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ORIGINAL ARTICLE

Process optimization and oxidative stability of omega-3 ice cream fortified with flaxseed oil microcapsules

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Abstract Microencapsulated flaxseed oil powder (MFOP) was supplemented for the fortification of α-linolenic acid (ALA, ω-3 fatty acid) in ice cream. Processing parameters were optimized in terms of the stage of homogenization of ice-cream mix, level of fortification (3, 4 and 5%) and flavors (vanilla, butter scotch and strawberry). Data revealed that free fatty acids increased significantly during first 15 days in all the samples and then remained constant. Peroxide value and thiobarbituric acid value first increased up to 30 and 45 days, respectively; and then decreased followed by a gradual increase up to 120 days. Fatty acids profile showed 18.74–21.38% decrease in ALA content in fortified ice creams after 120 days. A serving of 100 g of freshly prepared functional ice cream was able to meet ~ 45% of the RDA (1.4 g ALA/day), which reduced to 35.37–36.56% on the end of storage i.e. 120 days. Overall, it can be concluded that MFOP was oxidative stable in ice-cream throughout the storage, which could be fortified successfully at 4% (w/w) level.

Keywords Flaxseed oil powder · Microencapsulation · Omega-3 fatty acid · Fortification · Oxidative stability · Ice-cream

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Introduction

Several clinical and epidemiological studies have demonstrated a strong positive correlation between the consumption of omega-3 (ω-3) polyunsaturated fatty acids (PUFAs) and prevention of various chronic diseases such as cardiovascular diseases, rheumatoid arthritis, hypertension, kidney diseases, etc. (Sharma et al. 2012; Mirfatahi et al. 2016). Omega-3 fatty acids have also been proved for improving cognitive functions, brain development of infants, immunomodulatory functions, mood disorders, HDL-cholesterol, age-related macular degeneration, etc. (Goyal et al. 2014). The most common source of ω-3 PUFAs (eicosapentaenoic acid: EPA and docosahexaenoic acid: DHA) are marine derived products i.e. fish and fish oil. Therefore, vegan population is at a direct disadvantage of not receiving ω-3 PUFAs in their regular diet; the consequences of which are noticeably in a large population worldwide. Goyal et al. (2016a) reported that the current ratio of ω-3:ω-6 fatty acids in regular diet of the population who do not consume fish and fish oils is 1:16–50. However, World Health Organization (WHO) has suggested that this ratio should be nearly 1:5 (FAO/WHO 2010). According to a recent report of WHO, 17.7 million deaths occurred due to cardiovascular diseases in 2015, which represents 31% of all global deaths (WHO 2017). Though suboptimal diet is not the only cause of cardiovascular diseases or other chronic diseases, it plays a pivotal role in reducing the risks of such non-communicable diseases. Flaxseed, also known as linseed, is an emerging oilseed and a sustainable plant-derived source of omega-3 fatty acid (α-linolenic acid, ALA). Physiologically, ALA can be converted into long chain PUFAs (EPA and DHA) in presence of delta-5-desaturase, but its conversion efficiency is very limited (< 15%). Therefore, National Institute of Health (NIH) has recommended a large amount of ALA in diet (~ 2.2 g/day) as compared to EPA plus DHA (400 mg/day). Though ALA can be obtained from other plant origin sources such as soybean oil, walnuts, rapeseed/canola oil in limited amount; flaxseed is the richest source having 50–60% ALA of total fatty acids (Goyal et al. 2015a, b). The major problem of using flaxseed oil as a cooking or ingredient oil is its oxidative instability. ALA is highly sensitive to high temperatures, oxygen and metal ions; and produces off- and rancid flavors compound during processing and storage. This nature of flaxseed oil limits its applications in food and dairy industry. Recently, several authors have worked on the stabilization of flaxseed oil using various technologies such as ultrasonication, microencapsulation; and developed emulsion (Kartal et al. 2016) and microcapsules (Goyal et al. 2015a, b). Other studies are also available in the literature, which shows that emulsion and microencapsulated form of flaxseed oil were further fortified in milk (Goyal et al. 2017; Nagarajappa and Battula 2017), dahi (Goyal et al. 2016a, b) and other food products.

Ice cream is a popular dairy product and consumed by almost all the age groups and preferred especially by children. Although per capita consumption of ice cream is very low in India, but the annual growth rate of ice cream market is very high i.e., 12–15%. The sales volume is expected to nearly double in India from 334.4 ml in 2016 to 657.2 ml by 2021 and the market is likely to be worth over $1.6 billion by then (Anon 2017); suggesting a wide scope of omega-3 fortification in such product. Previous studies have demonstrated that fortification is one of the best approaches to combat macro and micronutrient deficiencies (Sihag et al. 2016; Goyal et al. 2016a, 2017). A very few studies are available on the fortification of flaxseed oil in ice cream and frozen desserts. Goh et al. (2006) incorporated flaxseed oil up to 12% (w/w) in ice cream and observed high melt down rate and texture defects during storage. Similarly, Lim et al. (2010) developed ice creams by partially substituting milk fat with flaxseed oil at 2.5, 5.0 and 7.5% (w/w) levels and observed decreased sensory scores in terms of flavor, aroma, texture and overall acceptability due to a nutty flavor of flaxseed oil. Recently, Ullah et al. (2017) supplemented chia oil (rich in ALA) at the level of 5, 10, 15 and 20% (w/w) in ice cream and observed that the sensory scores declined just after 30 days of storage due to oxidation of PUFAs. It is clear from the previous studies that highly oxidative PUFAs could not remain oxidative stable in supplemented product and produced textural defects and rancid flavors during long term storage. To the best of our knowledge, no study has been conducted on the fortification of microencapsulated form of flaxseed oil i.e., microcapsules in ice-cream for the determination of oxidative stability of omega-3 fatty acid. We hypothesized that flaxseed oil microcapsules encapsulated by whey proteins are more physically as well as oxidative stable during heating, processing and storage. By keeping this point in view, the present study was planned to optimize the process conditions of addition of MFOP in ice cream and evaluate the oxidative stability of ALA during storage.

