Impact of Artichoke Puree in Frozen Yoghurt with Encapsulated and Free Lactic Acid Bacteria

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TREPTOCOCCUS thermophilus CH-1, Lactobacillus delbrueckii subsp. bulgaricus Lb-S12DRI-VAC and Lactobacillus rhamonsus B- 445 encapsulated or free were successfully used with artichoke puree in ratios 5, 10 and 15 % to formulate frozen yoghurt mixtures. Chemical composition, inulin, antioxidant activity 1, 1-diphenyl-2-picrylhydrazyl (DPPH), pH values, melting rate, viscosity, sensory evaluation were determined and counts of Lb. bulgaricus, S. thermophilus and Lb. rhamnosus were detected when fresh and during storage up to 90 days. Inulin content exhibited different values for the different treatments. The data showed that treatments with added artichoke puree of encapsulated cells had DPPH content higher than that of free cells including the control. Encapsulated treatments exhibited lower pH values than that of free cells. The Control treatment of encapsulated bacteria had higher pH than other encapsulated treatments. The same trends were found in all treatments of free cells. The more increase in added artichoke puree ratio, the more decreased in the melting rate either in encapsulated or in free cells treatments. Treatments of encapsulated cells exhibited melting rate lower than that of free cells at the same ratios including the control. Treatments of free cells had lower viscosity than that of encapsulated cells at the same ratios of the added artichoke puree including controls. This decrease in viscosity values may be attributed to the fact that, the product with lower acidity will have lower viscosity values. For the sensory evaluation, encapsulated samples had better preference than that of free samples. Encapsulated samples have pleasant milky-slight sour taste with natural yoghurt flavor. Inulin caused improvement in flavor, texture and total acceptability. The encapsulated cells sample of 10 % artichoke puree has the most preference evaluation among all treatments even at the end of storage.

Keywords: Frozen yoghurt, Artichoke puree, Lb. bulgaricus, S. thermophilus, Lb. rhamnosus.

Introduction

Frozen yoghurt is a complex fermented frozen dairy dessert that combines the physical characteristics of ice cream with sensory and nutritional properties of fermented milk products. This elaboration results in a nutritious product with a refreshing taste and storage stability significantly longer than that of yoghurt (Guven and Karaca, 2002). Frozen yoghurt supplemented with probiotics provides additional health benefits. The frozen yoghurt environment is not optimum for survival of bacteria (Davidson et al., 2000). Probiotic microorganisms in aerated and frozen products such as ice cream and frozen yoghurt can be injured when subjected to oxygen and freezing temperature. Acidity of yoghurt also can be a harmful factor for probiotic

viability (Magarinos et al., 2007). In particular, artichoke-based foods provide great advantages upon consumption as they contain the prebiotic inulin and other indigestible organic molecules that reach the colon and can be used by the intestinal microbiota (Gon'i et al., 2005) and (Roberfroid, 2007). Inulin being significant by its unique molecular structure, which confirms the bifidogenic effect of inulin as a functional food ingredient. A protective action is performed by the micro-architecture of artichoke components surface which sustains adequate populations of viable bacteria and their anchorage, improving bacterial survival in gastrointestinal digestion (Valerio et al., 2006). The protective action exerted by these food matrices has been demonstrated by the performance of selected Lactobacilli - Lactobacillus plantarum and Lactobacillus paracasei during a simulated gastrointestinal digestion and in vivo trials (Valerio *et al.*, 2010).

Encapsulation is one of the most effective methods that improve the viability and stability of probiotic bacteria (Heidebach et al., 2010 and Dolly et al., 2011). The application of polysaccharideprotein matrix for probiotics encapsulation is a relatively new strategy and can be seen as a promising alternative approach developed for probiotic. Whey protein is a suitable wall material for encapsulation, and the core release properties can be altered by addition of sodium alginate (Gunasekaran, et al., 2007). Alginate is a naturally occurring anionic and hydrophilic polysaccharide. It is one of the most abundant biosynthesized materials (Narayanan et al., 2012) and (Skjak-Braerk et al., 1989). Shah and Ravula (2000) found that the survival of probiotic bacteria in fermented frozen desserts improved with encapsulation. Furthermore, these microorganisms must present good technological properties showing good multiplication in the milk, promoting sensorial properties suitable in the product and being stable and viable during storage, so they can be manipulated and incorporated in food products without losing the viability that must be at least of $10^6 - 10^7$ cfu g⁻¹ Etchepare *et al.*, (2015).

So, this study has investigated to produce functional frozen yoghurt by adding artichoke puree in different ratios using *Lb. bulgaricus*, *S. thermophilus* and *Lb. rhamnosus* encapsulated or free with acceptable quality attributes flavor, body & texture and total preference. This study has also been done to determine the survival of these micro-organisms under the conditions of manufacture and storage even after 90 days.

Materials and Methods

Materials

Fresh cow's milk was procured from Animal Production Research Institute, Agriculture Research Center, Dokki, Egypt. Whey protein concentrate (WPC) 80 % were obtained from Agrimark, USA. Sodium alginate (SA) was procured from Sigma Chemical Co., USA. Carboxymethyl cellulose (CMC) was obtained from BDH chemicals Ltd Poole, England. Emulsifier mono & diglyceride 60 % was obtained from Misr for Food Additives (MIFAD), Giza, Egypt. Artichoke, fresh cream, sugar and vanilla were purchased from the local market, Cairo, Egypt.

Bacterial strains

Lactobacillus delbrueckii subsp. bulgaricus Lb-12 DRI-VAC and Lactobacillus rhamnosus B-445 were provided by Northern Regional Research Laboratory (NRRL), Illinois, USA. Streptococcus thermophilus CH-1 was obtained from Chr. Harsens's Lab., Denmark.

Methods of manufacture

Preparation of Artichoke (Cynara scolymus L.) *puree:*

Artichoke (*Cynara scolymus* L.) fresh puree was prepared using artichoke heads. Artichoke heads were heat treated by holding in boiled water for 5 min to inhibit the oxidative enzymes and to prevent colouring changes. Artichoke treated heads were cut into small portions to be suitable for the milling machine (Braun mincer, Germany), then puree was filtrated to separate unmilled portions.

