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ORIGINAL PAPER

Formulation and assessing characteristics of probiotic ice cream fortified with free and encapsulated iron

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Abstract

Ice cream was supplemented with 50 mg per kg free and encapsulated forms of iron and *Lactobacillus casei* (a probiotic). Physicochemical characteristics such as pH, acidity, overrun, viscosity, melting rate, texture, and lipid oxidation of the enriched ice cream were determined over 90 days at − 18 °C. Furthermore, the survival of *Lactobacillus casei* and sensory attributes were also examined during and at the end of storage. No adverse effect was observed on hardness, adhesion, cohesion, and acidity but viscosity dropped during this period. A significant increase in the lipid oxidation rate was observed, especially in samples supplemented with the free iron. Some reduction in the probiotic counts was observed, however the value was maintained above the minimum threshold of 10^6 cfu/g at the end of 90 days.

Keywords Ice cream · Iron · *Lactobacillus casei* · Microencapsulation · Physicochemical · Survival

Introduction

There is a high demand for novel food products due to their improved taste and health impacts. In recent years, the need for functional foods which supplemented with minerals, bioactive compounds as well as probiotics is on the rise. Functional foods are generally described as edibles that provide essential nutrition and promote health [1, 2]. These foods have demonstrated physiological benefits when consumed in the usual diet. Functional foods mainly include beneficial compounds such as probiotics [3, 4]. Probiotics are defined as microorganisms which provide benefits to the host by delivering beneficial impact on intestinal infection, lactose intolerance, and higher serum cholesterol levels [5, 6]. They also improve the balance of intestinal microflora or microorganisms in the gastrointestinal tract and enhance mucosal defenses against pathogens in the human body [7]. The viability and metabolic activity of probiotic microorganisms must be maintained in all steps of food processing, and they should be able to survive in the gastrointestinal tract [8].

Dairy products such as fermented and non-fermented ice cream are a good vehicle for delivering probiotic bacteria in the human diet [9–11]. Ice cream is a favorite snack amongst all ages which is rich in nutrients and can be enriched further with various functional components. Furthermore, ice cream may be a good candidate to act as a carrier for probiotic bacteria [12]. Iron is a crucial element for the nutritional system of animals and humans. It is an essential component in the structures of cytochrome and several enzymes as well as a part of heme in hemoglobin and myoglobin, where it plays an indispensable role in transporting, storing, and using oxygen. Dairy products are excellent sources of calcium and protein but low in iron. Lack of appropriate quantity of iron may reduce the iron density in our diets particularly when we use high proportion of dairy products [13]. Anemia is a typical nutritional problem among the populations of developing countries. This may be problematic particularly for pregnant women and children who constitute about 30 percent of the world's population. It is said that the anemia and iron deficit may be responsible for more than half of the maternal deaths, worldwide. Absence of iron in

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children's diet may account for poor cognitive development, decreased future learning, reduced resistance to illness, increased perinatal risks for mothers, and abnormal neonatal development [14, 15]. Therefore, it is believed that food fortification with iron could be an appropriate strategy to tackle these shortcomings [16]. Ice cream is a very popular product among all age groups particularly children and adolescents. Therefore, increasing the iron density and its bioavailability in dairy products specially ice cream without importing adverse sensory attributes, can benefit all consumers [17]. However, it is important to realize that the iron compound used in food fortification should be highly bioavailable, does not change the taste and be stable during processing and storage [17]. It is anticipated that free iron fortification of dairy products introduces oxidized off-flavours and color changes due to the lipid pro-oxidation of milk fat.

Microencapsulation is a process that involves the small packaging of solid particles in liquids or gaseous materials. The packaging occurs in the form of miniature sealed capsules. The particles formed can release their contents at controlled rates under the influence of specific conditions [17]. The process creates a shield for the materials it contains – a barrier against chemical reactions used as a tool to protect bioactive compounds that are susceptible to degradation [18]. The process of microencapsulating of targeted functional or sensitive components will to a great extent provide protection of these compounds in many processed food products [19]. It is believed that use of encapsulated iron as a food ingredient may prevent its reactions with other food components and therefore inhibits the change in appearance or taste of food [20].

This objective of the present work was to formulate and produce various ice creams containing free and microencapsulated iron in addition to a known probiotic, *Lactobacillus casei*. Additionally, all formulae were evaluated for their physicochemical, rheological, texture profile, organoleptic characteristics. Moreover, the survival rate of *Lactobacillus casei* in each formula was also examined.