Materials and methods

Raw materials

Fresh and raw buffalo milk (6% fat, 8.5% SNF), skim milk [0.5% fat, 9% solids-not-fat (SNF)] and cream (55–60% fat) were collected from Experimental Dairy, ICAR-National Dairy Research Institute (NDRI), Karnal, India. Spray dried skim milk powder was procured from Modern Dairies Limited, Karnal, India, with 3.5% moisture, 1.5% fat and 95% SNF. Sugar obtained from the Experimental Dairy, ICAR-NDRI, was used as a sweetener in the ice cream formulation. It was assumed to contain 100% total solids. Whey protein concentrate-80 (WPC-80) was donated by Ace International LLP, New Delhi, India. The company (Davisco, USA) claimed that WPC-80 contained 0.2% moisture, 82.5% protein, 7.5% lactose, 6.4% fat and 2.4% ash content. Stabilizer and emulsifier mixture *Cremodan samporna* was obtained from Danisco, Gurgaon, India.

Packaging material

Polystyrene cups (100 ml) were obtained from the Experimental Dairy, ICAR-National Dairy Research Institute (NDRI), Karnal, India. Carton with LDPE layer on the inner side for one liter packaging were procured from the local market, Karnal, India.

Flaxseed oil microcapsules

Flaxseed oil microcapsules (microencapsulated flaxseed oil powder: MFOP) were prepared in Dairy Chemistry Division, NDRI, Karnal, India, by a method developed in-house and under patent (Application number: 2030/DEL/2014). This powdered form of microencapsulated flaxseed oil was fortified for developing the ice cream. The proximate composition of flaxseed oil microcapsules is given in Online Resource 1.

Methods

Preparation of fortified ice-cream

Ice-cream was manufactured as per standard method as narrated by De (1977). Fat and total solids content in ice cream mix were standardized using Pearson square method. Ice cream prepared with 10% milk fat and 36% total solids served as control. In test samples (T_1, T_2 and T_3), milk fat was partially replaced with flaxseed oil microcapsules at 3, 4 and 5% levels, respectively, by keeping total fat 10% and total solids 36% after supplementation. Initially numerous preliminary trials were conducted with different formulations of MFOP and its fortification levels. Finally, the trial consisting of MFOP encapsulated with whey proteins was selected at 3, 4 and 5% fortification levels. The selected trial was conducted in triplicates and single measurement was taken from each sample for each analysis. Flow diagram for the development of MFOP fortified ice-cream is shown in Online Resource 2. In brief, all the dry ingredients (except MFOP) and wet ingredients were mixed to prepare ice-cream mix at 60–70 °C followed by homogenization at 2500 psi pressure and 65 ± 2 °C temperature. MFOP was added in two stages: (1) Before the homogenization, and, (2) After the homogenization. Thereafter, the mixture was batch pasteurized at 85 ± 2 °C for 5 min followed by immediate cooling to 4 °C. After aging of ice-cream mix for 24 h, freezing and packaging was done at − 5 to − 7 °C followed by hardening and storage at − 18 ± 2 °C. Proximate composition of control and fortified ice-cream is given in Online Resource 1.

Optimization of processing conditions to prepare MFOP fortified ice-cream

Optimization of processing parameters e.g. stage of homogenization of ice-cream mix (before and after the addition of MFOP), levels of fortification (3, 4, 5%) and flavors (vanilla, strawberry and butter scotch) was achieved through evaluating the sensory attributes which were measured by a panel of ten trained judges of Dairy Chemistry and Dairy Technology Divisions, ICAR-National Dairy Research Institute, Karnal, India. Scoring card given by BIS (IS:2802 1964) was used for sensory evaluation of the product. A 9-point hedonic scale was used to document the panels' scores and provide quantifiable data. The scale comprises a series of nine verbal categories ranging from 'dislike extremely' to 'like extremely' and is described as such in various sensory studies (Goyal et al. 2016a, b, 2017). For subsequent quantitative and statistical analysis, the verbal categories are generally assigned numerical values, ranging from 'like extremely' as '9' to 'dislike extremely' as '1'. In the present study, ice-cream samples were evaluated for color and appearance, flavor, body and texture and mouthfeel (Sivakumar 2017). In taste and flavor characteristics, rancid/oxidized flavor was emphasized in ω-3 ice-cream.

Physico-chemical analyses

Moisture, fat, proteins and total solids content

Moisture content was measured by reference method of AOAC (2005). Fat and protein content was determined by Rose Gottlieb (Mojonnier extraction) Method (AOAC 2005) and Kjeldahl method (AOAC 2005), respectively. Total solids content of the ice cream mix was determined by gravimetric method (IS: 2802 1964).

Melting rate (g/min)

Ice-cream sample of known weight was placed on a wire mesh, which is placed on a pre-weighed measuring cylinder (100 ml capacity) with a glass funnel (10 cm dia.) The whole assembly was kept undisturbed at 25 ± 1 °C for 45 min. The weight of the melted samples collected in the measuring cylinder was noted and melting rate was determined by the formula given below:

$$\text{Melting rate (g/min)} = \frac{\text{Wt. of melted ice} - \text{cream (g)}}{\text{Time (min)}} \times 100$$

Free fatty acids (FFAs)

Accurately 1 g of extracted fat was dissolved in 5 ml of absolute ethanol and 15 ml of petroleum ether. Ten drops of an absolute alcoholic phenolphthalein solution (1%) was added, and the solution was titrated to the first definite colour change with 0.01 M potassium hydroxide in absolute alcohol using a 5 ml micro-burette. Results of the titration are expressed in terms of acid degree: ml of 1 M base required to titrate 100 g of fat.

Peroxide value (PV)

Oil was extracted from the sample using Soxhlet extraction method (AOCS 2000), which was analyzed for peroxide value as per IDF standard method 74A:1991 (IDF 2005).

Thiobarbituric acid value (TBA)

Thiobarbituric acid (TBA) value was determined as per the method used by Goyal et al. (2016a, b).

p-Anisidine value (p-AV)

p-Anisidine value was evaluated as per AOCS official method Cd 18–90 (AOCS 1993).

