Fortification of *dahi* (Indian yoghurt) with omega-3 fatty acids using microencapsulated flaxseed oil microcapsules

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Abstract The objective of the study was to develop and characterize omega-3 *dahi* (Indian yoghurt) through fortification of microencapsulated flaxseed oil powder (MFOP). Four different formulations of MFOP were fortified in *dahi* @ 1, 2 and 3 % levels and the level of addition was optimized on the basis of sensory scores. *Dahi* fortified at 2 % level was observed comparable to control, which was further studied for titratable acidity, syneresis, firmness, stickiness, oxidative stability (peroxide value), α -linolenic acid (ALA, ω -3) content and sensory attributes during 15d of storage. MFOP fortified *dahi* showed significantly ($p < 0.05$) higher acidity and percent syneresis after 12d of storage. However, peroxide value remained well below (~ 0.41) to the maximum permissible limit (5 meq peroxides/kg oil) prescribed by Codex Alimentarius Commission (1999). Gas-liquid chromatography profile showed ~ 21 % decrease in ALA content in fortified *dahi* after 15d of storage. Overall, it can be concluded that

flaxseed oil microcapsules could be successfully incorporated in *dahi*; which could serve as a potential delivery system of omega-3 fatty acids.

Keywords *Dahi* (Indian yoghurt) · Flaxseed oil microcapsules · Oxidative stability · Physico-chemical characteristics · Omega-3 fortification

Introduction

The traditional diet-heart paradigm, developed in 1960s and 1970s, provided the convincing evidences that consumption of saturated fats increases the risk of coronary heart diseases (CHD) by raising total and low-density lipoprotein (LDL) cholesterol levels (Mozaffarian 2011); and should be replaced with vegetable oils containing polyunsaturated fatty acids (PUFAs, ω -6) (FAO/WHO 2010). However, recently several researchers have challenged it on the basis of outcomes of epidemiological and clinical studies, and concluded that linoleic acid (LA: ω -6) was not involved in reduction of CHD, and may possibly increase it (Ramsden et al. 2010; Komprda 2012) due to production of 2- and 4-series prostaglandins and prostacyclin compounds. Indeed several risk factors such as physical inactivity, suboptimal diet, smoking, stress and increased use of alcohol are attributable to chronic diseases. Nonetheless, suboptimal diet is the leading cause of poor health in the world. It is interesting to note that vegan and non-fish consuming meat eater's diet is deficient in omega-3, which are essential fatty acids and critical for appropriate growth and development in humans. As such, it is not surprising that ω -3 fatty acids have also been implicated in the prevention of systemic and chronic diseases including hypertension, hypercholesterolemia, cancer (including colon, breast and prostate), inflammatory bowel diseases, diabetes and

Highlights

1. Omega-3 *dahi* fortified with 2 % flaxseed oil powder was comparable to control during storage.
2. Approximately 31.00 % of the RDA of omega-3 fatty acids could be met by developed fortified *dahi* (200 mL).
3. Fortified *dahi* remained oxidative stable for at least 15 days at low temperature.

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neurodegenerative disorders (Goyal et al. 2014; Sharma et al. 2012). It is reported that ω -6: ω -3 ratio in current Indian urban and Western diet is about 38–50:1 and 16:1, respectively over the recommended ratio of 5:1 (Singh et al. 2010, 2011). This high ω -6 to ω -3 ratio could be attributed to the higher consumption of ω -6 rich vegetable oils; and insufficient dietary intake of sea foods in vegan diet. Flaxseed (linseed) oil is the richest plant source of ω -3 fatty acid (α -linolenic acid, ALA: C18:3) and becoming popular due to its various health benefits; which have been comprehensively reviewed by several authors (Gogus and Smith 2010; Goyal et al. 2014). Thus, flaxseed oil could be utilized as a functional ingredient for the preparation of potential delivery systems to fulfill the nutritional gap of ω -3 fatty acids in regular diet.