Preparation of whey protein concentrate (WPC) and sodium alginate (SA) solution

Whey protein concentrate (WPC) 80 % were hydrated in distilled sterilized water (10 % w/v). The solution was agitated using magnetic stirrer for 1 h at room temperature and rest for 2 h in order to ensure a good protein hydration. Whey protein concentrate (WPC) solution was adjusted at pH 7 and heated to 80 °C for 45 min to denature the proteins. The solution was cooled overnight at room temperature. Then, Sodium alginate (SA) was added to the denatured WPC solution (3 % w/v) and stirred to complete dissolution.

Preparation of encapsulated lactic acid bacteria (LAB)

Different strains of lactic acid bacteria (Lb. rhamnosus, Lb. bulgaricus and S. thermophilus) that used in manufacturing of frozen yoghurt were encapsulated according to the method described by Gbassi et al. (2009). Lactobacilli cultures were transferred individually into MRS broth and incubated at 37 °C for 24 hr under anaerobic conditions. Streptococci culture was transferred into M17 broth and incubated aerobically at 37 °C for 24 h. Lactic acid bacteria cells strains were harvested by centrifugation at 4 °C, 5000 rpm for 15 min. Cells pellets were washed twice by sterile saline solution and re-centrifuged. Freshly harvested cells concentrates (1:1:1) of different lactic acid bacteria strains were mixed with (WPC + SA) mixture solution as (25 g / 100 ml) to obtain cells concentration 25 %. Sterilized syringe was used to drop-wise the mixture into calcium chloride solution (0.5 ml Mol). To obtain LAB beads magnetic stirring was maintained during encapsulation process, and then the resulted beads were rinsed with sterile saline solution.

Preparation of Frozen yoghurt mixtures

Frozen yoghurt was prepared according to methods described by Schmidt (2004). Frozen yoghurt base mixture was prepared to contain 12 % milk solids non-fat (MSNF), 8 % fat, 15 % sucrose, 0.2 % stabilizer (CMC) and 0.1 % emulsifier. Powder contents added to the fresh milk to obtain the whole mixture. The mixture was divided into four portions. Artichoke puree was added in ratios of 0, 5, 10 and 15 % to obtain mixtures of different treatments including the control. All mixtures were heat treated at $80 \pm 1^{\circ}$ C for 30 Sec. and then cooled to 4 °C. After that every mixture was divided into 2 portions for adding 2 % of LAB (Lb. bulgaricus, S. thermophilus and Lb. rhamnosus) strains encapsulated or free. Mixtures were incubated up to 3 hr at 40 °C, and then cooled for 2 hr at 4 °C. To obtain frozen yoghurt, mixtures were whipped in the ice cream maker (Model: BL1380) for 30 min., frozen yoghurt as an end product was collected at an exit temperature of -5.5 °C, packaged in plastic cups, and frozen stored at -20 ± 2 °C.

Methods of analysis

Chemical analysis

Fresh Frozen yoghurt mix samples were chemically analyzed for total solids, fat and ash content according to AOAC (2007). Total protein content was determined using semi micro-Kjeldal method as mentioned by Ling (1963). Antioxidant activity 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity (DPPH) was determined according to Zheng and Wang (2001).

Physico-chemical properties

pH values

pH values were measured using a digital laboratory Jenway 3510 pH meter, UK. Bibby Scientific LTD. Stone, Stafford shire, ST 15 OSA.

Apparent viscosity

Frozen yoghurt mix samples were gently stirred 5 times in clockwise direction prior to viscosity measurements. Apparent viscosity was measured at 7 °C using a Brookfield digital viscometer (Middleboro, MA 02346, USA). The sample was subjected to shear rates ranging from 2.0 to 20.000 S⁻¹ for upward curve. Viscosity measurements were performed in triplicate and expressed as centipoise (cP).

Overrun and specific gravity

The overrun percent of frozen yoghurt was calculated as described by Marshall et al. (2003) by weight according to the equation of:

$$0 \text{ verrun} \qquad \frac{[(\text{Weight of unit volume of mix})}{-(\text{Weight of unit volume of ice cream})]} \times 100$$

$$\frac{(\text{Weight of unit volume of ice cream})}{[(\text{Weight of unit volume of ice cream})]} \times 100$$

Specific gravity of frozen yoghurt mixtures and the final frozen product were measured at 20 °C according to Winton (1958).

Melting properties

Melting properties of frozen yoghurt was determined according to Arndt and Wehling (1989) by carefully cutting the foamed frozen yoghurt samples (~ 70 gm). Samples were placed to drain onto wire mesh over a glass funnel fitted on conical flask at 30 °C. The drained amount of frozen yoghurt were weighed every 10 min. until the entire sample had completely melted.

Microbiological analysis

Enumeration of encapsulated and free bacterial cells

Viable counts of LAB strains *Lb. bulgaricus*, *S. thermophilus* and *Lb. rhamnosus* encapsulated or free were enumerated. Encapsulated strains were enumerated as follows: 1 g of the capsules were re-suspended in 10 ml of phosphate buffer pH 7.4 and stirred for 5 min using an electric vortex. Saline solution was used to prepare serial dilutions of the bacterial strains. Log Colony Forming Unit/g (log cfu g⁻¹) were detected by count plating as follows:

Lb. bulgaricus count

Lactobacillus bulgaricus counts were detected using De Man-Rogosa - Sharpe (MRS) agar according to De Man *et al.* (1960). The plates were incubated at 37 °C for 48 hr under anaerobic conditions.

S. thermophilus count

Streptococcus thermophilus counts were detected aerobically at 37 °C for 48 hr using M17 agar according to Terzaghi and Sandine (1975).

Lb. rhamnosus count

Lactobacillus rhamnosus counts were enumerated using MRS agar plus vancomycin (MRS V) according to Saccaro *et al.* (2012). The plates were incubated anaerobically at 37 °C for 48 hr.

Lactic acid bacteria (LAB) survival rate

Survival rate of LAB different strains was calculated according to El-Shenawy *et al.* (2016) by the equation of :

Survival (%) = [log cfu g^{-1} at the end of storage / log cfu g^{-1} at fresh time] X 100.

Sensory properties evaluation

Samples of frozen yoghurt were sensory evaluated after hardening at -20 ± 2 °C for 24 hr by regular scoring panel members at Dairy Department, National Research Center. Scores were carried out according to Arbukle (1986), using scale of (45) points for flavour, (35) points for body & texture, (10) points for melting properties and (10) points for colour.