Materials and methods

Low-fat sterile milk (1.5% fat) and milk powder were kindly provided by (Khoshmaze Ice Cream Company, Shiraz, Iran). Other ingredients including sugar (Marvdasht Sugar Company, Fars, Iran), Hydrogenated vegetable oil (Ladan Company, Iran) food grade sodium carboxymethyl cellulose (CMC) (Shandong Yulong Cellulose Technology Co., Ltd., China), Ferrous sulfate (Rouz Daru Company, Iran), vanilla powder (Attarak Co., Iran), panisol (Denisco, Denmark) were used in the formulation of ice creams. Man, Rogosa, and Sharpe (MRS) agar, NaOH, hydrochloric, thiobarbituric, and trichloroacetic acids were all from Merck (Darmstadt, Germany.

Experimental design

Six ice cream formulae were prepared. They were control, free iron fortified (IR), microencapsulated iron fortified (IRME), probiotic (P), probiotic with free iron (IRP), and probiotic with microencapsulated iron (PIRME). The ingredients in each formula is summarized in Table 1. 0.1% *Lactobacillus casei* and/or 50 mg iron (free or encapsulated) per kg were added to respective formulae. The current recommended dietary allowance (RDA) for iron in the United States is 11 mg for men and 15 mg for women per day based on the Food and Nutrition Board and Institute of Medicine (2001) [21].

The proximate analysis of reconstituted milk was performed. Fat and proteins were analyzed using the Gerber and

Table 1 The composition of ice cream formulations

Ingredients	Trials					
	Control	IR	IRME	P	IRP	PIRME
Water (%)	63.8	63.8	63.8	63.8	63.8	63.8
Dry milk(%)	8.5	8.5	8.5	8.5	8.5	8.5
Carboxymethyl cellulose (%)	0.2	0.2	0.2	0.2	0.2	0.2
Stabilizer-emulsifier (%)	0.4	0.4	0.4	0.4	0.4	0.4
Vanilla (%)	0.1	0.1	0.1	0.1	0.1	0.1
Hydrogenated vegetable oil(%)	9	9	9	9	9	9
Sugar (%)	18	18	18	18	18	18
ferrous sulfate (mg/kg)	–	250	250	–	250	250
Lactobacillus casei	–	–	–	+	+	+

Control, (IR): ice cream contains free iron, (IRME): ice cream contains encapsulated iron, (P): ice cream contains 10^9 cfu/g *L. casei*, (IRP): ice cream contains free iron and 10^9 cfu/g *L. casei*, (PIRME): ice cream contains encapsulated iron and 10^9 cfu/g *L. casei*

Kjeldahl method, respectively [22]. The dry matters of milk were determined by drying the milk samples at 100 °C for 3.5 h using a laboratory oven. Lactose content was quantified by subtracting the fat, protein, and total ash percentages from the total solids content [22].

Encapsulation of iron

Alginate beads were produced using a modified extrusion technique [23]. The sodium alginate solution 2.5% was prepared in distilled water. 250 mg of ferrous sulfate (containing at least 50 mg iron) was added to the sodium alginate solution, and sterilized at 121 °C for 15 min. This solution was stored in a refrigerator overnight so that the alginate particles absorb water thoroughly. The suspension was injected by a needle (0.11 mm diameter) into sterile 0.1 mol L^{-1} $CaCl_2$. After 30 min of gelification in $CaCl_2$, the beads were washed and kept in sterile 0.1% peptone solution at 4 °C. The encapsulation efficiency was determined by the atomic absorption spectroscopy system according to Eq. (1) (ASS) (Shimadzu AA-670, Japan) [24, 25].

$$\text{Encapsulation efficiency} = \frac{(\text{iron levels released from the beads})}{(\text{initial iron content})} \times 100 \quad (1)$$

Probiotic culture activation

The probiotic culture of *Lactobacillus casei* (ATCC 39,392, Lyophilized) was obtained from CHR-Hansen (Horsholm, Denmark). The activation was done by inoculating the MRS (Man Rogosa Sharpe) broth culture at 37 °C for 24 h. The probiotic cells were centrifuged (75,005,286 EA, Thermo Fisher Scientific Inc. USA) and thereafter washed in a sterile saline solution with the same centrifugation process. Probiotic bacteria were inoculated into the ice cream. The cells concentration was adjusted at 10^9 cfu/mL. The ice cream mix was frozen at −4 to −5 °C and stored at −20 °C for hardening [2].