α-Linolenic acid (% ALA) measured by gas–liquid chromatography (GLC)

The concentration of ALA was evaluated by GLC method at 0 day and every month till 4 months of storage. First oil was extracted from the sample as mentioned above and converted into fatty acid methyl esters by the method given by DeMan (1964). The operating conditions of GLC are mentioned in our previous study (Goyal et al. 2017).

Statistical analyses

Data is reported as mean ± standard deviation (n = 3). To determine the effect of MFOP fortification on oxidative stability, sensory scores as well as other parameters, Tukey's Multiple Comparison Test was used at $p < 0.05$ (otherwise stated) using GraphPad Prism software (version 5.01 for Windows, San Diego California, USA).

Results and discussion

Effect of stage of homogenization of ice-cream mix (before and after the addition of MFOP) on sensory acceptability

MFOP was added in ice cream mix in two different ways: (1) before the homogenization, and (2) after the homogenization. Results (Fig. 1) indicated that there was no significant difference ($p < 0.05$) among the sensory scores for color and appearance of ice cream prepared by the addition of MFOP before as well as after the homogenization. The scores for color and appearance, remained almost similar to control irrespective of the stage of homogenization and MFOP addition level in ice cream, indicating that MFOP has no significant ($p < 0.05$) effect on color attribute. Literature pertaining to ice cream fortified with flaxseed oil microcapsules is not available; hence the results have been compared with the literature available on ice cream enriched with flaxseed oil, algal oil or chia oil. Lim et al. (2010) observed the effect of flaxseed oil supplementation for the preparation of reduced fat ice cream and reported that the amount of flaxseed oil addition was positively correlated with yellowness of ice cream. However, in present investigation, non-significant difference was observed in color attributes of omega-3 fortified ice creams which may be attributed to the microencapsulated nature of flaxseed oil and non-availability of free/surface oil in MFOP preparation used for the fortification of ice cream. The body and texture scores were also not affected significantly as a result of the stage of homogenization of the ice cream mix. However, the minimum scores were recorded for the ice cream samples containing MFOP at 5%.

It is evident from the Fig. 1 that stage of homogenization did not affect mouthfeel and flavor attributes of control significantly ($p < 0.05$). However, homogenization affected the mouth feel and flavor of omega-3 fortified ice cream to a significant ($p < 0.05$) level. The effect was more pronounced in the samples, wherein MFOP was added before the homogenization. In the present study, the size of the flaxseed oil microcapsules was in the range of 5.82–10.01 µm as shown in our previous study (Goyal et al. 2015a, b). Homogenization disrupts the particles and thus, reduces the particles' size to 0.2–2 µm. Therefore, lower sensory scores in the samples where MFOP was fortified before the homogenization can be explained due to the release of flaxseed oil from the microcapsules and subsequent production of off-flavors due to its oxidation. Among all studied level of addition of MFOP, 3% level showed the sensory scores comparable to the control. Thus, the stage, which was selected for the addition of flaxseed oil powder in ice cream, was 'after the homogenization process' of the ice cream mix. Therefore, the optimized level of MFOP was at 3%, which should be added after homogenization of the ice cream mix to develop vanilla flavored omega-3 fortified ice cream.

Effect of flavor and MFOP fortification level on sensory attributes of ice cream

As discussed above, only 3% fortification level was acceptable with vanilla flavor. Therefore, to enhance the fortification level, and thus the ALA content in the ice-cream, two more flavors i.e. butter scotch and strawberry were also studied, so that, the oily flavor could be masked in the fortified ice-cream.

Results showed that omega-3 fortified butter scotch flavored ice cream was not significantly different ($p < 0.05$) than that of control even on fortifying the ice

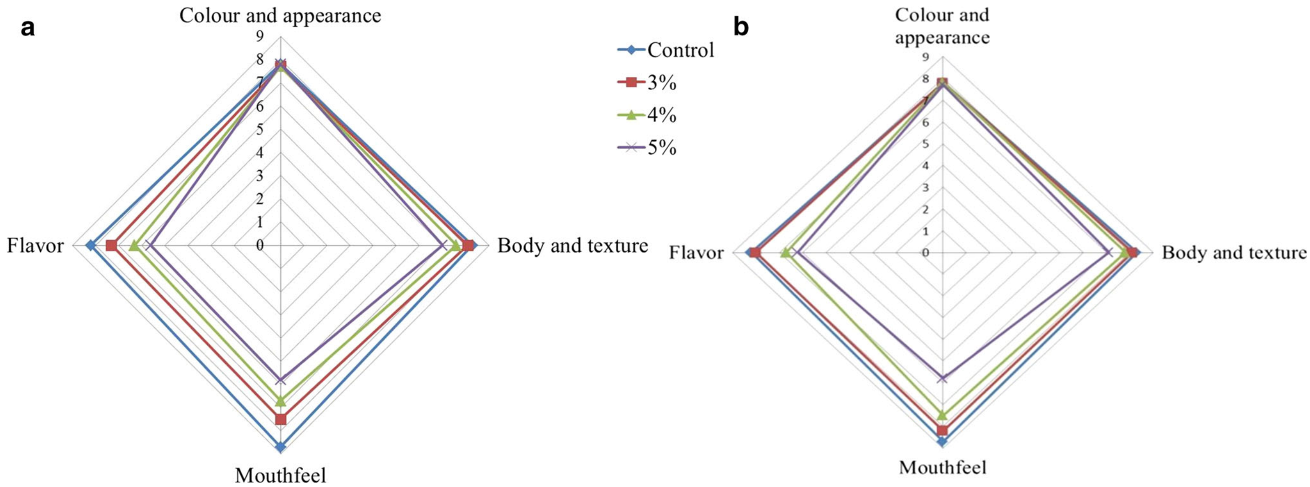

Fig. 1 Spider graphs for sensory scores of omega-3 fortified ice cream prepared by adding MFOP before (**a**) and after (**b**) the homogenization of ice cream mix

cream with higher concentration of MFOP (4%). Similar results were obtained for body and texture, mouth feel and flavor attributes except that of sample fortified with MFOP at 5%. Higher content of free oil, which might be present on the surfaces of microcapsules, could be a probable reason for lower sensory scores in 5% MFOP fortified ice-cream (Goyal et al. 2015a, b). Strawberry flavored omega-3 fortified ice cream showed a sensory behavior similar to the butter scotch flavored ice-cream. It can be concluded that on incorporating either of the flavors, higher fortification level (4%) could be achieved, which was 3% when vanilla flavor was used in the development of omega-3 fortified ice-cream. In all, on the basis of sensory scores, it can be concluded that the MFOP preparation could be added successfully after the homogenization of mixture during the preparation of ice-cream. The maximum supplementation level of MFOP, which was optimized on the basis of sensory scores, was up to 4% with both the flavors (butter scotch and strawberry).