Statistical analysis

Statistical analysis was performed according to MINITAB INC. (2005) Using General Linear Model (GLM). Duncan's multiple range was used to separate among means of three replicates of samples.

Results and Discussion

Chemical composition

Chemical Composition of frozen voghurt artichoke puree manufactured with and encapsulated or free LAB are shown in Table 1. The table indicated that total solids for treatments of control, 5, 10 and 15 % artichoke puree with encapsulated LAB were ranged from 37.78 -36.97% and that with free cells ranged from 37.75 - 36.89 %. Encapsulated treatments have slightly higher TS content than that of free cells. This variation in TS among all treatments could be due to the presence of capsules and the differences in material weight when mixtures formulas preparation.

As for, total nitrogen content in the control encapsulated sample was 0.87 % where it was 0.86 % in the control of free cells. For the treatments of artichoke puree, total nitrogen content in encapsulated treatments were ranged from 0.70 to 0.60 %. Meanwhile; it ranged from

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0.68 to 0.58 % in the treatments with free cells at the same added ratios. The significant variations in encapsulated treatments could be as a result of capsules materials which contain WPC.

Table 1 illustrated that, fat content of frozen yoghurt samples recorded 8.48, 8.32, 8.15 and 7.87% for encapsulated treatments and recorded 8.37, 8.33, 8.25 and 7.84% for treatments of free cells. These variations could be due to the differences in material weight when mixtures formulas preparation.

The ash content in control encapsulated sample was 1.21%, where it was ranged from 1.41 to 1.62% for samples contained artichoke puree. Nevertheless, it was 1.23% for the control of free cells; ash content was ranged from 1.40 to 1.64% for samples of added artichoke puree. These insignificant variations in ash content could be due to the added ratio of artichoke puree contains high ratio of minerals (EL-Sohaimy, 2013).

Inulin content exhibited different values for the different treatments. Control treatments of encapsulated or free LAB have no inulin content. For the other treatments, the more increase of added puree ratio the more inulin content. For the other treatments, the more increase of added puree the more inulin content even for the encapsulated or free LAB. Treatments with added puree of encapsulated cells were having inulin content slightly higher than that of free cell (Table 1).

As indicated from Table 1, treatments with added puree of encapsulated cells were having scavenging activity DPPH higher than that of free cells including the control. Variations in DPPH among treatments were significant. These results could be related to the effect of proteins content of the capsules (Zivkovic *et al.*, 2009), and the higher growth of bacterial strains. From the table it is clear that, the higher added ratio of artichoke puree the higher content of DPPH, this might be attributed to the antioxidant activity of artichoke as reported by (EL-Sohaimy, 2013) and (Gaafar *et al.*, 2013).

pH values

Table 2 illustrates pH values of frozen yoghurt with artichoke puree and encapsulated or free LAB. The data show that all treatments of microencapsulated LAB have pH values lower than that of free cells including the control. Among treatments of encapsulated LAB, it is clear that

the pH of control treatment were higher than the other treatments. pH values showed a decrease by increasing the added ratio of artichoke puree. The same trends were found in all treatments of free cells. No significant differences among treatments were recorded. Nevertheless, the more added ratio of artichoke puree, the more decrease in pH values, this could be due to enhancing the bacterial growth (Rezaei, *et al.*, 2014). pH values decreased significantly in all treatments during storage up to the end, the decrease were more pronounced in frozen yoghurt capsulated cells treatments. These results are in agreement with that of Ahmadi *et al.* (2014).

TABLE 1. Chemical composition, Inulin and antioxidant scavenging activity (DPPH) content of frozen
yoghurt with artichoke puree and encapsulated (C) or free (F) LAB.

			%			
Treatments*	TS	Fat	TN	Ash	Inulin	⁻ DPPH (mg/100 g)
			Encaps	ulated LAB		
CC	37.78 ^A	8.48 ^A	0.87 ^A	1.21 ^A	0.000 ^A	6.62 ^G
C5	37.68 ^{AB}	8.32 ABC	0.70 ^B	1.41 ^A	0.157 ^A	18.85 ^E
C10	37.42 ^{AB}	8.15 ^{ABC}	0.66 ^{BC}	1.53 ^A	0.314^{AB}	23.87 ^c
C15	36.97^{AB}	7.87 ^{BC}	$0.60 ^{\text{DE}}$	1.62 ^A	0.471 ^B	30.90 ^A
			Fre	ee LAB		
CF	37.75 AB	8.37 ^{AB}	0.86 ^A	1.23 ^A	0.000 ^A	5.58 ^H
F5	37.55 AB	8.33 ABC	0.68 ^{BC}	1.40 ^A	0.155 ^A	16.53 ^F
F10	37.35 AB	8.25 ABC	0.64 ^{CD}	1.51 ^A	0.313^{AB}	22.75 ^D
F15	36.89 ^B	7.84 ^c	0.58^{E}	1.64 ^A	0.469 ^B	28.47 ^в

5: Artichoke added puree 5 % 15: Artichoke added puree 15 %

A, B, C: Means with the same letter among treatments at the same character are not significantly different.

TABLE 2. pH value of frozen yoghurt with artichoke puree and encapsulated (C) or free (F) LAB when fresh and during storage.

T			Stora	ge period (da	ys)		
Treatments*	0	15	30	45	60	75	90
			Encapsulated	LAB			
CC	6.33 ^{Aa}	6.31 ^{Aa}	6.24 ^{Aa}	6.20 ^{Aa}	6.13 ^{Aa}	6.10 ^{Aa}	5.35 ^{Ab}
C5	6.30 ^{Aa}	6.27 ^{Aa}	6.16 ^{Aa}	6.10 ^{Aa}	5.98 Aab	5.53 Abc	5.29 ^{Ac}
C10	6.28 Aa	6.22 Aa	6.19 ^{Aa}	6.15 ^{Aa}	6.11 ^{Aa}	5.98 ^{Aa}	5.32 ^{Ab}
C15	6.26 ^{Aa}	6.23 Aa	6.19 ^{Aa}	6.13 ^{Aa}	5.91 Aa	5.86 ^{Aa}	5.30 ^{Ab}
			Free LAI	3			
CF	6.39 ^{Aa}	6.30 ^{Aab}	6.27 ^{Aab}	6.19 ^{Aab}	6.17 ^{Aab}	6.15 Aab	5.91 Ab
F5	6.38 Aa	6.21 Aa	6.18 ^{Aa}	6.14 ^{Aa}	6.10 ^{Aab}	5.93 Aab	5.58 ^{Ab}
F10	6.32 ^{Aa}	6.28 ^{Aa}	6.24 Aa	6.18 ^{Aa}	6.12 ^{Aa}	5.89 ^{Aab}	5.43 Ab
F15	6.29 ^{Aa}	6.25 Aa	6.22 Aa	6.10 ^{Aa}	5.94 Aab	5.79 Aabc	5.54 Abc

* See Table 1.

