed a survival rate ranged from 80 to 91% on average after being subjected to aging, freezing and a storage period of 60 days at -18°C compared to non-fermented mixtures (survival rate, 15.2% on average). Thus, adding fermented milk to regular ice milk mixture as manufacturing technique can provide the initial number of probiotic bacteria and the recommended number in the final product. The final number of *Lactobacillus casei* 01 was 18.3×10^7 cfu/g of $F_{30/15}$- ice milk with very light probiotic taste. This viable cell number was higher than that recommended number by the International Dairy Federation (10^7cfu/g). Therefore, this product can represent a good source of probiotic bacteria and is very acceptable for infants and adults who dislike fermented milks.

REFERENCES

Abghari. A.; Sheikh-Zeinoddin, M. and Soleimanian-Zad, S. (2011). Nonfermented ice cream as a carrier for *Lactobacillus acidophilus* and *Lactobacillus rhamnosus*. Int. J. Food Sci. Technol., 46: 84–92

Akalin, A.S. and Erişir, D. (2008). Effects of inulin and oligofructose on the rheological characteristics and probiotic culture survival in low fat probiotic ice cream. J. Food Sci., 73: 184–188

Akin, M.B.; Akin, M.S. and Kirmaci, Z. (2007). Effects of inulin and sugar levels on the viability of yogurt and probiotic bacteria and the physical and sensory characteristics in probiotic ice cream. Food Chem., 104: 93–99

Akin, S. (2005). Effects of inulin and different sugar levels on viability of probiotic bacteria and the physical and sensory characteristics of probiotic fermented ice cream. Milchwisssenschaft, 60: 297–301

Alamprese, C.; Foschino, R.; Rossi, M.; Pompei, C. and Corti, S. (2005). Effects of *Lactobacillus rhamnosus* CG addition in ice cream. Int. J. Dairy Technol., 58: 200–206

Alamprese, C.; Foschino, R.; Rossi, M.; Pompei, C. and Savani, L. (2002).Survival of *Lactobacillus johnsonii* La1 and influence of its addition in retail-manufactured ice cream produced with different sugar and fat concentrations. Int.Dairy .,12:201–208

A.O.A.C. (1990). Official Methods of Analysis.15th Ed. Association of Official Analytical Chemists,Inc., USA.

Arslan, A. A.; Goser, E. M. C.; Demir, M.; Atamer, Z.; Hinrichs, J. and Kücükcetin, A. (2016). Viability of *Lactobacillus acidophilus* ATCC 4356 incorporated into ice cream using three different methods. Dairy Sci. & Technol., 96: 477-487

Burke, A.K. (1947). Practical ice cream making. Olsen Publishing Co.,Milwauke, WI., USA.

Casarotti, S.N and Penna, A.L.B. (2015). Acidification profile, probiotic in-vitro gastrointestinal tolerance and viability in fermented milk with fruit flours. Int. Dairy J., 41: 1–6

Champagne, C.P. and Rastall, R.A. (2009). Some technological challenges in the addition of probiotic bacteria to foods. In: Charalampopoulos D, Rastall RA (eds) Prebiotics and probiotics science and technology. Springer, Berlin, pp: 763–806

Christiansen, P.S.; Edelten, D.; Kristiansen, J.R. and Nielsen, E.W. (1996). Some properties of ice cream containing *Bifidobacterium bifidum* and *Lactobacillus acidophilus*. Milchwissenschaft, 51: 502–504

Cruz, A.G.; Antunes, A.E.C.; Sousa, A.L.O.P.; Faria, J.A.F. and Saad, S.M.I. (2009). Ice-cream as a probiotic food carrier. Food Res. Int., 42: 1233–1239

Dello Staffolo, M.; Bertola, N.; Martino, M. and Bevilacqua, Y. A. (2004). Influence of dietary fiber addition on sensory and rheological properties of yogurt. Int. Dairy J. 14: 263–268

Di Criscio, T.; Fratianni, A.; Mignogna, R.; Cinquanta, L.; Coppola, R.; Sorrentino, E. and Panfili, G. (2010). Production of functional probiotic, prebiotic, and synbiotic ice creams. J. Dairy Sci., 93: 4555–4564

Doleyres, Y.; Fliss, I. and Lacroix, C. (2004). Increased stress tolerance of *Bifidobacterium longum* and *Lactococcus lactis* produced during continuous mixed-strain immobilized-cell fermentation. J. Appl. Microbiol., 97: 527–539

Egyptian standards 1185-1 (2005). Egyptian Organization for standardization and Quality, ARE.