Manufacture of ice cream

The ice cream mixes were made in 3 kg batches. The liquid milk was heated to 40–45 °C before mixing the solid ingredients. All of the required ingredients were mixed with the milk thoroughly. The mixes were pasteurized at 85 °C for 15 min and dispersed with stirring for 5 min at 45 °C using a simple mixer (Model 6790; Tefal, Rumilly, France). The ice cream mixes cooled down to room temperature and were stored overnight at 4 °C. Prior to freezing, vanilla powder, *Lactobacillus casei,* free or encapsulated iron were added to the formulae, as appropriate. Then, the mixtures were whipped and frozen using a 3 kg capacity batch ice cream making unit at constant speed (52 rpm) for about 20 min (Model: BQL-12Y; Shanghahi Lisong, Shanghai, China). After freezing, the temperature of soft ice cream reached −5 °C. Each formula then was packaged in a disposable HDPE container; hardened at −30 °C for 2 h, and stored at −18 °C for further analysis [1].

Physicochemical analyses

Physicochemical analyses were performed for 3 days after production. The total solid content of ice creams was determined using an oven set to 102 ± 2 °C (over 4 h) [26]. The pH of the melted samples was measured using a digital pH meter (Model 350 pH meter, Jenway, Dunmow, UK) at room temperature. Acidity was assessed using phenolphthalein as an indicator by titration with 0.1 N sodium hydroxide (NaOH). Acidity was expressed as the percentage of lactic acid [26]. The overrun of the final product was calculated using Eq. (2) [27].

$$\text{Overrun}(\%) = \frac{\textit{Weight of a known volume of mix} - \textit{weight of an equal volume of ice cream}}{\text{Weight of equal volume of ice cream}} \times 100 \quad (2)$$

The viscosities of ice creams were determined at 25 °C using a viscometer (Model RVT; Brookfield Engineering Laboratories, Stoughton, MA, USA) [22]. The melting rates of ice creams were evaluated using a 50 g ice cream sample stored at −18 °C. Briefly, the ice creams were placed on a 0.2 cm wire-mesh screen above a pre-weighed beaker at room temperature (24 °C). The mass of melted ice cream collected in the beaker was recorded every 10 min for 60 min. The texture of the samples was measured using a Texture analyzer (CT3 Texture Analyzer; Brookfield Engineering Laboratories), equipped with a stainless cylindrical probe (6.0 mm diameter, 35 mm height). The sample was compressed twice with the probe to 50% of its height at 2 mm/s rate [28].

The lipid oxidation was evaluated by TBA test according to Benjakal, and Baure (2001) with slight modifications [29]. A mixture of 1gr of ice cream sample, 9 ml of 0.25 N HCl solutions containing 0.375% TBA (Sigma- Aldrich, St. Louis, MO), and 15% trichloroacetic acid (Merck) was heated in a boiling water bath for 10 min. Then, samples were cooled to room temperature and centrifuged at 3500 g for 15 min. The absorbance was measured at 532 nm (a JENWAY 6305 UV/Vis. Spectrophotometer). The standard curve of malonaldehyde was used to calculate the TBA value. The results were expressed as milligrams of malonaldehyde per kilogram of a sample.

Enumeration of probiotic microorganism

Counting the probiotic microorganisms was performed at 1, 30, 60, and 90 days of storage at − 18 °C. Accordingly, 25 g of each ice cream sample were diluted in 225 ml phosphate buffer (pH 7.5) and homogenized for 5 min. Several dilutions were performed using peptone water (0.1%). The *L. casei* was counted by spread-plating 0.1 ml of each dilution in Lp-MRS agar (MRS agar supplemented with 2 g/L lithium chloride and 3 g/L sodium propionate) and incubation at 37 °C for 72 h [30].

Sensory evaluations

30 panelists performed the organoleptic assessment of ice creams after 1 and 90 days of storage at − 18 °C. The flavor, color, texture, and mouthfeel of the samples were evaluated. Score 4 indicated 'excellent,' and 0 showed 'unacceptable'. The panelists were asked to rank the ice creams by scores between 1 and 4.

Statistical analyses

The data were analyzed using SPSS version 20 (SPSS Inc., Chicago, IL, USA). Analysis of variance (ANOVA) was used to determine significant differences among treatments that were likely to affect physicochemical parameters about the viability of the probiotic microorganisms. The Duncan post hoc test was applied to compare mean values when the effect was significant. The sensory data were analyzed by the nonparametric Kruskal–Wallis test, followed by the Mann–Whitney U-test to identify and quantify the contrasts ($p < 0.05$).

