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Original Article

Changes in the viability of free and microencapsulated *Lactobacillus rhamnosus* during storage of ice milk fortified with sweet cherry

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A B S T R A C T

Four ice milk samples were produced, one of them served as control (C, no added sweet cherry pulp (SCP), and containing free cells of *L. rhamnosus*) and the other three treatments containing 30 % SCP and free cells (T_1), encapsulated cells using sodium alginate-skim milk (T_2), or encapsulated cells using sodium alginate-denaturated whey protein (T_3). The fresh ice milks were subjected to sensory evaluation, physicochemical properties (overrun, and meltdown tests) while the changes in pH, *L. rhamnosus* viability, total phenolic content (TPC), and antioxidant activity (AOA) have been monitored at 1, 15, and 30 days of frozen storage. SCP had higher TSS (17.5%), total sugar (13.24%), higher TPC (112 mg GAE/100g), and AOA (84.16%) while it had a lower pH value (4.14). Unlike the effect of having probiotic bacteria, adding SCP increased overrun, and decreased pH and melting rate of ice milks. Also, pH decreased over time, and the lowest value was observed for T_3. *L. rhamnosus* was strongly protected (had the highest survival rate of 95.94% for T_3) using alginate-denaturated whey protein followed by alginate-skim milk (90.04% for T_2). Adding SCP increased TPC and AOA of ice milks and their values were decreased during storage. Regarding sensory properties, all samples were acceptable. It could be concluded that encapsulation using sodium alginate blended with skim milk or denaturated whey protein improved the viability of *L. rhamnosus* in ice milk.

Introduction

Nowadays, probiotic dairy foods received more attention due to their enhanced functionality. Probiotics are defined as a group of microorganisms in a viable form that can improve the host's health when existing in sufficient numbers, higher than 10^6 CFU/g or mL (Terpou *et al.*, 2018).

Ice cream is amongst the most popular and consumable dairy foods by populations of all ages (Karaman *et al.*, 2014). Also, ice milk is another frozen dessert refer to a standardized frozen dessert class with fat content not less than 3% and does not reach 5%. This definition of ice milk fully complies with the Egyptian standard No. 1185-1/2005.

Besides its acceptable and popularity, fruit-fortified ice cream is characterized by its higher functionality and antioxidative properties due to the presence of polyphenols, pigments, and antioxidants. Due to its preferable delicious taste, color, and higher antioxidant activity (Piccolella *et al.*, 2008), sweet cherry fruit represents a suitable to be incorporated into ice milk and ice cream.

Despite the suitability of ice cream as a suitable medium for delivering probiotics to humans, probiotic viability is declined during processing and storage. This effect result from the stress conditions (formulation, freezing, overrun, storage, and thawing) existing during the processing and storage of ice cream (Mohammadi *et al.*, 2011). In particular, probiotic bacteria are exposed, during freezing and melting steps, to various degrees of damage, ranging from lethal effect (bacterial death) to sublethal effect (inhibition of growth and metabolic activity) (Davies and Obafemi 1985). Furthermore, probiotic viability could be partially lost during their passage along the gastrointestinal tract especially under acidic conditions reported for the stomach (Song *et al.*, 2012).

Recently, microencapsulation represents a successful strategy that protected probiotics against the harsh conditions during food production and storage (Rokka and Rantamaki, 2010) keeping them in viable form and sufficient numbers by being trapped inside specific encapsulating agents (Abd El-Salam and El-Shibiny, 2015). The extrusion method represents the simplest technique used for encapsulation (Lee *et al.*, 2019). The incorporation of some polymers such as whey protein can improve the microencapsulation efficiency

and therefore probiotics protection (Abd El-Salam and El-Shibiny, 2015). Accordingly, *L. acidophilus* viability has been improved by 40% through the encapsulation using whey protein isolate-alginate polymer (Dehkordi *et al.*, 2019).

Given the above-mentioned, the purpose of the present study aimed to the evaluation of the effectiveness of encapsulating biopolymers formulated from sodium alginate blended with skim milk or denaturated whey protein in protecting the viability of microencapsulated *L. rhamnosus* (as compared to free cells) added to ice milks fortified with sweet cherry pulp (as a probiotic delivery system model).