Effect on MFOP fortification on free fatty acids (FFAs) in ice-cream

As evident from Table 1, FFAs content was 0.65 and 0.77–0.91% in control and butter scotch flavored omega-3 fortified ice-cream, respectively. FFAs content was highest in 4% MFOP fortified ice cream, while lowest in control. However, 3% MFOP fortified ice cream found to be containing FFAs in between the two. There was a significant ($p < 0.05$) difference among the FFAs content of omega-3 fortified ice-cream and control throughout the storage period. Higher FFAs in omega-3 fortified ice-cream could be due to the presence of free/surface oil in MFOP preparations as well as initial high levels of FFAs in the flaxseed oil used for microencapsulation (Goyal et al. 2015a, b). Data showed that FFAs content increased continuously during the storage of 120 days in all the ice-cream samples. Similar trend was observed for strawberry flavored fortified ice-cream as shown in Table 1. This increase could be due to the hydrolysis of unprotected/free oil present in the MFOP used in the preparation of omega-3 fortified ice creams (Ullah et al. 2017). Another reason of continuous increase in FFAs during storage could be due to the contamination of metal ions (pro-oxidans), enzymatic activity and/or presence of moisture in the system (Mc Sweeny and Fox 2003). Ice cream mix was homogenized; the milk fat globule membrane was broken down and plenty of water was also available to support the hydrolytic activities of lipase enzyme (Nadeem et al. 2015). Other workers have also reported a gradual increase in FFAs of ice cream supplemented with olein fraction and soymilk during storage (Abdullah et al. 2003; Nadeem et al. 2015). Recently, Ullah et al. (2017) characterized the physico-chemical properties of ice cream supplemented with olein fraction of chia (*Salvia hispanica* L.) oil (as a partial replacement of milk fat) at 5, 10, 15 and 20% concentrations. They observed that FFAs of all the treatments and control ranged from 0.08 to 0.12%, which slowly increased to 0.16 and 0.14%, respectively, after a storage period of 60 days. In the present investigation, FFAs of ice-cream fortified with various levels of MFOP were less than the maximum permissible limit of 0.2% (Ullah et al. 2017).

Effect of MFOP fortification on melt down rate of ice-cream

Results showed that on 0 day, resistance for the melting was significantly ($p < 0.05$) higher in control (39.0 min/50 g) than that of omega-3 fortified ice-cream (35.9–37.0 min/50 g) (Table 2). It can also be observed

Table 1 Effect of MFOP fortification and storage on free fatty acids (%FFAs) of butter scotch and strawberry flavored ice cream

Storage period (days)	Butterscotch flavored			Strawberry flavored		
	Control	3% MFOP fortified	4% MFOP fortified	Control	3% MFOP fortified	4% MFOP fortified
0	0.65 ± 0.10^{a}	0.77 ± 0.02^{b}	0.91 ± 0.10^{c}	0.71 ± 0.00^{a}	0.88 ± 0.00^{b}	0.96 ± 0.03^{b}
15	0.93 ± 0.05^{a}	1.23 ± 0.15^{b}	1.42 ± 0.05^{c}	1.11 ± 0.05^{a}	1.42 ± 0.02^{b}	1.51 ± 0.03^{b}
30	0.94 ± 0.05^{a}	1.25 ± 0.01^{b}	1.45 ± 0.05^{c}	1.15 ± 0.07^{a}	1.44 ± 0.01^{b}	1.58 ± 0.05^{b}
45	0.96 ± 0.01^{a}	1.28 ± 0.15^{b}	1.48 ± 0.01^{c}	1.17 ± 0.05^{a}	1.49 ± 0.02^{b}	1.62 ± 0.03^{b}
60	0.98 ± 0.05^{a}	1.35 ± 0.03^{b}	1.52 ± 0.05^{c}	1.21 ± 0.01^{a}	1.52 ± 0.07^{b}	1.65 ± 0.03^{b}
75	1.04 ± 0.02^{a}	1.41 ± 0.02^{b}	1.54 ± 0.05^{c}	1.23 ± 0.02^{a}	1.56 ± 0.02^{b}	1.70 ± 0.02^{b}
90	1.11 ± 0.02^{a}	1.45 ± 0.02^{b}	1.56 ± 0.01^{c}	1.25 ± 0.07^{a}	1.57 ± 0.05^{b}	1.73 ± 0.05^{b}
105	1.14 ± 0.02^{a}	1.48 ± 0.02^{b}	1.63 ± 0.02^{c}	1.28 ± 0.01^{a}	1.60 ± 0.02^{b}	1.79 ± 0.05^{b}
120	1.16 ± 0.02^{a}	1.52 ± 0.11^{b}	1.67 ± 0.01^{c}	1.31 ± 0.02^{a}	1.62 ± 0.02^{b}	1.82 ± 0.02^{b}

Values are mean ± SD (n = 3). Values with different superscripts (a, b, c) differ significantly within the rows for each flavored ice-cream at $p < 0.05$

MFOP microencapsulated flaxseed oil powder

Table 2 Effect of MFOP fortification and storage on melt down rate (min/50 g sample) of butter scotch and strawberry flavored ice cream