Results and discussion

Physicochemical properties

The chemical composition of the reconstituted milk in the ice cream formula was comprised of lactose 4.31 ± 0.01%, protein 2.99 ± 0.01%, dry matter 7.56 ± 0.03%, and fat 0.20 ± 0.02%. In the current study, the encapsulation efficiency of 92% was achieved.

Values of pH, titratable acidity (TA), and overrun

Changes to pH and acidity that occurred during 90-days storage are shown in Fig. 1 and 2, respectively. The reduction in pH and increase in the acidity occurred in all samples

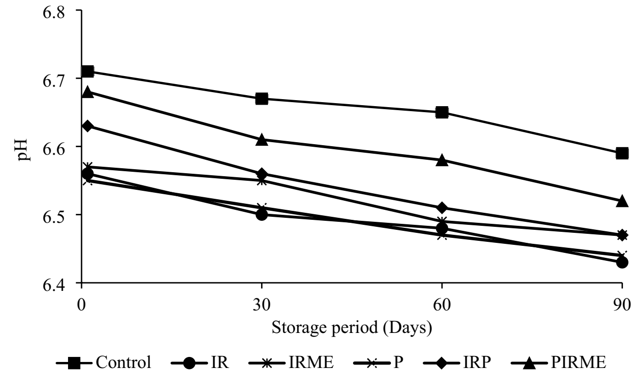

Fig. 1 The pH value of the ice cream control, (IR): ice cream contains free iron, (IRME): ice cream contains encapsulated iron, (P): ice cream contains 10^9 cfu/g *L. casei*, (IRP): ice cream contains free iron and 10^9 cfu/g *L. casei*, (PIRME): ice cream contains encapsulated iron and 10^9 cfu/g *L. casei*

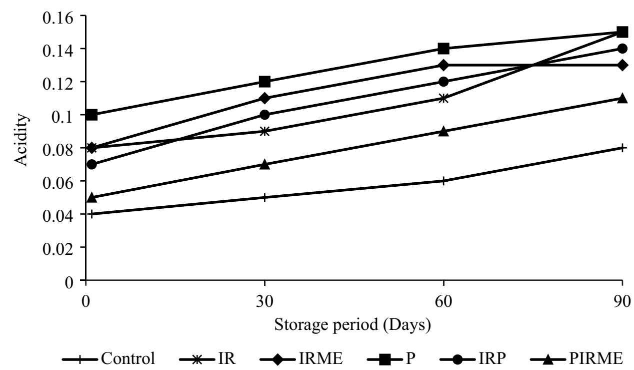

Fig. 2 The acidity (% Lactic acid) of the ice cream samples: control, (IR): ice cream contains free iron, (IRME) ice cream contains encapsulated iron, (P) ice cream contains 10^9 cfu/g *L. casei*, (IRP) ice cream contains free iron and 10^9 cfu/g *L. casei*, (PIRME) ice cream contains encapsulated iron and 10^9 cfu/g *L. casei*

during storage at − 18 °C. The lowest pH was observed in the formulae fortified with iron. Adding iron in its free and encapsulated forms to ice cream decreased the pH and increased the acidity. This may be attributed to the acidities of iron solution and the exchange between iron ions and the micellar bound H^+. These results agree with those reported in the literature [24, 31, 32]. The work carried out by Darwish et al. [15] showed a significant decrease in pH values for products stored for 21 days. The lowest pH value corresponds to products fortified with free iron on the 21st day of storage. An increase in acidity was also observed in iron-fortified yogurt produced by Askari and Bolandi [24] after 21 days storage in a refrigerated condition. In the same study, present of *L. casei* had a similar effect on the pH and acidity during 90 days of storage. The reason for the low pH of products after 90 days storage are attributed to production of lactic acid or acetic acid by probiotics through the

Embden-Meyerhof-Parnas pathway [24]. Presence of probiotic in our formulae also reduces the pH and increase the acidity. These findings were in good agreement with those previously reported in the literature [1, 31, 33]. The results herein were in accordance with Sabet-Sarvestani et al. [34], who produced a probiotic ice cream containing *Lactobacillus casei*/*Lactobacillus plantarum* and showed it had a lower pH than the unfermented ice cream. Also, their results show that the acidity of the fermented ice creams was higher than the unfermented samples due to the conversion of lactose to lactic acid due to the fermentation of LAB. Balthazar et al. [12] and Akalin and Erisir [22] also reported similar results [24].

The values of overruns are displayed in Fig. 3. Addition of iron and the probiotic did not influence the overrun values. Cruz et al. [35] indicated that adding probiotic microorganisms had no effect on the overrun value and did not cause any modification in the firmness of ice cream.