2. Materials and methods

Chemicals, reagents, and fruit

Fresh sweet cherries (*Prunus avium*) were obtained from a local market in Giza, Egypt. Fresh skim milk (0.3% fat and 9 % SNF) and cream (68% fat) were obtained from the Dairy Technology Unit, Faculty of Agriculture, Cairo University. Skim milk powder (97% TS) was obtained from Dairy America™, USA. Sugar was obtained from a local market in Giza, Egypt. Carboxymethyl cellulose (CMC) was obtained from Misr Food Additives Company (MIFAD), Egypt. 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, Folin-Ciocalteu reagent were purchased from Sigma Chemical Co. (Saint Louis, MO, USA). Sodium alginate and hydrochloric acid pure (35–38%) were purchased from Loba Chemie, Pvt Ltd - Mumbai, India. Whey protein concentrate (80%) was obtained from milkiland Intermarket, Poland. The culture media (MRS broth and MRS agar) were obtained from CONDA (Spain) while peptone was purchased from S D Fine-Chem Limited (Mumbai, India). Sodium chloride, sodium bicarbonate, trisodium citrate, and sodium hydroxide were purchased from El Nasr pharmaceutical chemicals, Cairo, Egypt. Other chemicals used were of analytical grade.

Sweet cherry pulp (SCP) preparation

The fruit was well-washed using tap water and the seeds have been removed using a knife. The cut fruit was well-mixed by a blender (Ultra Turrax blender IKA, Merc, Germany) at 1200 rpm for almost 1 min. After that, it was filtered using double layer-cheese clothes and then frozen stored until use.

Propagation of microbial culture

Lactobacillus rhamnosus was kindly provided by Food Science and Human Nutrition Dept. (NUTBRO group), Faculty of Veterinary Sciences, University of Murcia, Spain. This strain has been anaerobically activated using MRS broth for 48 h at 37℃ and subcultured thrice to obtain high biomass. After that, the harvested cells, by centrifugation at 4 ℃ for 20 min at 4000 rpm, were washed using saline solution (0.9% w/v, NaCl). Under the same centrifugation conditions, the cell biomass was recovered and suspended in a suitable volume of saline solution (Fareez *et al.*, 2015) and was subsequently microencapsulated using alginate biopolymer (combined with skim milk or denaturated whey protein) or used as non-encapsulated (free) cells.

Alginate biopolymers preparation

During the present study, two combinations of alginate biopolymers have been prepared in which sodium alginate, represents the main biopolymer. Sodium alginate has been combined with skim milk (named Alg-SM) or denaturated whey protein (named Alg-DWP). The first biopolymer named Alg-SM was prepared as described by Shi *et al.* (2013) by mixing two parts of 3 % (w/v) sodium alginate solution with one part of sterilized skim milk while the second biopolymer named Alg-DWP was prepared according to Rajam *et al.* (2012) by mixing one part of 3 % (w/v) sodium alginate solution with one part of 10 % sterilized freshly prepared denatured whey protein solution.

Procedure of microencapsulation

According to Feucht and Kwak (2013), cell suspensions have been microencapsulated by the extrusion method using the two freshly prepared biopolymers of Alg-SM or Alg-DWP. Four parts of the biopolymers were blended with the cell suspension (one part) with stirring for 10-20 min. Extrusion of the mixture has been conducted using a sterile syringe (0.5 mm, 25 mL) into a solution of $CaCl_2$ (0.2 M) which served as a hardening solution with moderate stirring for 30 min to assure full gellification. The created microcapsules have been harvested, through filtration, washed using sterile saline solution, and kept at 5ºC until use.

Encapsulation efficiency

The viable probiotic counts entrapped in alginate microcapsules (beads) have been dissolved in tri-sodium citrate solution (2%) and the samples were subjected to serial dilution up to 10 fold and poured plated on MRS agar. The plates were anaerobically incubated for 72 h at 37ºC. The numbers of colonies were counted and recorded as a colony-forming unit (CFU) per gram of microcapsule. The encapsulation efficiency (EE) was calculated as described by Kailasapathy (2006). EE = N / N0 × 100 where N: number of colonies from beads while N0: number of colonies from the free cell suspension.