Storage period (days)	Butterscotch flavored			Strawberry flavored		
	Control	3% MFOP fortified	4% MFOP fortified	Control	3% MFOP fortified	4% MFOP fortified
0	38.0 ± 1.0^{a}	36.0 ± 1.0^{b}	34.3 ± 1.5^{b}	38.3 ± 2.1^{a}	36.3 ± 1.2^{b}	34.7 ± 1.3^{c}
15	38.1 ± 1.0^{a}	36.1 ± 1.0^{b}	35.1 ± 1.8^{b}	38.3 ± 2.1^{a}	36.4 ± 1.1^{b}	34.9 ± 1.5^{c}
30	40.6 ± 2.0^{a}	34.6 ± 0.5^{b}	34.6 ± 0.5^{b}	38.0 ± 1.0^{a}	36.7 ± 0.5^{b}	35.5 ± 1.0^{b}
45	39.6 ± 0.5^{a}	35.0 ± 1.0^{b}	35.1 ± 1.0^{b}	39.0 ± 1.0^{a}	36.3 ± 0.5^{b}	35.3 ± 0.5^{b}
60	40.3 ± 2.0^{a}	35.0 ± 1.0^{b}	34.9 ± 1.0^{b}	40.0 ± 1.0^{a}	36.3 ± 0.5^{b}	35.1 ± 1.2^{b}
75	38.0 ± 1.0^{a}	36.0 ± 1.0^{b}	34.6 ± 1.5^{c}	33.0 ± 1.5^{a}	37.0 ± 1.0^{b}	34.9 ± 1.0^{c}
90	39.6 ± 1.5^{a}	36.3 ± 1.5^{b}	35.5 ± 1.5^{b}	39.7 ± 1.2^{a}	37.0 ± 1.0^{b}	35.8 ± 1.2^{c}
105	39.6 ± 2.0^{a}	36.6 ± 0.5^{b}	34.9 ± 1.5^{b}	39.3 ± 1.2^{a}	36.7 ± 1.2^{b}	34.9 ± 1.0^{c}
120	38.6 ± 1.5^{a}	36.3 ± 1.5^{b}	34.7 ± 1.0^{c}	40.0 ± 1.0^{a}	37.7 ± 0.5^{b}	34.1 ± 1.0^{c}

Values are mean ± SD (n = 3). Values with different superscripts (a, b, c) differ significantly within the rows for each flavored ice-cream at $p < 0.05$

MFOP microencapsulated flaxseed oil powder

that 4% MFOP fortified ice-cream showed faster meltdown followed by 3% MFOP fortified ice-cream. There was no significant change in melt down rate of the control as well as omega-3 fortified ice-cream during the storage of 120 days. There are several factors which affect the melt-down rate of ice cream such as amount and nature of fat, total solids, amount of protein, emulsifier and stabilizers, degree of whipping, freezing and storage temperature, etc. In our study, over-run and amount of fat were same in all the ice-cream samples but the nature of the fat was different from the control. In omega-3 fortified ice-cream, milk fat was partially replaced by microencapsulated flaxseed oil. Results suggested that microencapsulated powder remained stable to pasteurization, freezing and storage at − 18 to − 20 °C. Fats with more unsaturated fatty acids and lower melting point give ice-creams which melt more rapidly. Goh et al. (2006) characterized the ice-cream containing flaxseed oil and observed a significant increase in melt-down rate and a subsequent decrease in hardness with an increase in amount of flaxseed oil in ice cream. It can be noticed here that though there was a significant difference in melt down rate between the control and MFOP fortified ice-cream, but the difference was not as high as reported by other workers, who have used free oil (Goh et al. 2006; Lim et al. 2010). As discussed above, melt-down rate depends upon the type, nature and amount of added fat/oil in the ice-cream. During churning and freezing process, the milk fat gets better flocculated and stabilizes the air cells, thus, resulting in higher stability and melting resistance. As observed by Goh et al. (2006), partial replacement of milk fat by flaxseed oil resulted in minimal fat flocculation, decreased air cells stability and a softer ice cream displaying higher meltdown rate. In the present study, lower melt-down rate in MFOP fortified ice-creams when compared to formulations developed by other workers (Goh et al. 2006; Nadeem et al. 2009) could be attributed to encapsulated form of fortified flaxseed oil, which might have increased the flocculation and hence, showed better structure. Nadeem et al. (2009) reported that melting rate increased significantly with increase in palm-olein amount in ice-cream. Similarly, Adhikari and Arora (1994) also found an increase in melting rate on partial replacement of butter oil with ground nut oil for the preparation of ice cream.

Effect of MFOP fortification on peroxide value (PV)