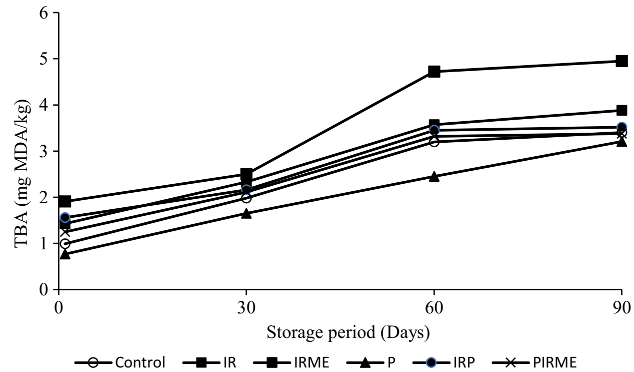

Fig. 4 TBA value (mg MDA/kg) of different groups of ice creams stored at −18 °C for 3 months, (absorbance at 532 nm). Control, (IR): ice cream contains free iron, (IRME): ice cream contains encapsulated iron, (P): ice cream contains 10^9 cfu/g *L. casei*, (IRP): ice cream contains free iron and 10^9 cfu/g *L. casei*, (PIRME): ice cream contains encapsulated iron and 10^9 cfu/g *L. casei*

TBA test during storage

The values of TBA changed during the three months of storage at − 18 °C (Fig. 4). The results showed that TBA values increased in all samples during this period. The highest TBA corresponds to iron fortified ice creams. Previous studies show that iron fortification results in several changes to milk and yogurt. This is to the interaction between iron and casein, resulting in iron-casein complexes; with O_2 molecules acting as a pro oxidant. Moreover, iron can also act as a catalyst and increase fats' oxidation. Therefore, lipid oxidation in dairy products in the presence of iron is not unexpected. The data showed that the oxidation process in formulae containing free iron was faster than those fortified by encapsulated iron. As previously stated, the barrier encapsulation creates, reduces the fat oxidation rates in the products. This was in a good agreement with data reported by Kim et al. [36]. It was reported that TBA absorbance was significantly lower in yogurt when fortified with encapsulated iron than yogurt samples fortified with free iron. Other researchers reported similar results [17, 19].

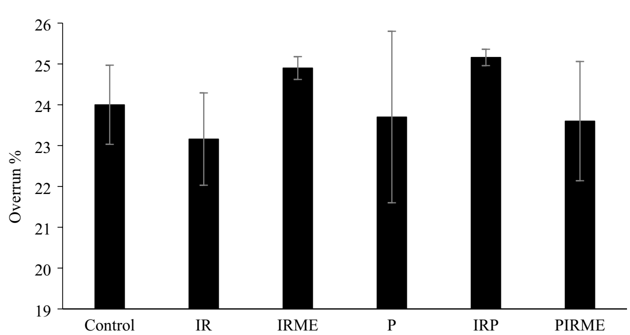

Fig. 3 Mean ± SD of overrun in the ice cream samples on the first day (%)

The lowest TBA value was in the ice cream enriched with the *Lactobacillus casei* alone. The probiotic bacteria can prevent the formation of reactive oxygen species or eliminate oxygen in oxidation reactions catalyzed by an NADH oxidase. This was a confirmation of data previously published by Gheisari et al. [33] and by Kwak et al. [37] for milk samples fortified by iron. The TBA absorbance was significantly lower in encapsulated groups, hence fat stability in dairy products was better maintained [17, 38].

Bacterial population

The *L. casei* counts in various formulae during the three months of storage at − 18 °C indicated in Fig. 5. The probiotic count in all samples decreased during the storage. This reduction if probiotic microbial count may be attributed to the mechanical stresses of the mixing and forming ice crystals during the freezing process [35, 39, 40]. Our results are in line with numerous recent studies on fortification of dairy products with various microbial strains [1, 2, 33, 36, 41]. Balthazar et al. [12] found similar results, who induced the effect of inulin and *L. casei* on probiotic and symbiotic ice cream properties. Their results showed that the bacterial count in ice cream samples were decreased during the storage.