Ice milk processing

The ice milk was manufactured according to the slightly modified procedure described by Karthikeyan (2013). Four ice milk mixes (1 kg each) were prepared and standardized to contain, 4% fat, 11% solids not fat (SNF), 12% sugar, and 0.2% stabilizer. The formulation of ice milk mixes is presented in Table 1. All ingredients were well-mixed until no clumps were present, heat-treated to 80 °C for 1 min then cooled to 5º C. *L. rhamnosus* strain have been incorporated into the ice milk mixes at a rate of 1% for the free cell suspension or 2% of alginate beads (adding 1 of free cell suspensions and 2% of microencapsulated cells yields the same viable counts of *L. rhamnosus* added to the products mixes). For improving the whipping properties of the mix, each mix has been undergone to aging process at 5º C overnight where this process cools it down before freezing, enables the partial crystallization of milk fat, and provides more time for the stabilizer for better hydration. Afterward, the aged mix was frozen in an ice cream freezing machine (Taylor-mate model 165, Italy). The resultant ice milk samples were packaged in 100mL-plastic cups, stored at - 20 °C for 24 h to harden, and stored for 30 days.

The different ice milks tested in the current study were the following:

C: the control ice milk sample without added sweet cherry pulp and contains 1% of free cells of *L. rhamnosus*; T1: ice milk with added sweet cherry pulp (30%) and 1% of free cells of *L. rhamnosus*; T2: ice milk with added sweet cherry pulp (30%) and 2% of microencapsulated *L. rhamnosus* using sodium alginate-skim milk; and T3: ice milk with added sweet cherry pulp (30%) and 2% of microencapsulated

L. rhamnosus using sodium alginate-denaturated whey protein.

Ice milk samples have been subjected to sensory evaluation, overrun, meltdown test at the first day of storage while changes of pH, viable counts of *L. rhamonosus*, total phenolic content (TPC), and antioxidant activity (AOA) have been estimated at 1, 15, and 30 days of storage time. All analyses were conducted in three replicates.

Analytical determination

Chemical composition

Total soluble solids (TSS) content (as Brix) in SCP was measured at 20 º C by refractometer (Abbe Hergestellt in der DDR, Germany). Total sugars of SCP were estimated according to AOAC (2012). pH values were measured in SCP and ice milk samples by pH meter (Hanna, digital pH meter, Spain).

Functional properties

HPLC analysis of SCP individual polyphenols

The individual polyphenols were estimated as described by Schieber *et al.* (2001) using HPLC Agilent 1200 series equipped with a C18 column reverse phase (Zorbax ODS 5 pm 4.6 x 250 mm) maintained at 35 °C, auto-sampler, solvent degasser, ultraviolet (UV) detector set at 280 nm and quarter HP pump (series 1050). The mobile phase is composed of solvent A (water/acetic acid, 98:2) and solvent B (methanol/acetonitrile, 50: 50) at a flow rate of 1 ml/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (5% B); 0–25 min (30% B); 25-35 min (40% B); 35-40 min (52% B); 40-50 min (70% B) and 50-55 min (100% B). The injection volume was 10 µl. The identification and quantification of all polyphenols have been conducted through the comparison with the peak area recorded for external standards.

TPC estimation by Folin–Ciocalteu assay

For extracting the phenolic compounds, methanol was well-mixed with ice milk (1:10 w/v) or SCP (1:100 w/v). The mixture was then subjected to centrifugation for 10 min at 1300 g. The obtained supernatant has been used in TPC determination as described by Singleton and Rossi (1965) slightly modified by Vital *et al.* (2018). The obtained data have been recorded as mg gallic acid equivalent (GAE) per 100g of ice milk or SCP. The ice milk was analyzed at 1, 15, and 30 days of storage.

DPPH assay

An aliquot of the methanolic extract of SCP and ice milk, obtained for TPC determination, has been used in the DPPH assay according to the method of Brand-Williams *et al.* (1995) slightly modified by Li *et al.* (2009).