Peroxide value of omega-3 fortified ice-cream was measured till 120 days of storage at an interval of 15 days. PV of control and fortified ice-cream was 0.19 and 0.23–0.28 meq peroxides/kg oil on 0 day, respectively. Peroxide value of MFOP fortified ice-cream was not significantly different ($p < 0.05$) than that of control till first 15 days of storage at all fortification levels. As the storage period progressed, PV increased and reached to the maximum on 30th day of storage indicating the formation of hydroperoxides to the maximum level. The value was statistically highest in the ice-cream sample fortified with 4% of MFOP as compared to control and 3% MFOP fortified samples. It is interesting to note that PV decreased gradually from 45th day till 60th day of storage and then again increased till the end of storage period in butter-scotch flavored ice-cream samples irrespective of the fortification level (Fig. 2a). It is well known that hydroperoxides can be converted into secondary oxidation products such as low molecular weight aldehydes and ketones. The decrease in PV after 30 days could be due to the simultaneous conversion of hydroperoxides into secondary oxidation products thereby showing a decrease in the PV up to 60th day of storage. From 75th day onwards, PV again started to increase and continued till the end of 120 days of storage. The increase in this period was very slow as compared to the increase observed up to 30 days of storage. It can be noted that PV differed significantly ($p < 0.05$) for control and fortified sample from 45th day of storage. 4% MFOP fortified ice-cream showed higher PV than that of 3% MFOP fortified sample as well as control, which might be due to higher free oil present in the powder and subsequently in the product (Goyal et al. 2015a, b). Enhancement of unsaturated fatty acids in milk fat by the fat modification strategies may enhance the autoxidation process (Ashes et al. 1997). Rudnik et al. (2001) also reported that oils which contain high content of unsaturated fatty acids lead to reduced oxidative stability. A gradual increase in PV of MFOP fortified ice cream could be attributed to the relatively higher amount of unsaturated fatty acids than that of control. It can be noted that the initial PV of flaxseed oil used in the form of MFOP was 0.99 meq peroxides/kg oil, which increased to 1.88 and 2.32–2.73 meq peroxides/kg oil in control and fortified ice cream, respectively. Similar kind of observations was recorded in case of omega-3 fortified strawberry flavored ice-cream shown in Fig. 2b. Ullah et al. (2017) studied the oxidative stability of ice cream supplemented with chia oil. They reported that PV increased from 0.31 ± 0.02 to 1.84 ± 0.14 meqO_2/kg within 60 days in ice cream supplemented with 20% olein fraction of chia oil. In another study conducted by Nadeem et al. (2015), cream was fractionated at three different temperatures (25, 15, and 10 °C) and all the low melting fractions were individually incorporated into ice cream by keeping fat content constant (10%). They observed PV of 0.22 and 0.20–0.30 for control and all the treatments, which increased to 1.2 and 2.0–3.9 meq O_2/kg, respectively, after 6 months of storage. Oxidative stability of ice cream prepared by the addition of flaxseed oil was inferior to original milk fat (Lim et al. 2010). Though PV of omega-3 fortified ice-cream increased as a function of storage but did not increase beyond that level (5 meq peroxide/kg), which is prescribed by Codex Alimentarius Commission (1999). In present investigation, high oxidative stability of omega-3 fortified ice-cream obtained in the present study might be correlated to the antioxidative properties of whey proteins used for the preparation of flaxseed oil microcapsules (Goyal et al. 2015a, b). Acidic amino acids of whey proteins show metal binding behavior and thus, act as anti-oxidants (Cervato et al. 1999).

Effect of MFOP fortification on thiobarbituric acid (TBA) value

As discussed above, primary oxidation products (hydroperoxides) are converted into secondary oxidation products during storage, which cause off-color and rancid flavor of the products. Malonaldehyde is the main component among the secondary oxidation products, which is reported carcinogenic and mutagenic agent. When these oxidation products react with TBA, colored-reaction products are formed. TBA value of control and butter scotch flavored fortified ice cream was 0.0 and 0.03–0.04 mmol malonaldehyde/kg oil on 0 day, respectively (Fig. 2c). There was a significant ($p < 0.05$) difference between TBA value of MFOP fortified ice cream and control throughout the storage period. However, statistically, a non-significant ($p < 0.05$) difference was observed between TBA value of 3 and 4% MFOP fortified ice-cream

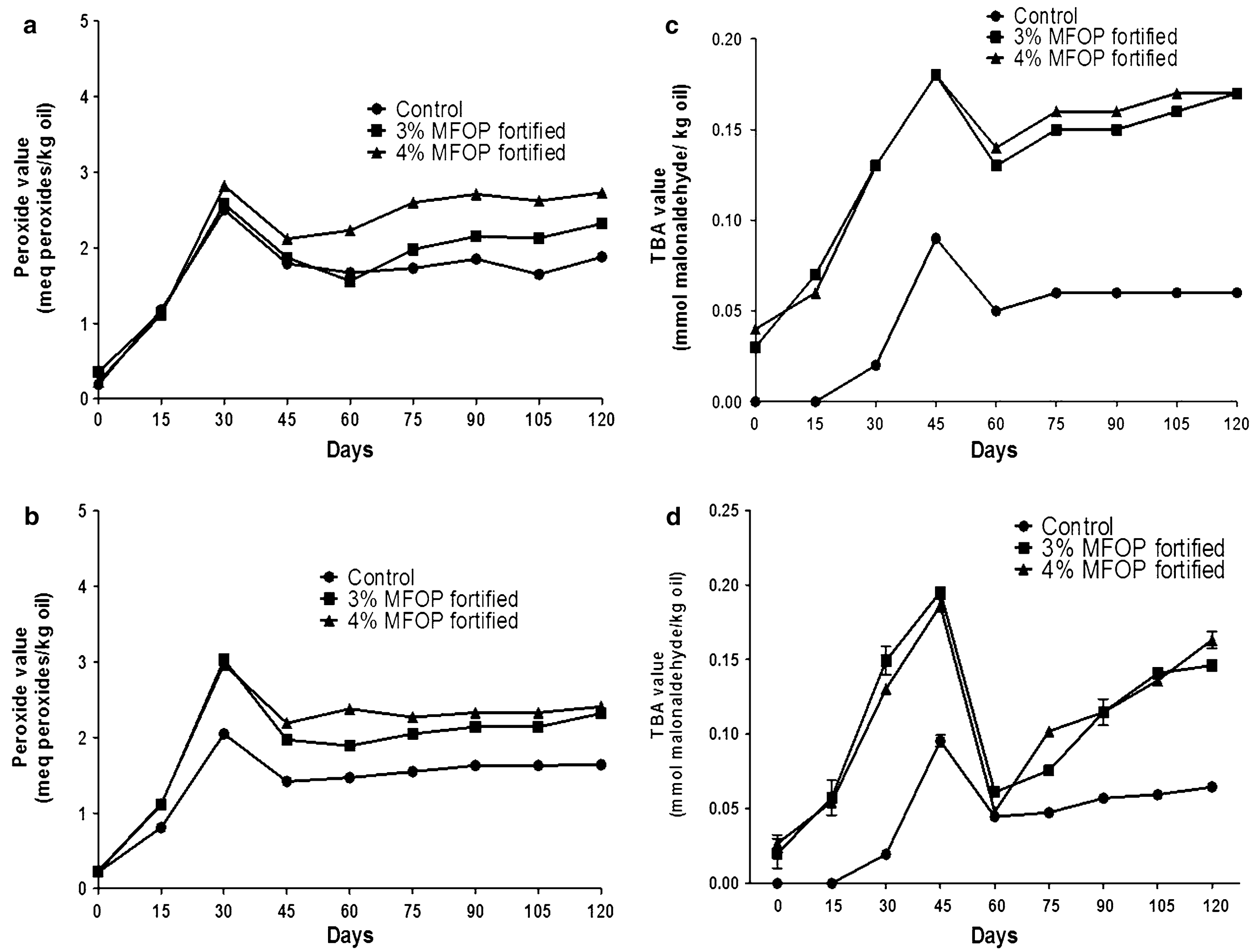

Fig. 2 Effect of MFOP fortification and storage on peroxide value (**a** butter scotch flavored; **b** strawberry flavored) and thiobarbituric acid value (**c** butter scotch flavored; **d** strawberry flavored) of ice-cream. *MFOP* microencapsulated flaxseed oil powder