However, a higher mean value of counts in iron-fortified ice creams indicated that iron fortification, in general, increased the growth of probiotic bacteria. This increase was higher when adding free forms of iron compared to encapsulated iron after 60 days of storage. However, after 90 days of storage, no significant difference was observed between these two iron groups ($p < 0.05$). Jayalalitha et al. [19] showed that iron does not affect the survival of

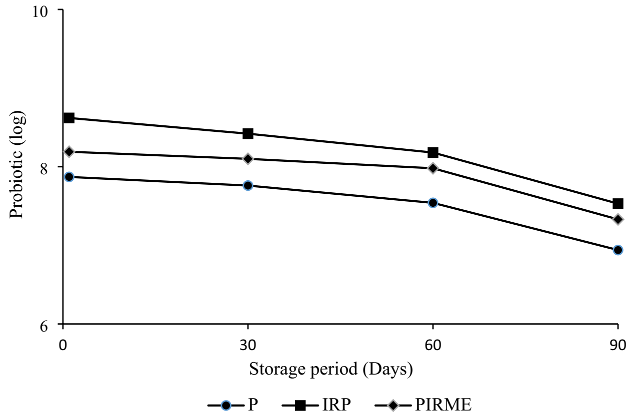

Fig. 5 Survival of *Lactibacillus casei* in ice cream samples: (P) ice cream contains 10^9 cfu/g *L. casei*, (IRP) Ice cream contains free iron and 10^9 cfu/g *L. casei*, (PIRME): ice cream contains encapsulated iron and 10^9 cfu/g *L. casei*

L. acidophilus in yogurt. In their study, probiotic count decreased in all samples after 14 days of storage. Fortifying yogurt with iron did not influence the growth and survival of *Pseudomonas fluorescence* and *Escherichia coli* in work reported by Hekmat and McMahon, 1997 [41]. El-kholy et al. [42] reported that the fortification of yogurt with ammonium ferric sulfate and ferrous ammonium sulfate did not significantly affect the total lactic acid bacteria in all treatments during 10 days of storage at 4 ± 2 °C. On the contrary, in a study on yogurt fortified with iron, a significant decrease was observed in the populations of *Lactobacillus delbrueckii* ssp., *Bulgaricus, and Streptococcus salivarius* ssp, and *thermophiles* after 21 days of storage at 4–5 °C. They attributed this decrease to the metabolic enzymatic activity of the yogurt starter culture that increases the acidity and reduces the pH [17].

A minimum of 10^6–10^7 cfu.g^{-1} of probiotic bacteria in food products could benefit health [39]. Their beneficial effects include improving intestinal microflora's balance and mucosal defenses against pathogens, improved lactose intolerance, and Anti-carcinogenic activity [7]. In our study, despite reductions in viable bacterial numbers during the three months of storage of ice cream, *L. casei* count was well above 10^6 cfu.g^{-1} as recommended for a product to be termed as "a probiotic".

Sensory analysis

The results of sensory scores of the ice cream samples during three months of storage at − 18 °C are shown in Fig. 6. The sensory scores show a reducing trend in all