Overrun and meltdown tests

The overrun of the obtained ice milk was estimated according to Akin *et al.* (2007). The meltdown test has been carried out as reported by Muse and Hartel (2004) under specific and fixed temperature (25℃). During one hour, the melted volume has been recorded every 15 min intervals. Results were expressed as time (min) against drained volume (%) and the slope of the curve was taken as the melting rate.

Probiotic survivability

The viable counts of *L. rhamnosus* were determined in ice milk samples at 1, 15, and 30 days according to Terpou *et al.* (2017). In brief, 10 g of ice milk were aseptically collected at different sampling points and diluted in 90 mL of 2% tri-sodium citrate (in case of the samples containing the alginate microcapsule) or 90 mL of sterilized saline solution (in case of samples containing the free cells) and then were vortexed. After that, serial dilutions have been prepared up to ten-fold using saline solution. The viable counts of *L. rhamnosus* were counted using pour plate technique on MRS agar under anaerobic conditions for 72 h at 37ºC. The viable counts of *L. rhamnosus* have been recorded as log CFU/g and also as survival rate percent (Magarinos *et al.*, 2007).

Sensory properties

According to Di Criscio *et al.* (2010), all ice milks remained for 5 min at 20℃ before they were subjected to a sensory evaluation which takes place the next day after production and storage at -18℃. Ice milk samples have been sensory evaluated according to Pimentel *et al.* (2013) with slight modifications. A 20 g serving has been assessed by ten qualified panelists belonging to Dairy Research Dept., Food Technology Research Institute, Giza, Egypt. Each sample was presented in a 50-mL disposal plastic cup and coded by using 3-digit random numbers. The samples were evaluated for color (15), flavor (45), texture (40), and overall acceptability (100 points).

Statistical analysis

The data were expressed as mean ± SD of three independent replicates. All data (except that obtained on the first day of ice milk storage) have been statistically analyzed with two-way ANOVA to identify the significant differences between the means of samples and storage period. Tukey test was used for the comparison between means at confidence interval set at 95%.

Results and discussion

Chemical composition and individual polyphenols of SCP

Sweet cherry fruit is known for its higher content of soluble solids content, and sugars (Table 1). The results showed in Table 1 displayed that this fruit had higher TSS (17.50 %), and total sugars content (13.24%). Also, the pH value of cherry pulp was 4.14. Great variations have been observed regarding these parameters of cherry fruit in the literature. The reason for these variations could be mainly due to the variation in its cultivar, which is considered the main contributor factor for that variations. For instance, Serradilla *et al.* (2012) obtained similar values (15 – 20 °Brix) of TSS for three cultivars of sweet cherry. Also, higher values (19.5 - 21.4 °Brix) were obtained by Bernalte *et al.* (1999) for other cherry cultivars. Regarding pH values, almost similar wide values of pH ranged between 3.76 and 4.36 have been obtained by Serradilla *et al.* (2012). Concerning Total sugars content, Nawirska-Olszanska *et al.* (2017) displayed that total sugars content in sweet cherries ranged between 7.7–26.5%.

Also, the data exist in Table 1 displayed higher content of TPC and AOA (112 mg GAE/100g FW, and 84.16 %, respectively) for SCP. Rotrigues *et al.* (2011) reported that TPC of sweet cherry fruit was ranged between 95.14 – 170.35 mg GAE/100 g FW. The higher TPC content might be reflected in a higher antioxidant activity which can exert health-promoting activities upon consumption (Rotrigues *et al.*, 2011). In this regard, the antioxidant activity of SCP was 84.16 % (Table 1). A wide range of antioxidant activity

ranged between 13 – 90% obtained by Skrzyński *et al.* (2016) was associated with the variation in the fruit cultivars. Also, results present in Table 1 and Fig. 1 demonstrated that gallic acid was the predominant phenolic compound (297.14 µg/g) followed by chlorogenic acid (99.81 µg/g), rutin (40.35 µg/g), syringic acid (23.74 µg/g), catechin (20.23 µg/g), ferulic acid (11.91 µg/g), methyl gallat (3.65 µg/g), coumaric acid (2.78 µg/g), and cinnamic acid (0.56 µg/g).