on a particular day of analyses. As in case of PV, TBA value first increased till 45 days in all ice-cream samples followed by a gradual decrease up to 60 days. This decrease in TBA value could be attributed to the further degradation of secondary oxidation products (Bhattacharya et al. 1988). After showing a decrease in TBA value, ice-cream samples (both control and omega-3 fortified) showed again a continuous but very slow increase till the end of storage period. It is evident that TBA value was 0.06 and 0.17–0.20 mmol malonaldehyde/kg oil at the end of studied period of 120 days in control and omega-3 fortified ice cream, respectively. The change in TBA values in strawberry flavored ice-cream followed a similar trend as in case of butter scotch flavored fortified ice cream (Fig. 2d). Despite a significant increase in TBA value of fortified ice-creams during storage, samples were acceptable in terms of sensory attributes. Though CODEX or any other regulatory agency did not prescribe the maximum permissible value of TBA in order to know the oxidized status of fats and oil; Fernandez et al. (1997) reported TBA value of 5 as the threshold for sensory acceptability. In the present study, TBA value remained well below to this threshold throughout the storage; and thus, it can be inferred that flaxseed oil in the form of spray dried powder remained oxdatively stable in the ice-cream for the studied period.

Effect of MFOP fortification on p-anisidine value (p-AV)

Anisidine value determines the secondary and tertiary stages of auto-oxidation. The effect of MFOP fortification and storage on p-anisidine value of butter scotch flavored ice-cream is shown in (Table 3). p-AV of MFOP fortified ice-creams did not differ significantly ($p < 0.05$) than that of control till 30 days of storage. On the contrary, 3 and 4% MFOP fortified ice-cream showed a significant difference in p-AV from 45th day onwards till the end of studied storage period. Continuous increase in p-AV in all the ice-

Table 3 Effect of MFOP fortification and storage on p-anisidine value (p-AV) of butter scotch and strawberry flavored ice-cream

Storage period (days)	Butterscotch flavored			Strawberry flavored		
	Control	3% MFOP fortified	4% MFOP fortified	Control	3% MFOP fortified	4% MFOP fortified
0	0.60 ± 0.30[a]	0.60 ± 0.45[a]	0.80 ± 0.36[a]	0.49 ± 0.27[a]	0.57 ± 0.21[a]	0.75 ± 0.23[a]
15	2.10 ± 0.88[a]	2.25 ± 1.17[a]	2.30 ± 0.11[a]	0.94 ± 0.16[a]	1.04 ± 0.69[a]	1.28 ± 1.77[a]
30	3.13 ± 0.78[a]	3.28 ± 0.57[a]	3.36 ± 0.76[a]	2.97 ± 1.19[a]	3.25 ± 2.57[a]	3.30 ± 1.14[a]
45	5.06 ± 0.86[a]	5.58 ± 0.77[b]	5.86 ± 0.54[b]	2.65 ± 1.36[a]	3.94 ± 0.19[b]	4.67 ± 0.81[c]
60	6.24 ± 0.30[a]	6.83 ± 0.14[b]	7.73 ± 0.33[c]	4.07 ± 0.18[a]	4.22 ± 0.93[b]	5.09 ± 1.15[c]
75	7.26 ± 0.24[a]	8.07 ± 0.49[b]	8.27 ± 0.21[b]	4.14 ± 0.93[a]	4.19 ± 0.21[a]	5.27 ± 1.36[c]
90	7.59 ± 0.78[a]	9.31 ± 0.45[b]	9.48 ± 0.57[b]	5.83 ± 0.81[a]	6.57 ± 0.27[b]	6.64 ± 0.34[b]
105	8.38 ± 0.90[a]	9.70 ± 0.90[b]	10.97 ± 2.30[c]	7.48 ± 0.43[a]	7.89 ± 1.14[b]	7.94 ± 1.14[b]
120	9.58 ± 0.51[a]	9.95 ± 0.36[b]	11.64 ± 0.80[c]	8.42 ± 0.21[a]	8.62 ± 0.33[a]	8.95 ± 0.18[b]

Values are mean ± SD (n = 3). Values with different superscripts (a, b, c) differ significantly within the rows for each flavored ice-cream at $p < 0.05$

MFOP microencapsulated flaxseed oil powder

cream samples could be correlated to the continuous generation of secondary oxidation products (Goyal et al. 2016a, b). It is clear from the Table 3 that 4% MFOP fortified ice-cream showed the highest p-AV followed by the 3% MFOP fortified and then control in butter scotch flavored ice-cream after 120 days of storage. Higher p-AV in 4% MFOP fortified sample could be attributed to the higher amount of unsaturated fatty acids, which were oxidized and yielded secondary and tertiary oxidation products to a greater extent. On the contrary, slightly different results were recorded in case of strawberry flavored omega-3 fortified ice cream as shown in Table 3, which indicated no significant ($p < 0.05$) difference between the p-AV of 3% MFOP fortified sample and control even after 120 days of storage. However, comparatively higher and significantly ($p < 0.05$) different value was observed in 4% MFOP fortified ice-cream than that of other samples. Ullah et al. (2017) prepared ice-cream by partially replacing the milk fat with olein fraction of chia oil at 5, 10, 15 and 20% concentrations (T1, T2, T3 and T4) and observed no significant effect of storage on p-AV of control and treatments till 30 days. However, a substantial increase was observed in T3 and T4 as compared to control and other treatments after 60 days of storage (Ullah et al. 2017). In another study conducted by Nadeem et al. (2015), milk fat was replaced by different olein fractions of milk fat separated at different temperatures (10, 15 and 25 °C) and observed a pronounced increase in p-AV in all the samples except control after 2 months of storage. In the present study, it is clear that p-AV was much lower in MFOP fortified ice cream even after 120 days of storage than the maximum value (20 meq/kg l) specified by CODEX (2013) for oils rich in PUFAs.