Milk and milk products are widely consumed by all age groups and play a key role in nutrition throughout life (Upadhyay et al. 2014). *Dahi*, which has an appearance similar to that of yoghurt, is a popular fermented dairy product in the Indian subcontinent (Rathi et al. 2015). It is prepared by fermenting milk from cows, buffalos or goats with mesophilic lactic cultures such as *Lactococcus lactis* ssp. *Lactis*, *Lactococcus lactis* ssp. *Diacetylactis* and *Lactococcus lactis* ssp. *Cremoris*. *Dahi* is easy to digest and possess therapeutic properties such as inhibition of cancer cell line (Agarwal and Bhasin 2002); improvement in appetite and vitality; curing of dyspepsia, diarrhoea and other intestinal disorders; removal of adverse effects of drugs; encouragement of thiamine synthesis, lowering the cholesterol level and easy digestion by lactose-intolerant people (Srinivasan 2011). In India, ~9 % of the total milk production is converted into fermented milk products, of which 77.8 % (7 % of total milk production) is used to prepare *dahi*, intended for direct consumption (Sarkar 2008).

Recently, a few researchers have worked on the development of omega-3 emulsions (Goyal et al. 2015a, 2015b; Kuhn and Cunha 2012) and fortification in milk (Benito et al. 2006), cheese (Bermudez-Aguirre and Barbosa-Canovas 2011), yogurt (Dawczynski et al. 2013) and *dahi* (Liutkevicius et al. 2010). However, negative impacts on sensory acceptability such as higher acidity, free fatty acids, peroxide value, off-taste and flavor during storage have been reported in flaxseed oil added milk (Divya et al. 2013) and whey beverages (Liutkevicius et al. 2007). Such defects might be the consequences of extremely oxidative nature of flaxseed oil, which could be attributed to its highly polyunsaturated nature (>75 %). Flaxseed oil produces rancid and off-flavor compounds in presence of oxygen & high temperatures during processing and storage. Thus, strategies aimed at stabilization and fortification of flaxseed oil could have profound influence in developing novel ω -3 rich functional foods. A wide range of literature suggested that spray drying is the most common technique to protect heat sensitive omega-3 oils using sodium

caseinate, whey protein concentrate, gum Arabic, maltodextrins, etc. as microencapsulating agents; and showed higher physical, oxidative & storage stability of microencapsulated form of omega-3 oils (Gallardo et al. 2013; Carneiro et al. 2013; Karaca et al. 2013; Goyal et al. 2015b), which could be utilized for the fortification of various food products.

As far as our best knowledge, little information is available on the supplementation of α -linolenic acid (ω -3) in the form of microencapsulated flaxseed oil in *dahi* (Indian Yoghurt). Therefore, the present study was aimed to study the effect of fortification of microencapsulated flaxseed oil powder (MFOP) on the physico-chemical and sensory characteristics of *dahi* (Indian yoghurt).

Materials and methods

Raw materials and ingredients

Milk and starter culture

Fresh raw buffalo whole milk and skimmed milk were procured from the Experimental Dairy of National Dairy Research Institute (NDRI), Karnal, India. *Dahi* culture NCDC-167 (mixed culture: *Lactococcus lactis* ssp. *Lactis*, *Lactococcus lactis* ssp. *Diacetylactis*, *Lactococcus lactis* ssp. *Cremoris*, *Leuconostoc* spp.) was obtained from National Collection of Dairy Cultures (NCDC), NDRI, Karnal, India. The starter culture was activated in autoclaved skim milk (10 g/100 mL) for 4–6 h for attaining high activity.