A, B, C: Means with the same letter among treatments in the same storage period are not significantly different.

a, b, c: Means with the same letter in the treatment during storage periods are not significantly different.

Overrun and specific gravity

Overrun of frozen yoghurt treatments are shown in Table 3. All treatments of added artichoke with encapsulated or free LAB have overrun values higher than control treatments. Overrun values were increased by increasing the added ratio up to 10 % and then tend to decrease as the added puree increased to 15 % either for encapsulated or free LAB treatments. These could be related to higher viscosity values of added artichoke treatments. The presence of fiber in the mixes could be affected the ability of each mixture to contain air cells during whipping (El-Shenawy et al., 2016). Artichoke treatments with free LAB recorded overrun values lower than that of encapsulated LAB, Pinto et al. (2012).

Specific gravity is the ratio of the density of component or a material at 20 °C compared to the density of water at the same temperature. Nevertheless, specific gravity of all artichoke treatments were higher than controls. Specific gravity decreased gradually when the added puree increased, that for mixtures and the frozen yoghurt encapsulated or free LAB treatments.

Melting rate

Melting rate of frozen yoghurt treatments with artichoke puree and encapsulated or free LAB at different times expressed as g / min are shown in Table 4. All treatments have no melting at 10

min, by increasing melting time up to 20 min the melting increased up to 1.0, 0.5, 0.3 and 0.2 g for the treatments of CC, C5, C10 and C15. For the treatments of CF, F5, F10 and F15 melting increased up to 2.0, 0.6, 0.3 and 0.2 g at the same time. CF sample has higher melting rate than that of CC that when compared by added puree treatments either of encapsulated or free LAB. This could be attributed to the lower heat-transfer rate of air trapped in the mixture as a result increase melting time. Melting is affected by factors such as air incorporated, ice crystals and network of fat globules formed during freezing, (Moeenfard and Tehrani, 2008). Melting of added puree treatments even of encapsulated or free LAB decreased by increasing the added puree, (Mohammadi et al., 2011). When the time increased up to 50 min, melting increased in all treatments in different rates. Treatments of encapsulated cells with different added ratios of artichoke puree exhibited melting rate lower than that of free cells at the same ratios including the controls. This could also be due to the more water retention by the capsules materials (Goh et al., 2012). Among treatments of artichoke with encapsulated LAB, for the treatment of C10 melting increased significantly from 60 up to 80 min and was the highest. Where, at the end of melting time, treatments of C15 and F15 % have the highest melting rate comparing with all other artichoke treatments.

TABLE 3. Overrun as (%) and specific gravity (mixes & end product) of frozen yoghurt with artichoke
puree and encapsulated (C) or free (F) LAB.

Treatments*	Overrun %	Spe	cific gravity
Treatments	Overrun 76	Mix	Frozen yoghurt
		Encapsulated LAB	
CC	37.39 EF	1.095 ^{BC}	0.797 ^A
C5	38.86 ^B	1.154 ^A	0.831 ^A
C10	39.29 ^A	1.138 AB	0.817 ^A
C15	38.10 ED	1.120 ^{BC}	0.811 ^A
		Free LAB	
CF	37.35 ^E	1.092 ^{BC}	0.795 ^A
F5	38.55 ^{CD}	1.150 ^A	0.830 ^A
F10	38.58 ^c	1.135в	0.819 ^A
F15	37.54 EF	1.110 ^{BC}	0.807^{A}

* See Table 1.

A, B, C: Means with the same letter among treatments at the same character are not significantly different.

Viscositv

Viscosity of frozen yoghurt with artichoke puree and encapsulated or free LAB at different share rates is illustrate in Fig. 1. It is clear that, treatments of free cells having lower viscosity values than that of capsulated cells at the same ratios of added artichoke puree including the controls. This decrease in viscosity values of different frozen yoghurt mixes may be attributed to the fact that, the product with lower acidity will have lower viscosity values (Ordonez et al., 2000). Variations in viscosity values could be explained by the inulin-milk protein interaction (Meyer et al., 2011). By comparing treatments of capsulated cells with that of free cells it can be found that, the treatment

C5 was at the same range of treatment F15 at the different shear rates. Moreover, treatment of encapsulated cells C15 has the highest viscosity among all treatments meanwhile; treatment FC has the lowest viscosity values. Gonzalez-Tomas et al. (2008), verified that inulin despite being more thermally stable than other types of fibers and tends to form microcrystals in milk which retain a great quantity of water, resulting in a more viscous gel. Mohammadi et al. (2011), reported that potential interaction between the inulin and milk proteins could also be present in the system. Inulin being highly hygroscopic, would bind water to form a gel-like network.

TABLE 4. Melting rate (g/min) of frozen yoghurt with artichoke puree and encapsulated (C) or free (F) LAB.