Effect of storage on % ALA content of omega-3 fortified ice-cream

Results showed that ALA content was 2.98, 6.84 and 8.79% in control, 3 and 4% MFOP fortified ice-cream on 0th day, respectively (Table 4). A significant ($p < 0.05$) difference among the %ALA content of control and MFOP fortified samples was due to the difference in levels of MFOP used in preparing ice-cream, and thus, flaxseed oil. It can be observed that ALA content decreased in all the samples during the storage probably due to auto-oxidation of PUFAs. In present investigation, heat was not a major factor responsible for ALA degradation as the product was stored at very low temperatures (− 18 to − 20 °C). Data revealed that ALA content reduced to the tune of 34.0, 23.10 and 21.38% in control, 3 and 4% MFOP fortified ice-cream, respectively, after 120 days of storage. Similarly, in case of strawberry flavored omega-3 fortified ice-cream, 30.8, 25.10 and 18.74% reduction was observed in ALA content of control, 3 and 4% MFOP fortified ice-cream, respectively. Observations of the present investigation were found in line with the findings of Lim et al. (2010), who reported a decrease of 6.2–6.94 in % ALA content in flaxseed oil added ice-cream after 42 days of storage. Our results are in good agreement with the findings of Ullah et al. (2017) also, who analyzed the fatty acids profile of ice-cream supplemented with olein fraction of chia (*Salvia hispanica* L.) oil, which is also one of the richest source of ALA. In their study ice-cream samples containing 5, 10, 15 and 20% olein fractions of chia oil showed approximately 9.88, 8.41, 8.24 and 5.81% ALA reduction, respectively.

Indian Council of Medical Research has recommended 1.6 g ALA/day for adults (ICMR 2010). Therefore, approximate 45% of the RDA can be met of ALA by 100 g

Table 4 Effect of storage on %ALA content of butter scotch and strawberry flavored ice-cream

Storage period (days)	Butterscotch flavored			Strawberry flavored		
	Control	3% MFOP fortified	4% MFOP fortified	Control	3% MFOP fortified	4% MFOP fortified
0	2.98 ± 0.12^a	6.84 ± 0.11^b	8.79 ± 0.17^c	2.59 ± 0.07^a	7.53 ± 0.13^b	8.27 ± 0.15^c
30	2.73 ± 0.17^a	6.34 ± 0.26^b	8.26 ± 0.13^c	2.46 ± 0.05^a	7.14 ± 0.12^b	7.92 ± 0.21^c
60	2.41 ± 0.08^a	5.90 ± 0.11^b	7.71 ± 0.09^c	2.31 ± 0.09^a	6.64 ± 0.13^b	7.56 ± 0.12^c
90	2.15 ± 0.12^a	5.55 ± 0.08^b	7.34 ± 0.13^c	1.92 ± 0.12^a	6.28 ± 0.17^b	7.10 ± 0.04^c
120	1.95 ± 0.11^a	5.26 ± 0.10^b	6.91 ± 0.10^c	1.79 ± 0.11^a	5.64 ± 0.10^b	6.72 ± 0.06^c

Values are mean ± SD (n = 3). Values with different superscripts (a, b, c) differ significantly within rows for each flavored ice-cream at $p < 0.05$

MFOP microencapsulated flaxseed oil powder

serving of developed omega-3 fortified butter scotch flavor ice-cream (fortified by 4% MFOP) on 0 day. As evident from the data that storage led to a decrease in the ALA content in ice cream, hence ice cream sample (fortified by 4% MFOP) stored for 120 days could meet 35.37% of the RDA. In case of strawberry flavored omega-3 fortified ice-cream (fortified by 4% MFOP) ~ 36.56% of RDA per 100 g serving of ice cream could be met after 120 days of storage.

Effect of storage on sensory acceptability of omega-3 fortified ice-cream

It is interesting to note that sensory attributes viz. color and appearance and body and texture of omega-3 fortified ice cream and control did not differ significantly throughout the storage period (Online resource 3). However, the scores for body and texture and mouthfeel decreased gradually for butter scotch flavored ice-cream samples during the storage. This decrease in scores could be attributed to the formation of ice crystals on the surface due to temperature fluctuation during storage. It can be observed that the difference among the flavor scores of control, 3 and 4% MFOP fortified ice-cream till 90 days of storage was non-significant ($p < 0.05$). These observations suggested that omega-3 fortification did not affect the flavor of ice-cream adversely up to the 90 days of storage. Thereafter, a significant decrease was observed in flavor scores of MFOP fortified ice-cream irrespective of the fortification level than that of control. The reason for decline in flavor scores of ice cream fortified with MFOP could be due to the generation of peroxides during the storage period (Ullah et al. 2017). Though the flavor scores were decreased during storage, the product was acceptable by the judges of sensory panel. Similar findings were obtained in strawberry flavored omega-3 fortified ice-cream. Ullah et al. (2017) reported no significant impact of addition of chia oil fraction on color, flavor and texture of fresh ice cream. Contrary to our results, they observed a significant deterioration in sensory scores of color, flavor and texture after 30 days of storage period due to the oxidation of unsaturated fatty acids.

Conclusions

Microencapsulated flaxseed oil powder used for the development of omega-3 fortified ice-cream remained oxidative stable for the studied period of storage. Sensory attributes of fortified ice-cream revealed that the developed omega-3 fortified ice cream (butter scotch and strawberry flavored) remained organoleptically acceptable till the studied period of storage. ALA content was reduced on account of storage. A serving of 100 g of developed functional ice-cream containing 4% MFOP could meet approximately ~ 45% of the RDA of ALA on 0 day of storage. Functional ice cream stored for 120 days could meet 35.37–36.56% of the RDA.

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Compliance with ethical standards

Conflict of interest Authors have no conflicts of interest to declare.

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