Chemicals and other ingredients

Refined flaxseed oil was procured from Kamani Oil Industries Pvt. Ltd., Khopoli, Maharashtra, India. Sodium caseinate and whey protein concentrate (WPC)-80 (Davisco, USA) were purchased from Ace International LLP, New Delhi, India. Whey protein concentrate (WPC) was claimed to contain 82.5 % protein (on dry basis), 6.4 % fat, 0.2 % moisture, 7.5 % lactose and 2.4 % ash content. Lactose was purchased from Fischer Scientific, Mumbai, India; and antioxidants were purchased from Sigma-Aldrich, Germany. Other chemicals were of analytical grade and were purchased from Sigma-Aldrich, Germany and Himedia, Mumbai, India. Microencapsulated flaxseed oil powder (MFOP) was used as a functional ingredient for omega-3 (ALA) fortification in the product, which was prepared in the lab of department of Dairy Chemistry, National Dairy Research Institute, Karnal, India, by the following method, which contained 3.76 ± 0.27 % moisture, 35.30 ± 0.84 % flaxseed oil, whey protein/sodium caseinate 25.70 ± 0.45 % and 35.26 ± 0.58 % lactose.

Preparation of microencapsulated flaxseed oil powder (MFOP)

Four different formulations of emulsions containing flaxseed oil, whey proteins concentrate (WPC)/sodium caseinate (NaCas) and lactose (1.25:1:1) were prepared. In the first formulation (FO/WPC), lactose and WPC were mixed in distilled water followed by addition of flaxseed oil. In the second formulation (FO-AO/WPC), ascorbyl palmitate was mixed in flaxseed oil. This mixture was further added to the pre-mixed solution of the dry-ingredients. In the third formulation (FO/WPC Δ), heat denatured WPC was mixed with lactose and flaxseed oil. In the last formulation (FO/NaCas), sodium caseinate was dissolved in warm water (55 ± 5 °C) followed by addition of lactose and flaxseed oil. All the formulations were mixed well and homogenized at low pressure [69 bar @ 20 l per hour (LPH)] to obtain coarse emulsions, followed by high pressure homogenization (241.31 bar @ 20 LPH; Goma Engineering Pvt. Ltd., Thane, India). The emulsions were spray dried by single stage spray drier with 10 Kg/h capacity (SSP Pvt. Ltd., Faridabad, India) equipped with rotary atomizer. Emulsions were pumped to the spray drier at a flow rate of 40 mL/min at room temperature (30–35 °C). The inlet and outlet temperatures of spray dryer were maintained at 170 ± 1 °C and 75 ± 1 °C, respectively. The developed spray dried preparations [microencapsulated flaxseed oil powder (MFOP)] were packed separately in an aluminum foil pack (thickness: 80–100 μ m) and stored at room temperature (30–35 °C) until further used for fortification. The general composition of MFOP is given in Table 1.

Development of MFOP fortified dahi (Indian yoghurt)

Dahi was prepared from MFOP fortified milk as per the standard method given by De (2012). Buffalo whole milk was standardized by Pearson square method in such a way that it contained total 3 % fat and 8.5 % solids-not-fat (SNF) after MFOP addition at 1, 2 and 3 % level. All the batches were homogenized at 2500 psi pressure followed by the addition of MFOP at different levels; pasteurized at 80 °C for 5 min and then cooled to 45 °C. The starter culture was inoculated at the rate of 2 g/100 ml milk to each batch and filled into 100 mL UV-sterilized plastic cups. Incubation was carried out at 45 ± 1 °C for about 5–6 h. Once the desired consistency was obtained, set dahi cups were transferred to refrigerated storage of 4–7 °C. The general composition of control and fortified dahi is given in Table 1.

Optimization of fortification level of MFOP preparations in dahi on the basis of sensory evaluation

Sensory evaluation was done by a panel of ten trained judges of the department of Dairy Chemistry and Dairy Technology, National Dairy Research Institute, Karnal, India. Fortified dahi

(fortified at 1, 2 and 3 % levels) were graded for any change in colour & appearance, flavour, sourness (acidity) and body & texture as compared to control. Composite scoring card for sensory analysis of pasteurized dahi as given by BIS (IS: 7768, 1980) was used, with slight modifications. In flavour characteristics, the main focus was on rancid and oxidized flavor in fortified dahi. Other mentioned flavour characteristics were excluded. Sensory scores were statistically analyzed by using one-way ANOVA (Tukey's test) and the most acceptable dahi (in terms of fortificant level) was further evaluated for its physico-chemical and sensory characteristics during the storage.