Fávaro-Trindade, C.S.; Bernardi, S.; Bodini, R.B.; de Carvalho Balieiro, J.C. and de Almeida, E. (2006). Sensory acceptability and stability of probiotic microorganisms and vitamin C in fermented acerola (Malpigha emarginata DC.) ice cream. J. Food Sci., 71: 492–495

Gheisari, H.R.; Ahadi, L.; Khezli, S. and Dehnavi, T. (2016). Properties of ice-cream fortified with zinc and *Lactobacillus casei*. Acta Sci. Pol. Technol. Aliment, 15: 367-377

Gill, C.O. (2006). Microbiology of frozen foods. In: Da-Wen Boca S (ed) Handbook of frozen food processing and packaging. CRC, Boca Raton, pp 85–100 Güven, M. and Karaca, O. B. (2002). The effects of varying sugar content and fruit concentration on the physical properties of vanilla and fruit ice-cream-type frozen yogurts. Int. J. Dairy Technol., 55: 27–31

Hagen, M. and Narvhus, J.A. (1999). Production of ice cream containing probiotic bacteria. Milchwissen schaft, 54: 265–268

Hekmat, S. and McMahon, D. J. (1997). Manufacture and quality of iron-fortified yogurt. J. Dairy Sci., 80: 3114–3122

Homayouni, A.; Azizi, A.; Ehsani, M.R.; Yarmand, M.S. and Razavi, S.H. (2008). Effect of microencapsulation and resistant starch on the probiotic survival and sensory properties of symbiotic ice cream. Food Chem., 111: 50–55

Homayouni, A.; Ehsani, M.R.; Mousavi, S.M.; Valizadeh, M. and Djome, Z.E. (2006). Improving the quality of low-fat ice cream by hydrolyzing of casein micelles with chymosin (II) – Sensory evaluation. Iranian J. Agricultural Sci., 36: 1507–1515

Lima, K. G. C.; Kruger, M. F.; Behrens, J.; Destro, M. T.; Landgraf, M. and Franco, B. D. G. M. (2009). Evaluation of culture media for enumeration of *Lactobacillus acidophilus, Lactobacillus casei* and *Bifidobacterium animalis* in the presence of *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus*. LWT – Food Sci. Techn., 42: 491–495.

Magariños, H.; Selaive, S.; Costa, M.; Flores, M. and Pizarro, O. (2007). Viability of probiotic microorganisms (*Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis ssp. lactis* Bb-12) in ice cream. Int. J. Dairy Technol., 60:128–134

Marshall, R.T.; Goff, H.D. and Hartel, W.R. (2003). Ice cream. 6th Ed. Kluwer Academic/Plenum Pub., New York, USA.

McBrearty, S.; Ross, R.P.; Fitzgerald, G.F.; Collins, J.K.; Wallace, J.M. and Stanton, C. (2001). Influence of two commercially available bifidobacteria cultures on cheddar cheese quality. Int. Dairy J., 11: 599–610

Mohammadi, R.; Mortazavian, A.M.; Khosrokhavar, R. and Da Cruz, A.G. (2011). Probiotic ice cream: viability of probiotic bacteria and sensory properties. Ann. Microbiol., 61: 411–424

Nousia, F.G.; Androulakis, P.I. and Fletouris, D.J. (2011). Survival of *Lactobacillus acidophilus* LMGP-21381 in probiotic ice cream and its influence on sensory acceptability. Int. J. Dairy Technol., 64: 130–136

Ordonez, G.A.; Jeon, I.J. and Roberts, H.A. (2000). Manufacture of frozen yogurt with ultrafiltered milk and probiotic lactic acid bacteria . J. Food Processing Preservation, 24: 163-176

Özer, B.; Robinson, R. K.; Grandison, A. S. and Bell, A. E. (1997). Comparison of Techniques for measuring the rheological properties of Labneh (Concentrated Yoghurt). Int. J. Dairy Technol., 50: 129–133.