Table 1. Formulation of ice milk mixes used in the current study (g/100g).

Ingredient (g)	C	T1	T2	T3
Fresh skim milk	80	52.8	52.8	52.8
Skim milk powder	1.9	1.3	1.3	1.3
Cream (68 % fat)	5.9	5.9	5.9	5.9
Sugar	12	8.1	8.1	8.1
Stabilizer	0.2	0.2	0.2	0.2
Sweet cherry pulp	----	30	30	30
***L. rhamnosus* (%)**	1	1	2	2

C: control ice milk mix without added sweet cherry pulp + 1 % free *L. rhamnosus*, T1: ice milk mix with added 30 % sweet cherry pulp + 1 % free *L. rhamnosus*, T2: ice milk mix with added 30 % sweet cherry pulp + 2 % encapsulated *L. rhamnosus* using alginate-skim milk; T3: ice milk mix with added 30 % sweet cherry pulp + 2 % encapsulated *L. rhamnosus* using alginate-denaturated whey protein.

Table 2. Some chemical composition, total phenolic content (TPC, mg GAE/100g), antioxidant activity (%), and individual phenolic compounds in sweet cherry pulp.

Parameters		Sweet cherry pulp
TSS (%)		17.50 ± 0.06
pH value		4.14 ± 0.01
Sugars (%)		13.24 ± 0.27
TPC (mg GAE/100g)		112 ± 2.65
AOA (%)		84.16 ± 0.75
Individual polyphenols (µg/g)	**Gallic acid**	297.14
	Chlorogenic acid	99.81
	Rutin	40.35
	Syringic acid	23.74
	Catechin	20.23
	Ferulic acid	11.91
	Methyl gallate	3.65
	Coumaric acid	2.78
	Cinnamic acid	0.56

Values are means ± SD of three independent replicates. TSS: total soluble solids, TPC: total phenolic content, GAE: gallic acid equivalent, AOA: antioxidant activity.

Physical properties of ice milk

The physical properties of ice milk samples are present in Fig. 2. Results displayed lower values (21.44 - 25.04%) of overrun for the tested samples and were similar to Pinto *et al.* (2012) and Bezerra *et al.* (2015). The current finding confirmed that adding probiotics (encapsulated or not) had no impact on overrun values. Similar results confirmed that overrun was not altered by the presence of lactic acid bacteria (Akalin and Erişir, 2008; Hashemi *et al.*, 2015). The data confirmed that fruit incorporation led to decreased overrun (22.89, 22.58, and 22.18% recorded for T_1, T_2, and T_3). Thus, the highest overrun value (25.04%) was recorded for control ice milk (Fig. 2A). A similar trend has been obtained by adding fig into ice cream formulation (Murtaza *et al.*, 2004).

Regarding the melting data, results indicated that the control ice milk was more slowly melted down while T3 sample had the highest melting rate (Fig. 2B). In agreement with the current data, Sofjan and Hartel (2004) exhibited that ice cream samples with a higher overrun level melt at a slower rate. This is because the air acts as a barrier to prevent heat transfer leading to reducing the melting rate. Thus, the decreased melting rate could be due to fruit addition while adding probiotic bacteria did not change the melting rate of ice milk samples. A similar tendency was found by Alamprese *et al.* (2005) who confirmed that incorporation of *L. rhamnosus* GG into ice cream mixes had no impact on ice cream melting resistance, alongside its minimal effect on overrun. Accordingly, there is an inverse association between the overrun level and the melting rate of ice milk.

Also, results shown in Fig. 2C indicated that pH values were higher in control ice milk and lower in added-cherry fruit ice milk treatments. This observation could be due to the naturally lower pH value of sweet cherry pulp (Table 2). Generally, the pH values slightly decreased over time.