					Treatments* (g)				
Time (min.)		Encapsula	ated LAB		Free LAB					
	CC	C5	C10	C15	CF	F5	F10	F15		
10	0.0^{Ai}	0.0^{Ah}	0.0^{Ah}	0.0 ^{Ag}	0.0^{Ah}	0.0^{Ah}	0.0^{Ah}	0.0^{Ah}		
20	1.0^{Bh}	$0.5^{\rm CDh}$	$0.3^{\rm DEh}$	0.2^{Eg}	2.0^{Ag}	0.6^{Ch}	0.3^{DEh}	0.2^{Eh}		
30	8.0^{Bg}	2.0^{Dg}	1.5^{Dg}	$0.3^{\rm Eg}$	$10.5^{\rm Af}$	3.5^{Cg}	3.2^{Cg}	1.74^{Dg}		
40	15.0^{Bf}	4.5^{Ff}	3.25^{Gf}	$2.0^{\rm Hf}$	20.0 ^{Ae}	11.5 ^{Cf}	$8.0^{\rm Df}$	$6.0^{\rm Ef}$		
50	20.0 ^{Ce}	10.0^{Ee}	7.5^{Fe}	6.5 ^{Ge}	28.5 Ad	25.5 ^{Be}	12.0^{De}	10.5^{Ee}		
60	$22.0^{\rm Dd}$	14.0^{Fd}	15.5^{Ed}	$10.0^{\rm Gd}$	40.5 Ac	38.0^{Bd}	24.5 ^{Cd}	22.0^{Dd}		
70	28.0^{Dc}	16.0 ^{Ge}	18.0^{Fc}	14.0^{Hc}	45.0 ^{Bb}	46.5 ^{Ac}	27.0^{Ec}	34.0 ^{°Cc}		
80	30.0^{Cb}	18.0 ^{Fb}	20.5^{Db}	19.5 Eb	47.5 Aa	47.5 Ab	32.5 ^{Bb}	47.0 ^{Ab}		
90	31.5^{Da}	$20.0{}^{\rm Ga}$	23.0^{Fa}	$30.0{}^{\rm Ea}$	47.5 ^{Ca}	49.0 ^{Ba}	47.0 ^{Ca}	51.0 ^{Aa}		

* See Table 1.

A, B, C: Means with the same letter among treatments at the same melting time are not significantly different.

a, b, c: Means with the same letter in the treatment during melting times are not significantly different.

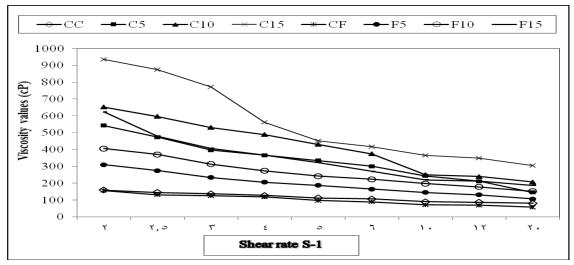


Fig. 1. Viscosity of frozen yoghurt with artichoke puree and encapsulated (C) or free (F) LAB. Artichoke added puree 10 % C : Control 10:15: Artichoke added puree 15 %

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5: Artichoke added puree 5 %

Lactobacillus bulgaricus count

Table 5 demonstrates that Lb. bulgaricus log cfu g-1 counts of frozen yoghurt with artichoke puree and encapsulated or free LAB when fresh were 10.20, 10.33, 10.45 and 10.55 log cfu g-1 for the treatments of CC, C5, C10 and C15 and were 10.08, 10.20, 10.30 and 10.43 log cfu g⁻¹ for the treatments of CF, F5, F10 and F15, consequently. It is clear that, the counts of *Lb. bulgaricus* in the capsulated treatments were significantly higher than that of free cells(EL-Sayed et al., 2015). The count was increased by increasing the added ratio of artichoke puree in encapsulated or free LAB treatments. By extending the frozen storage for 7 days numeration were tend to decrease in all treatments. This could be due to freeze shock (Rezaei et al., 2014). The decrease was more noticeable and significant after 30 days of storage; CC treatment recorded 8.90 log cfu g⁻¹. Meanwhile, CF treatment recorded 8.25 log cfu g-1. This decrease was higher when compared by the treatments of C15 or F15 that recorded 9.65 log cfu g⁻¹ for each. Results demonstrated that the viability of Lb. bulgaricus when encapsulated in alginate-whey protein microspheres was higher comparing by free cells during storage. These results are in agreement with Meng-Yan C. et al.

(2014). The decrease was continued up to 75 days of storage in different rates for all treatments. At the end of the storage period although, the numeration were decreased significantly in all treatments, treatments of encapsulated cells have higher numeration than that of free cells at all added ratios of artichoke puree even the controls.

Streptococcus thermophlius count

Counts of Streptococcus thermophilus (log cfu g-1) free or encapsulated in frozen yoghurt with added artichoke puree are shown in Table 6. Results of the samples when fresh illustrate that, the CC has the lowest numeration when compared by the other treatments of encapsulated cells. Free cells treatments exhibited lower numeration as log cfu g-1 at the different ratios of added artichoke including the control. Treatment of C15 has the highest numeration among all treatments. Encapsulation preserve cells from detrimental environmental factors such as low pH (Wenrong and Griffiths, 2000), and freeze shocks (Shah and Ravula, 2000). Numerations of all samples tend to decrease gradually and significantly by extending the storage up to the end. Results indicated that, encapsulated cells samples have higher numeration than that of free cells.

 TABLE 5. Counts of Lactobacillus delbrueckii subsp. bulgaricus (log cfu g⁻¹) of frozen yoghurt with artichoke puree and encapsulated (C) or free (F) LAB when fresh and during storage.

		Storage period (days)												
Treatments	Fresh	7	15	21	30	45	60	75	90					
				Encapsula	ted LAB									
CC	10.20^{FDa}	$10.05^{\rm CDa}$	9.75 ^{Сь}	9.15 ^{Dc}	8.90 ^{BCd}	8.15^{De}	7.20^{Ef}	6.90^{Fg}	6.25^{Fh}					
C5	$10.33 ^{\text{BCa}}$	10.12^{CDb}	10.00 ^{Bb}	9.50 ^{Cc}	9.10^{Bd}	8.70 ^{Ce}	8.30 ^{Cf}	8.00^{Dg}	7.75 ^{Ch}					
C10	10.45 ^{Aa}	10.30 ^{Aa}	10.15 ABab	9.75 ^{Bb}	9.50 ^{BCc}	9.12^{Cd}	8.70^{De}	8.40^{Ef}	8.00^{Fg}					
C15	10.55 ^{Aa}	10.40^{Aab}	10.25 ^{Ab}	9.80 ^{Bc}	9.65 Ac	9.25 Ad	8.85 ^{Ae}	8.60^{Af}	8.20^{Fg}					
				Free 1	LAB									
CF	10.08^{Da}	9.50^{Eb}	9.00^{Dc}	8.60^{Ed}	8.25 De	7.40^{Ff}	6.50^{Fg}	6.10^{Gh}	5.80 ^{Gi}					
F5	10.20^{CDa}	10.00^{Db}	9.80 ^{Cc}	9.00 ^{Dd}	8.80^{Ce}	$7.95^{\rm Ef}$	7.30^{Eg}	7.00^{Fh}	6.65^{Ei}					
F10	10.30^{BCa}	10.22^{BCa}	10.00^{Bb}	9.45 ^{Ce}	$9.00^{\rm Bd}$	8.20^{De}	7.85^{Df}	7.30^{Eg}	7.00^{Dh}					
F15	$10.43^{\rm ABa}$	10.30 ABab	10.20 ^{Ab}	10.00 ^{Ac}	9.65 ^{Ad}	9.00 ^{Be}	8.60^{Bf}	8.20 ^{Cg}	7.95^{Bh}					