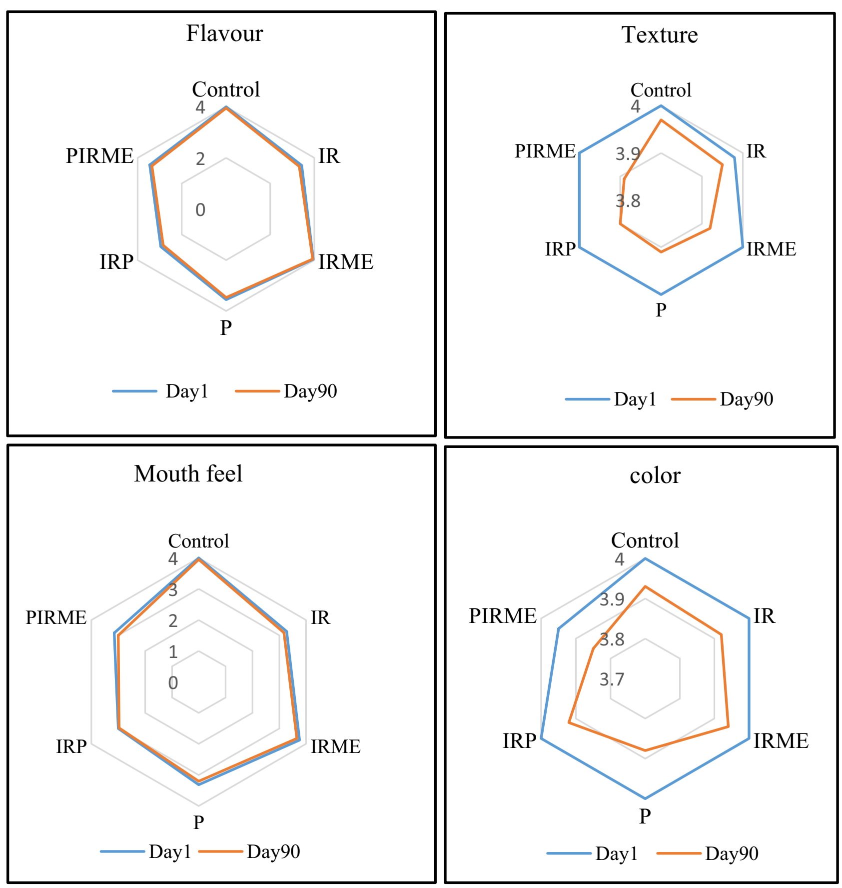

Fig. 6 Sensory evaluation of different groups of ice creams on days 1 and 90 of storage. Control, (IR): ice cream contains free iron, (IRME): ice cream contains encapsulated iron, (P): ice cream contains 10^9 cfu/g *L. casei*, (IRP): ice cream contains free iron and 10^9 cfu/g *L. casei*, (PIRME): ice cream contains encapsulated iron and 10^9 cfu/g *L. casei*

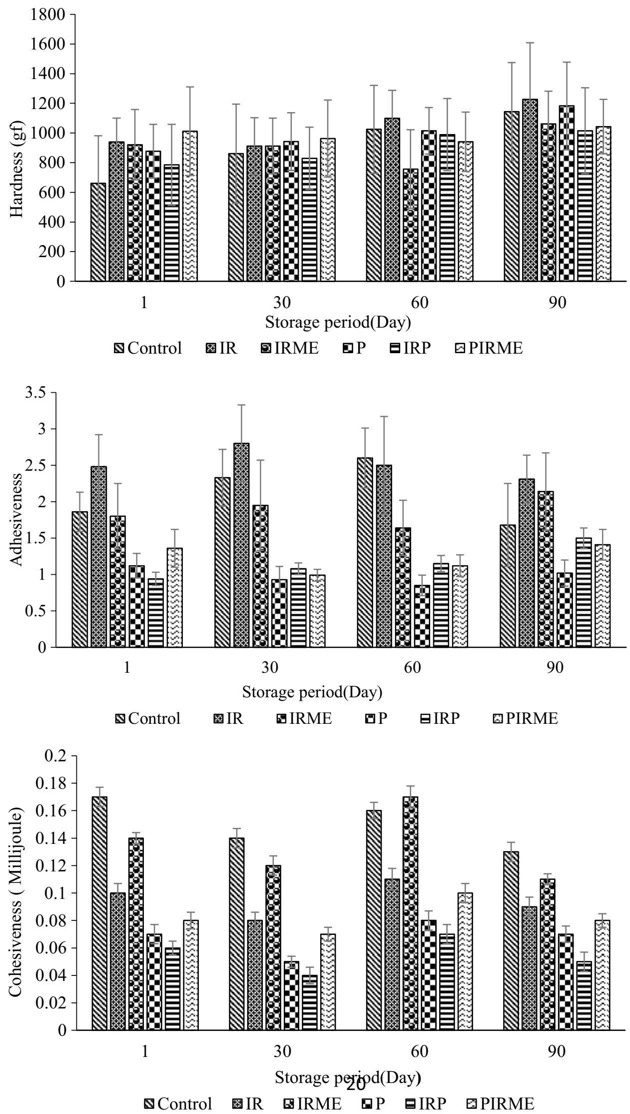

Fig. 7 The textural characteristics of the ice creams samples: control, (IR): ice cream contains free iron, (IRME): ice cream contains encapsulated iron, (P): ice cream contains 10^9 cfu/g *L. casei*, (IRP): ice cream contains free iron and 10^9 cfu/g *L. casei*, (PIRME): ice cream contains encapsulated iron and 10^9 cfu/g *L. casei*

samples over this period. The control group and the ice creams fortified with encapsulated iron showed the highest flavor scores. It seems addition of L. *casei* and free iron, negatively affected the taste and mouthfeel of the ice creams. This is attributed to the growth of probiotic bacteria and the generation of acidic flavor generated during the fermentation. The results herein were according to the previous studies by Sabet-Sarvestani et al. [34], Afzaal et al. [2], and Kataria et al. [43].