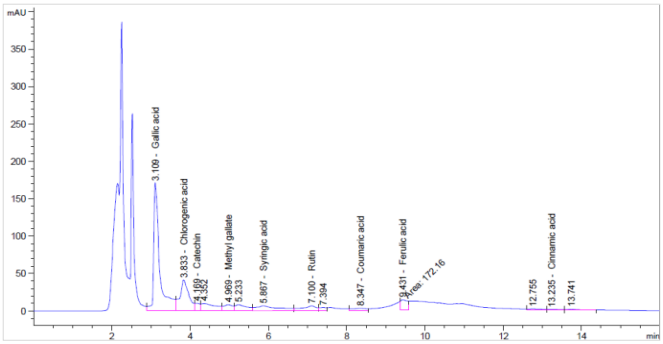

Fig. 1. HPLC chromatogram profile of the individual polyphenols of sweet cherry pulp at 280 nm using UV detector.

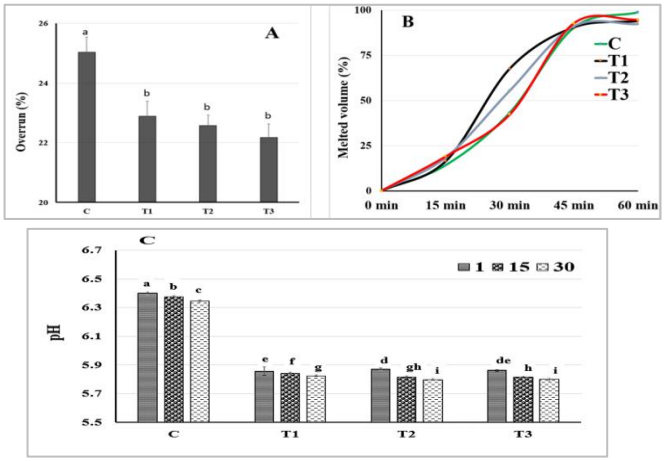

Fig. 2. Physical properties of ice milk samples fortified with sweet cherry pulp during frozen storage for 30 days: A) overrun (%), B) melted volume (%), and C) pH values. Values are means ± SD of three independent replicates. Means with different superscripts are significantly different ($p < 0.05$). C: control ice milk without added sweet cherry pulp + 1 % free *L. rhamnosus*, T1: ice milk with added 30 % sweet cherry pulp + 1 % free *L. rhamnosus*, T2: ice milk with added 30 % sweet cherry pulp + 2 % encapsulated *L. rhamnosus* using alginate-skim milk; T3: ice milk with added 30 % sweet cherry pulp + 2 % encapsulated *L. rhamnosus* using alginate-denaturated whey protein.

A similar pH trend has been obtained by adding acerola pulp (Fávaro-Trindade *et al.*, 2006) which had a naturally lower pH value. Also, Celik *et al.* (2006) observed that yoghurt with added higher content of cornelian cherry paste had a lower pH value due to the naturally lower pH of the added fruit.

Viability of *L. rhamnosus*

Results in Table 3 revealed that the viability of *L. rhamnosus* was strongly affected by the progress of frozen storage. However, the viable counts of this probiotic strain remained above the therapeutic level (10^6 CFU/g). The highest viable numbers have been observed for T3 treatment (ice milk + sweet cherry fruit + microencapsulated cells with Alg + DWP) as compared to other treatments including control ice milk. Also, the microencapsulated cells (T3, then T2) had a higher survival rate (95.94, and 90.04%, respectively) while the lowest survival rate was recorded for control ice milk (81.66%). The higher survival rates described for T3 and T2 demonstrated the importance of microencapsulation technology in protecting the probiotic bacteria from the harsh conditions associated with ice milk processing and storage. Besides, the higher survival rate of probiotic strain could also be partially explained by the lower overrun values recorded for T2 and T3 treatments. Moreover, probiotic bacteria could be able to survive more in food matrix containing phytochemicals as several probiotic bacteria capable of metabolizing it and thus avoiding the expected viability loss. Akalin & Erisir (2008) and Bezerra *et al.* (2015) reported similar findings regarding the effect of overrun and the presence of plant phytochemicals on probiotics viability. Besides, encapsulating agents play a pivotal role in maintaining the higher viability of probiotics. In this regard, the survival rate of *L. acidophilus* has been improved in the gastric fluid by 40% by adding whey protein isolate (Dehkordi *et al.*, 2019). It is worth noting that the encapsulation efficiency recorded in the present study was 94.87 and 92.47% resulted from using Alg-DWP, and Alg-SM, respectively.