* See Table 1

A, B, C: Means with the same letter among treatments in the same storage period are not significantly different.

a, b, c: Means with the same letter in the treatment during storage periods are not significantly different.

Lb. rhamnosus count

Lactobacillus rhamnosus count free or encapsulated in frozen yoghurt with added artichoke puree is demonstrate in Table 7. These data indicate that the control of free cells and that of encapsulated cells have the same numeration 10.30 log cfu g⁻¹ in the fresh sample. The samples of F10 and F15 have the same numeration as that of C5 recorded 10.40 log cfu g⁻¹. Sample of C15 has the highest numeration 10.60 log cfu g⁻¹ followed by that of C10 comparing by the other treatments. It is noticeable that, samples with added artichoke puree of free cells were having numeration lower than that encapsulated cells at the same added ratios. This could be due to that molecular oxygen induces cell death or poor viability of probiotics (Kieronczyk et al., 2006). Probiotic microorganisms in frozen products such as frozen yoghurt can be injured when subjected to oxygen and freezing temperature (Rezaei et al., 2014). Acidity also could be harmful factor for probiotic viability (Magarinos et al., 2007). By extending the frozen up to end of storage, numerations decreased in all treatments, the numeration decreased significantly from 10.30 to 5.85 log cfu g⁻¹ for the CF treatment. Meanwhile, it decreased significantly from 10.60 to 7.70 log cfu g-1 for the C15 treatment. The formation of ice crystals due to temperature fluctuations during storage may rupture and reduce viability of bacterial cells (Davidson et al., 2000). Ice crystal size and air cell size gradually increased with prolonged storage time at -18°C (Park et al., 2015).

Lactic acid bacteria (LAB) survival rate (%) Survival rate of encapsulated or free LAB

in frozen yoghurt with added artichoke puree is illustrated in Table 8. Encapsulated treatments having a survival rate higher than that of free cells. Capsulation has a protective action against freezing damage by ice crystals (Mohammadi et al., 2011) and pH decrease (Kailasapathy, 2006). Encapsulation with alginate protected viable cells significantly from harsh acidic condition of simulated gastrointestinal tract and gastric juice conditions into simulated intestine (Ayama et al., 2014). Among treatments of added artichoke puree, survival rate increased by increasing the added artichoke puree due to addition of inulin. The control treatments were have less survival rate than that of artichoke encapsulated or free. There are differences among treatments in survival rate of the Lb. bulgaricus, S. thermophilus and Lb. rhamnosus. These could be due to the initial count and the ability of each strain to resist the manufacture and storage conditions. Lb. bulgaricus have the highest survival rate in all treatments encapsulated or free. These could be has a correlation with the synergistic effect of S. thermophilus as an oxygen scavenger subsequently can reduce oxygen exposure to bifidobacteria, (Lourens-Hattingh and Viljoen, 2001). Also, Gosmann and Rehm (1986 & 1988) reported that micro-organisms aggregates could help to improve anaerobic conditions in the beads center. However, (Beunik et al., 1989) mentioned that, the protective effect of microencapsulation against oxygen toxicity could be due to that alginate restricts the diffusion of oxygen through the gel, creating anoxic regions in the center of the beads.

m	Storage period (days)											
Treatments*	Fresh	7	15	21	30	45	60	75	90			
				Enc	apsulated L	AB						
CC	10.25 ^{Ca}	9.90 ^{сь}	935 CDc	9.00 ^{Cd}	8.70 ^{Ce}	8.20^{Cf}	7.85 ^{Cg}	7.30^{Ch}	6.45 ^{Ci}			
C5	10.70^{Ba}	10.35 ^{Bb}	10.00^{Bc}	9.60 ^{Bd}	9.45 ^{Bd}	9.00 ^{Be}	8.55 Bf	8.00^{Bg}	7.40^{Bh}			
C10	10.80^{ABa}	10.45^{ABb}	10.20 ^{Ac}	9.85 Ad	9.60 ABe	$9.25{}^{\rm Af}$	8.65^{ABg}	8.20^{Ah}	7.60^{Ai}			
C15	10.89 ^{Aa}	10.60 ^{Ab}	10.25 Ac	9.95 Ad	9.70 ^{Ae}	9.40^{Af}	8.75 Ag	8.30^{Ah}	7.65 ^{Ai}			
					Free LAB							
CF	10.12 ^{Ca}	9.50 Eb	9.00 Ec	8.50^{Ed}	7.90^{Ee}	7.00^{Gf}	6.30 Gg	5.70^{Gh}	5.00^{Fi}			
F5	10.15 ^{Ca}	9.50 Eb	9.20 ^{Dc}	8.60^{DEd}	8.00^{Ee}	7.35^{Ff}	6.65 Fg	6.00^{Fh}	$5.30^{\rm Ei}$			
F10	10.17^{Ca}	9.70 ^{Db}	9.30 ^{CDc}	8.65^{DEd}	8.20^{De}	7.60^{Ef}	7.00^{Eg}	6.50^{Eh}	6.00^{Di}			
F15	10.20 ^{Ca}	9.85 CDb	9.40 ^{Cc}	8.75^{Dd}	8.30^{De}	7.90^{Df}	7.25^{Dg}	6.70^{Dh}	6.15^{Di}			

 TABLE 6. Counts of Strepococcus thermophilus (log cfu g⁻¹) of frozen yoghurt with artichoke puree and encapsulated

 (C) or free (F) LAB when fresh and during storage.