Patel, A.N.; Michaud, P.; Singhania, R.R.; Soccol, C.R. and Pandey, A. (2010). Polysaccharides from probiotics: new developments as food additives. Food Technol. Biotechnol., 48: 451–463

Salem, M.M.F.; Fathi, F.A. and Awad, R.A. (2005). Production of probiotic ice cream. Pol. J. Food Sci. Nutr., 55: 267–271

Sánchez, B.; De Los Reyes-Gavilán, C. G.; Margolles, A. and Gueimonde, M. (2009). Probiotic fermented milks: Present and future. Int. J. Dairy Technol. 62: 472–483

Steel, R.G.D., Torrie, J.H. and Dickey, D. (1997). Principles and Procedures of Statistics: A Biometrical Approach. 3rd Ed. McGraw Hill

Streit, F.; Delettre, J.; Corrieu, G. and Beal, C. (2008). Acid adaptation of *Lactobacillus delbrueckii subsp. bulgaricus* induces physiological responses at membrane and cytosolic levels that improves cryotolerance. J. Appl. Microbiol., 105: 1071–1080

Sultana, K.; Godward, G.; Reynolds, N.; Arumugaswamy, R.; Peiris, P. and Kailasapathy, K. (2000). Encapsulation of probiotic bacteria with alginate-starch and evaluation of survival in simulated gastrointestinal conditions and in yoghurt. Int. J. Food Microbiol., 62: 47–55

Winton, A. L. (1958). Analysis of Foods. 3rd Ed. John Wiley and Sons Inc., New York, USA.

الحفاظ علي حيوية بكتريا البروبيوتك *Lactobacillus casei 01* عند تصنيع المثلوج اللبني بطرق مختلفة

فاطمه على متولي رمضان ، محمد أحمد عزام ، أشواق عبد المنعم حسن و نجوى محمود عبد الحميد
قسم علوم وتكنولوچيا الألبان – كلية الزراعة – جامعة القاهرة

تهدف هذة الدراسة إلى تصنيع مثلوج لبني يحتوي على ثلاث تركيزات من السكر ١٣و١٥و١٧ % ومدعم ببكتريا *Lactobacillus casei* 01 . لذا صنعت ثلاث مجاميع من المخاليط ، المجموعة الاولي بدون تخمير (NF) حيث لقح المخلوط بـ *L casei* 01 مباشرة قبل تعتيقه ، والمجموعة الثانية (F_{37}) والثالثة (F_{30}) لقحت مخاليطهما بـ ٥٠٪ من كمية اللبن الفرز اللازمة لتكوينهما والتي لقحت بـ *L casei* 01 ثم التحضين على ٣٧°م حتى pH ٤,٥ في حالة المجموعة الثانية ، و التحضين على ٣٠°م حتى pH ٥,٥ في حالة المجموعة الثالثة ، ثم إضافة اللبن الفرز المتخمر لباقي المخلوط قبل التعتيق . قيمت الـ pH واللزوجة لجميع المخاليط ، والريع ومعدل الإنصهار للمثلوجات اللبنية الناتجة مقارنة بالكنترول (بدون *L casei* 01) . كما قيمت المثلوجات اللبنية الناتجة حسياً مقارنة بالكنترول . قدرت الأعداد الحية لبكتريا *L casei* 01 في المخاليط أثناء فترة التعتيق ، وفي المثلوجات اللبنية الناتجة أثناء التجميد والتخزين لمدة ٦٠ يوماً على درجة حرارة – ١٨°م . وقد أظهرت النتائج ما يلي - إنخفاض الـ pH وإرتفاع معنوي للزوجة مخاليط المجموعة الثانية F_{37} مقارنة بالمخاليط الأخري . - ليس هناك أي تأثير للبكتريا *L casei* 01 على الريع أو معدل الإنصهار للمثلوجات اللبنية الناتجة مقارنة بالكنترول. - إرتفاع معنوي للأعداد البروبيوتك *L casei* 01 في مجموعتي التخمر F_{37},F_{30} بالمقارنة بمجموعة التلقيح المباشرNF . - حصلت المثلوجات اللبنية لمجموعة F_{30} علي أعلى درجات القبول الحسي لدي المحكمين سواء بعد ١ أو ٦٠ يوم من التخزين علي درجة – ١٨°م . لذلك يوصي هذا البحث بتخمير اللبن الفرز المعد لصناعة المثلوج اللبني ببكتريا *L casei* 01 علي درجة حرارة ٣٠°م للمحافظة على أعداد هذه البكتريا البروبيوتك مساويةً أو أكثر من الأعداد الموصى بها في المنتج اللبني الوظيفي .