Table 3. Viable counts (log CFU/g) and survival rate (%) of *L. rhamnosus* in ice milk samples fortified with sweet cherry pulp during frozen storage for 30 days

Treatment	Viable counts of *L. rhamnosus* (log CFU/g)			Survival rate (%)
	1 day	15 day	30 day	
C	9.03 ± 0.02^{bc}	8.55 ± 0.12^{d}	7.38 ± 0.23^{g}	81.66 ± 2.67^{c}
T1	9.13 ± 0.03^{bc}	8.41 ± 0.05^{de}	7.91 ± 0.12^{f}	86.60 ± 1.55^{b}
T2	9.19 ± 0.03^{ab}	9.06 ± 0.05^{bc}	8.28 ± 0.12^{e}	90.04 ± 2.54^{b}
T3	9.33 ± 0.11^{a}	9.19 ± 0.01^{ab}	8.95 ± 0.09^{c}	95.94 ± 2.13^{a}

Values are means ± SD of three independent replicates. Means with different superscripts are significantly different ($p < 0.05$). CFU: colony-forming unit, C: control ice milk without added sweet cherry pulp + 1 % free *L. rhamnosus*, T1: ice milk with added 30 % sweet cherry pulp + 1 % free *L. rhamnosus*, T2: ice milk with added 30 % sweet cherry pulp + 2 % encapsulated *L. rhamnosus* using alginate-skim milk; T3: ice milk with added 30 % sweet cherry pulp + 2 % encapsulated *L. rhamnosus* using alginate-denaturated whey protein.

Phenolic compounds and antioxidant activity in ice milk

The total phenolic content (TPC) and antioxidant activity (AOA) of ice milk are shown in Fig. 3. TPC and AOA values have been increased by adding sweet cherry fruit. The highest TPC and AOA values were detected on the first storage day while the lowest values were observed on the final storage day. T3 sample had the highest TPC (276.61, 216.71, and 208.54 mg GAE/100g) and AOA values (80.30, 68.85, and 59.94%) at 1, 15, and 30 days of storage time.

The sweet cherry pulp is characterized by its higher content of polyphenols (Table 2) which in turn is reflected in its increased antioxidant activity. Thus, cherry fruit-ice milk treatments had higher values of TPC and AOA as compared to ice milk not containing this fruit. Interestingly, the observed variations between T1, T2, and T3 samples could result from the used encapsulating agents. In this regard, denaturated whey protein might have a role in the increased AOA observed for T3 sample. This could be explained by the presence of antioxidative peptides within the whey protein chain (Mohan *et al.*, 2015) which increase the free radical scavenging activity of whey proteins (Gad *et al.*, 2011). During storage, the decreased stability of polyphenols could be attributed to several factors including pH, enzymes, oxygen, storage time, temperature, and light (Bkowska *et al.*, 2003). Hence, frozen storage strongly affected the polyphenols stability, and therefore phenolic compounds in ice milk deeply degraded during storage. Freezing of acerola pulp provoked a decline in the content of flavonoids and anthocyanins (Lima *et al.*, 2002). Similarly, a reduction in TPC and AOA values has been observed during storage (Fig. 2A, and B). It is well-known that the degradation of polyphenols is necessarily followed by a corresponding decrease in antioxidant activity. These results are in general accordance with that reported by Karaaslan *et al.* (2011) who recorded a similar tendency in yoghurt fortified with grape and callus extracts.