The texture and color of ice creams did not vary significantly amongst 6 formulae prepared in the present study ($p < 0.05$). However, it is worth mentioning that

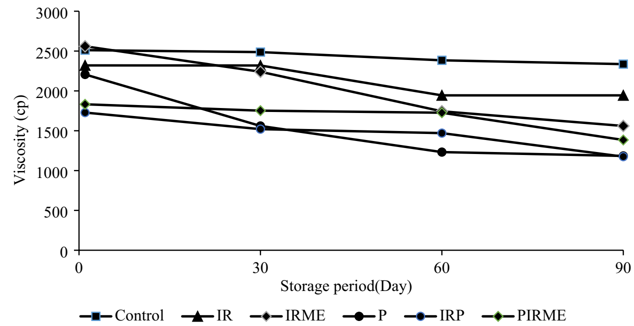

Fig. 8 Viscosity value (cP) of ice cream samples: (C) Control ice cream without iron and *L. casei*, (IR): ice cream contains free iron, (IRME): ice cream contains encapsulated iron, (P): ice cream contains 10^9 cfu/g *L. casei*, (IRP): ice cream contains free iron and 10^9 cfu/g *L. casei*, (PIRME): ice cream contains encapsulated iron and 10^9 cfu/g *L. casei*

encapsulated iron formulae scored higher sensory values than those containing free iron-fortified ice creams. Gaucheron (2000) reported that microencapsulation techniques could avoid oxidized and metallic flavors and color changes in fortified iron samples [13]. The available literature indicates bitterness and metallic flavor values in yogurt fortified with encapsulated iron. The results obtained in the present study supported those reported by Jayalalitha et al. [19], Kwak et al. [37], and Subash et al. [17]. Total evaluations in color, texture, and flavor in 6 groups of ice cream in the present study were qualitatively acceptable with no marked off-flavor during the storage period.

Rheological measurements

Results pertaining to texture analysis of ice cream samples are given in Fig. 7. The results showed that iron fortification did not significantly influence the texture properties of the ice cream samples. Adhesiveness, hardness, and the cohesiveness of the all samples did not experience any significant alteration during the 90 days of storage at − 18 °C. Interestingly, no significant difference was observed in values of viscosity and melting rate for ice creams in different groups (Fig. 8 and Table 2). Our results are in a good agreement with those published by Gheisari et al. [33] who fortified ice creams with probiotic and zinc. They also reported that no significant variations were observed in the adhesiveness, hardness, and cohesiveness. However, their findings indicate a reducing trend over the period of storage at − 18 °C in viscosity and melting point of ice creams fortified by probiotic and zinc. The viscosity of ice cream fortified with zinc however was not significantly different compared with the control group [33].

Table 2 Mean ± SD of melting rate (g/min) in ice creams stored at −18 °C for 90 days

Treatment groups	Storage period (days)			
	1	30	60	90
Control	0.82 ± 0.013^{Ba}	0.81 ± 0.017^{Ba}	0.70 ± 0.050^{Aa}	0.65 ± 0.020^{Aa}
IR	0.83 ± 0.017^{Ba}	0.81 ± 0.010^{Ba}	0.78 ± 0.060^{Ba}	0.75 ± 0.006^{Ab}
IRME	0.84 ± 0.003^{Ba}	0.83 ± 0.011^{Ba}	0.71 ± 0.040^{Aa}	0.68 ± 0.002^{Aa}
P	0.83 ± 0.010^{Ba}	0.81 ± 0.010^{Ba}	0.70 ± 0.040^{Aa}	0.68 ± 0.010^{Aa}
PIR	0.83 ± 0.010^{Ba}	0.82 ± 0.009^{Ba}	0.76 ± 0.050^{Ba}	0.72 ± 0.009^{Bb}
PIRME	0.82 ± 0.004^{Ba}	0.81 ± 0.010^{Ba}	0.77 ± 0.001^{ABa}	0.74 ± 0.008^{Ab}

Mean values in the same column that are denoted by different small letters are significantly different ($p < 0.05$). Mean values in the same row that are denoted by different capital letters are significantly different ($p < 0.05$)

Conclusion

Development of ice cream fortified with iron and a probiotic such as *Lactobacillus casei* could be an effective means to boost nutritional and functional values of ice cream. However, studies on the characteristics and storage stability of such product is a key parameter in its successful production. In the present study, 6 formulae for ice cream containing free and encapsulated iron and a probiotic strain (*L. casei*) were developed. Presence of these functional ingredients did not exhibit any adverse effects on ice creams' physicochemical, rheological and sensory characteristics. In general, ice creams formulated with encapsulated iron yielded better sensory scores than those containing free-iron. Encapsulated iron can be used effectively in the ice creams without entailing oxidized off-flavor and color. The microbial population of *L. casei* in ice creams maintained above the threshold value of 10^6 cfu/g even after 90 days storage at − 18 °C.

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Declarations

Conflict of interest Authors declare that there is no conflict of interest.

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