* See Table 1

A, B, C: Means with the same letter among treatments in the same storage period are not significantly different.

a, b, c: Means with the same letter in the treatment during storage periods are not significantly different.

Tuestments*	Storage period (days)											
Treatments*	Fresh	7	15	21	30	45	60	75	90			
				Enc	apsulated	LAB						
CC	10.30 ^{Aa}	10.00 ^{Cb}	9.75 ^{Cc}	$9.45 ^{\text{BCd}}$	8.50^{Ee}	7.85^{Df}	7.50^{Cg}	6.65^{Eh}	$6.15^{\rm Ei}$			
C5	10.40 ^{Aa}	10.20^{Bb}	10.00^{Bc}	9.35^{CDd}	8.90^{De}	$8.45^{\rm BCf}$	$8.00^{\rm \ Bg}$	7.50^{Ch}	$7.00^{\rm Ci}$			
C10	10.50^{Ba}	10.25^{ABb}	$10.15^{\rm ABb}$	9.60^{ABc}	9.20^{Bd}	8.60^{Be}	8.20^{Bf}	$7.65 ^{\mathrm{BCg}}$	$7.33^{\rm \ Bh}$			
C15	10.60^{ABa}	10.40 Aa	10.20^{Aa}	$9.70^{\rm Ab}$	9.50 ^{Ab}	9.00 ^{Ac}	$8.65^{\rm Ad}$	8.00 ^{Ae}	$7.70^{\rm Af}$			
					Free LAB							
CF	10.30 ^{Aa}	9.80 ^{Db}	9.20 ^{Dc}	8.90^{Ed}	8.00^{Fe}	$7.65^{\rm Ef}$	6.70^{Dg}	6.25 ^{Fh}	5.85 ^{Fi}			
F5	10.35 ^{Aa}	10.12^{BCb}	10.00^{Bb}	9.20 ^{Dc}	8.60^{Ed}	7.75^{DEe}	7.20^{Cf}	6.90^{Dg}	6.50^{Dh}			
F10	10.40 ^{Aa}	10.20^{Ba}	10.10^{ABa}	9.35 ^{CDb}	9.00 ^{CDb}	8.50^{Bc}	8.00^{Bc}	7.55 ^{Cd}	7.25 ^{Bd}			
F15	10.40 ^{Aa}	10.20^{Bb}	$10.10^{\rm ABb}$	9.70 ^{Ac}	9.10^{BCd}	8.30 ^{Ce}	$8.00^{\rm Bf}$	$7.80^{\rm \ Bg}$	$7.40^{\rm \ Bh}$			

TABLE 7. Counts of *Lb. rhamnosus* (log cfu g⁻¹) of frozen yoghurt with artichoke puree and encapsulated (C) or free (F) LAB when fresh and during storage.

* The same as Table 1

A, B, C: Means with the same letter among treatments in the same storage period are not significantly different.

a, b, c: Means with the same letter in the treatment during storage periods are not significantly different.

Treatments*	Lb. rhamnosus	Lb. bulgaricus	S. thermophilus
		Encapsulated LAB	
CC	59.70874	61.27451	62.92683
C5	67.30769	75.0242	69.15888
C10	69.80952	76.55502	70.37037
C15	72.64151	77.72512	70.3125
		Free LAB	
CF	56.79612	57.53968	49.40711
F5	62.80193	65.19608	52.21675
F10	69.71154	67.96117	58.99705
F15	71.15385	76.22244	60.29412

 TABLE 8. LAB Survival rate (%) of frozen yoghurt with artichoke puree and encapsulated (C) or free

 (F) LAB at the end of storage period.

* See Table 1

Sensory evaluation

Sensory evaluation of frozen yoghurt with artichoke puree and encapsulated or free LAB is shown in Table 9. From the data, fresh samples exhibited the highest score for C10 followed by C5, F5and CC which have recorded scores 44, 43, 42 and 42 respectively. Meanwhile, the lowest recorded scores were 40 and 39 for C15

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and F15. It is clear that, encapsulated samples having better preference than that of free samples. Encapsulated samples have pleasant milky-slight sour mouth feel with natural yoghurt flavor. The characteristics of flavour are due to artichoke flavour and fermentation compounds such as lactic acid, acetaldehyde and other carbonyl compounds. It could be also related to

the presence of inulin that improve mouth feel of frozen yoghurt samples, Ismail et al. (2013). Body & texture exhibited scores 32, 32, 33 and 30 for each fresh encapsulated and free samples. There are no significant differences in B & T between encapsulated and free samples at the same levels of added artichoke puree even for the controls. Melting point for encapsulated or free LAB showed that, samples with added puree at all levels were better and more resistant than the controls. It could be due to the capacity of inulin, act as a stabilizer for imbibing water, as a result water molecules become immobilized and unable to move freely among other molecules in the mix (Akalin and Erisir 2008). Colour recorded scores showed that no significant differences among treatments of encapsulated or free LAB at the same levels of added artichoke puree including the control. The treatments of C15 and F15 have

the lowest score comparing by the others. These could be related to the own colour of artichoke puree, has an effect on samples colour especially at the high level. Table 9 also showed that encapsulated cells samples have total preference higher than free cells at the same levels of added puree. The sample of C10 has the most preference evaluation scores among all treatments. Inulin caused improvement in flavor, texture and total acceptance properties (Rezaei et al., 2014) and (Kip et al., 2006). Flavour and B & T properties were decreased gradually by storage for 60 and 90 days. Storage has no noticed effect on melting point up to the end. Colour properties tend to decrease in free cells treatments after 60 days of storage up to the end more than encapsulated treatments. Encapsulated sample of 10 % added artichoke puree (C10) were the most preferred at all by the end of storage.

TABLE 9. Sensory properties of frozen yoghurt with artichoke puree and encapsulated (C) or free (F) LAB when fresh and during storage.