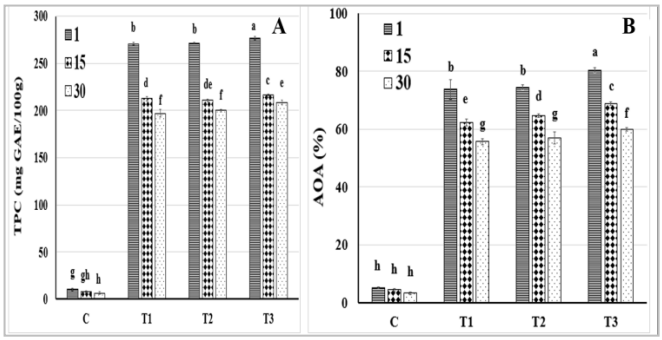

Fig. 3. A) Total phenolic content (TPC, mg GAE/100g); and B) antioxidant activity (AOA%) in ice milk samples fortified with sweet cherry pulp during frozen storage. Values are means ± SD of three independent replicates. Means with different superscripts are significantly different ($p < 0.05$). C: control ice milk without added sweet cherry pulp + 1 % free *L. rhamnosus*, T1: ice milk with added 30 % sweet cherry pulp + 1 % free *L. rhamnosus*, T2: ice milk with added 30 % sweet cherry pulp + 2 % encapsulated *L. rhamnosus* using alginate-skim milk; T3: ice milk with added 30 % sweet cherry pulp + 2 % encapsulated *L. rhamnosus* using alginate-denaturated whey protein. GAE: gallic acid equivalent.

Sensory evaluation

Table 4 showed the sensory attributes of ice milks. The findings exhibited that all ice milk samples acquired high scores and were acceptable. The control ice milk gained the highest overall acceptability (94.83) followed by ice milk samples fortified with cherry fruits (94, 94, and 94.33 for T1, T2, and T3, respectively). Although there were insignificant differences between control and ice milk treatments in color and texture, significant changes were observed regarding flavor descriptor between control and T1 which contains free

cells of *L. rhamnosus* and 30 % sweet cherry pulp. Similar lower flavor scores were gained by frozen yoghurt samples containing probiotic bacteria and jambolan fruit (Bezerra *et al.,* 2015) or acerola fruit (Favaro-Trindade *et al.,* 2006). This tendency of consumers may result from their domesticity with the flavor of dairy products produced using the conventional yoghurt bacteria. Additionally, the great change in product flavor could result from the formation of organic acids and ethanol by the incorporated microorganisms. The current findings are comparable with those reported by Jalili *et al.* (2009). Regarding ice milk texture, no significant changes were observed by adding cherry fruit and probiotic bacteria. The panelists did not recognize any granules of microcapsules of *L. rhamnosus* and therefore the texture of ice milk samples was preferable without any impact on the consumer's scores. The most preferable texture was observed for T3. This observation is mostly explained by the presence of whey protein concentrate and sodium alginate as encapsulating agents which could improve the texture of the ice milk sample. Overall, all ice milk samples were organoleptically acceptable and gained high scores. This observation is in general agreement with that reported by Delikanli and Ozcan (2014).

Table 4. Sensory properties of fresh ice milk samples fortified with sweet cherry pulp

Treatment	Color (15)	Texture (40)	Flavor (45)	Overall acceptability (100)
C	13.50± 0.50^{a}	38.00± 1^{a}	43.33± 0.58^{a}	94.83± 2^{a}
T1	13.33± 0.58^{a}	38.67± 0.58^{a}	42.00± 0.50^{b}	94.00±1.32^{a}
T2	13.00± 1^{a}	38.67± 0.58^{a}	42.33± 0.58^{ab}	94.00± 1^{a}
T3	13.00± 1^{a}	39.00± 1^{a}	42.33± 0.58^{ab}	94.33±1.53^{a}

Values are means ± SD of three independent replicates. Means in the same column with different superscripts are significantly different ($p < 0.05$). C: control ice milk without added sweet cherry pulp + 1 % free *L. rhamnosus*, T1: ice milk with added 30 % sweet cherry pulp + 1 % free *L. rhamnosus*, T2: ice milk with added 30 % sweet cherry pulp + 2 % encapsulated *L. rhamnosus* using alginate-skim milk*;* T3: ice milk with added 30 % sweet cherry pulp + 2 % encapsulated *L. rhamnosus* using alginate-denaturated whey protein.

Conclusion

Given the above-mentioned, the current study revealed that adding sweet cherry fruit, an excellent source of phenolic compounds and antioxidant activity, led to developing valuable ice milk with high antioxidative properties. Also, the incorporation of *L. rhamnosus* in the microencapsulated form increased its viability by protecting it against the stress factors that exist during ice milk production and storage.

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