		Encapsulated LAB Free LAB									
Assessed		CC	C5	C10	C15	CF	F5	F10	F15		
						Fresh					
	F	42 BCa	43 ABa	44 ^{Aa}	40^{DEa}	41^{CDa}	$42 ^{\text{BCa}}$	43 ABa	39 E		
В&Т		32 Aa	32 Aa	33 Aa	30 ^{Ba}	32 Aa	32 Aa	33 Aa	30 ^B		
M. P		8 Aa	9 Aa	9 Aa	9 Aa	8 Aa	9 ^{Aa}	9 ^{Aa}	9 Aa		
C		9 Aa	9 Aa	9 Aa	8 Aa	9 Aa	9 ^{Aa}	9 ^{Aa}	8 Aa		
Г		91^{DEa}	93 BCa	95 ^{Aa}	87 ^{Fa}	90 ^{Ea}	92^{CDa}	94^{ABa}	86 ^F		
					3	0 Days					
	F	41 ^{ABCa}	42 ABab	43 Aab	39 BCa	40 ^{BCa}	41 ABCa	42^{ABa}	38 ^c		
В&Т	-	32 Aa	32. Aa	33 Aa	30 Ba	32 Aa	32 Aa	33 Aa	30 E		
M. P		8 ^{Aa}	9 Aa	9 Aa	9 Aa	8 Aa	9 Aa	9 Aa	9 Aa		
C		9 Aa	9 Aa	9 Aa	8 Aa	9 Aa	9 Aa	9 Aa	8 Aa		
C T		90 DEa	92 ^{BCa}	94 ^{Aa}	86 ^{Fa}	89 ^{Ea}	91 CDa	93 ^{ABa}	85 F		
					6	0 Days					
	F	39 Bb	41 ^{Abc}	42 Abc	37 ^{CDb}	38 ^{BCb}	39 Bb	39 Bb	36 ^D		
В&Т	-	31 ABa	31 ABab	32 Aab	29 CDa	30 ^{BCb}	30 BCb	31 ABb	28 ^D		
M. P		8 Aa	9 Aa	9 Aa	9 Aa	8 Aa	9 Aa	9 Aa	9 Aa		
С		9 Aa	9 Aa	9 Aa	8 ABa	9 Aa	8 ABab	8 ABab	7^{Bal}		
T		87 ^{Сь}	90 ^{Bb}	92 Ab	83 Eb	85 ^{Db}	85 ^{CDb}	87 ^{Сь}	80 F		
					9	0 Days					
	F	37 ^{Bc}	40 ^{Ac}	41 Ac	35 CDc	36 ^{BCc}	37 ^{Bc}	37 ^{Bc}	34 ^D		
В&Т	-	29 BCb	30 ^{ABb}	31 Ab	27^{DEb}	28 ^{CDc}	29 ^{BCb}	30 ^{ABb}	26 ^E		
M. P		- 8 Aa	9 Aa	9 ^{Aa}	9 Aa	- 8 Aa	-9 ^{Aa}	9 Aa	9 Aa		
C		8 Aa	8 Aa	8 Aa	7^{ABa}	8 Aa	7 ^{ABb}	7 ^{ABb}	6 ^{Bb}		
Ť		82 ^{Ce}	87 ^{Bc}	89 Ac	78 ^{Ec}	80 ^{Dc}	82 ^{Ce}	83 ^{Ce}	75 F		

F : Flavour

B & T : Body & Texture

C: Colour

M. P: Melting Properties T: Total score

A, B, C: Means with the same letter among treatments in the same storage period are not significantly different.

a, b, c: Means with the same letter in the treatment during storage periods are not significantly different.

Conclusion

Functional frozen yoghurt was successfully manufactured by adding artichoke puree in different ratios. The most preferable is that of 10 % added puree using encapsulated LAB (*Lb. bulgaricus, S. thermophilus* and *Lb. rhamnosus*) with acceptable quality attributes flavour, body & texture and total preference. This study also approved that, the added artichoke puree up to 10, 15 % increased the survival of encapsulated LAB under the conditions of manufacture and storage even after 90 days.

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صناعة الزبادى المجمد بإستخدام مهروس الخرشوف وبكتريا حمض اللاكتيك الحرة أو في كبسولات

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امكن صناعة مخاليط مختلفة من الزبادي المجمد بإستخدام مهروس الخرشوف بنسب 5 , 10 , 15 ٪ مع بكتريا Streptococcus thermophilus, Lactobacillus delbrueckiisub sp. Bulgaricus, Lb. rhamonsus سواء في كبسولات أو حرة. تم تقدير التركيب الكيماوي ، الأنيولين ، مضادات الأكسدة ، قيم الـ Hp ، القابلية للإنصبهار، اللزوجة وكذلك التقييم الحسى والعدد الميكروبي لكل المعاملات سواء طازجة أو أثناء التخزين لمدة 90 يوم. وقد أوضحت النتائج أن المعاملات المضاف لها مهروس الخرشوف ذات البكتريا سواء المكبسلة أو الحرة كانت مستويات مضادات الأكسدة بها أعلى عن عينات المقارنة. وقد كانت الإختلافات فى مستويات مضادات الأكسدة بين المعاملات معنوية. كما أوضحت النتائج أنه كلما زادت نسبة إضافة مهروس الخرشوف كلما زادت نسبة المواد المضادة للأكسدة. بالنسبة للمعاملات المضاف لها مهروس الخرشوف ، كلما زادت نسبة إضافة المهروس كلما انخفضت نسبة قابلية المعاملات للإنصهار وذلك حتى للمعاملات ذات البكتريا المكبسلة. وقد لوحظ أن العينات ذات البكتريا المكبسلة كانت نسب قابليتها للإنصبهار أقل من العينات ذات البكتريا الحرة عند كل نسب الإضافة وحتى العينة المقارنة. كما وجد أن المعاملات ذات البكتريا الحرة كان لها قيم لزوجة أقل من العينات ذات البكتريا المكبسلة عند نفس نسب الإضافة من وعند اجراء التقيم .pH المهروس وحتى العينة المقارنة، هذا الإنخفاض في قيم اللزوجة له علاقة بقيم الـ الحسى للعينات وجد أنَّ العينات ذات البكتريا المكبسلة لها طعم لبني حامضي خفيف وقد ساعد مهروس الخرشوف على تحسين الخواص الحسية من حيث النكهة والقوام والتركيب وكذلك الخواص الكلية. وتشير نتائج هذه الدراسة الى أن إضافة مهروس الخرشوف بنسبة 10 ٪ اثناء تصنيع الزبادي المجمد بإستخدام بكتريا

حمض اللاكتيك في كبسولات يؤدى الى إنتاج منتج لبني ذو فوائد صحية كبيرة وذو صفات حسيه مفضلة