Physico-chemical analyses

Effect of MFOP fortification on titratable acidity (% lactic acid)

The titratable acidity was determined by the method given by AOAC (2005). Approximately 20 g dahi sample was weighed in a conical flask and diluted with twice its volume with double distilled water. Two ml of phenolphthalein indicator was added and the contents were titrated with 0.1 N NaOH to persistent pink colour. Acidity was calculated by the following formula (Eq. 1) and reported as % lactic acid by weight.

$$\text{Acidity (\% lactic acid)} = \frac{9 NV1}{V2} \quad (1)$$

Where, V1 = Volume (mL) of 0.1 N NaOH required for titration, N = Normality of NaOH used, V2 = Volume (mL) of milk taken for test.

(1 ml 0.1 N NaOH = 0.009 g lactic acid).

Effect of MFOP fortification on spontaneous syneresis in undisturbed set dahi

Spontaneous syneresis of undisturbed set dahi was determined using a siphon method used by Amatayakul et al. (2006). In this study, a cup of set dahi was taken from the refrigerator, weighed and kept at an angle of approximately 45° to allow the whey on the surface to collect on the side of the cup. A needle connected to a syringe was used to siphon the liquid whey from the surface of the sample and the cup of dahi was then re-weighed. The siphoning was carried out within 10 s to prevent further leakage of whey from dahi. The syneresis was expressed as the percentage weight of the whey over the initial weight of the dahi sample according to the formula given below (Eq. 2).

$$\% \text{Syneresis} = \frac{W1 - W2}{W1} \times 100 \quad (2)$$

Where, W1 is the initial wt. of dahi cup; W2 is the final wt. of dahi cup (After whey removal).

Effect of MFOP fortification on texture profile

The firmness of *dahi* prepared from control and fortified milk was determined using texture analyzer fitted with 5 kg load cell. For texture analysis, *dahi* samples were prepared from 80 ml milk in 100 ml glass beakers, the set samples were kept in immersion chamber maintained at 25 °C before the analysis. The samples were subjected to mono-axial compression of 20 mm distance on the texture analyzer by the crosshead speed of 2 mm/s. The test conditions maintained were as under: Probe P25 cylindrical (diameter 25 mm), distance =20 mm, load cell =25 kg, Pre-test speed =2 mm/s, Test speed =1 mm/s, Post-test speed =2 mm/s, Single TPA i.e. single time penetration. A minimum of five replicates were run for texture analysis.

Effect of MFOP fortification on oxidative stability (peroxide value)

To determine oxidative stability of microencapsulated flaxseed oil in fortified *dahi*, oil was extracted using Soxhlet extraction method given by AOCS (2000). In brief, 20 g *dahi* sample was mixed with 10 g cellulose powder to make a thick paste, and then filled in extraction thimble (30 × 80 mm) followed by extraction with chloroform at 50 °C for 3 h. After extraction of oil, the solvent was evaporated using rotary flash evaporator under vacuum at 40 °C temperature and the extracted oil was used to determine the PV of flaxseed oil according to the IDF standard method 74 A:1991 (IDF, International Dairy Federation 2005).

Effect of storage on α -linolenic acid in MFOP fortified *dahi* measured by Gas-liquid chromatography (GLC)

α -Linolenic acid content in MFOP fortified *dahi* was determined at every third day till 15 days of storage

by GLC method. Fat was extracted from fortified *dahi* according to the method described above. After fat extraction, fatty acid methyl esters were prepared as follows:

Preparation of fatty acid methyl esters (FAMES) of extracted fat/oil

Methylation of extracted oil samples was done by using the sealed tube method of DeMan (1964) and modified by the method of Luddy et al. (1968). Incubation temperature of 75 °C was used instead of 60 °C. Approximately 0.1 g of the melted fat sample was introduced into a freeze drying tube by means of pasture pipette directly to the bottom without sticking fat inside the inner wall of tubes followed by the addition of approximately 0.5 ml of 0.2 N sodium methoxide. The tube was heat sealed and placed in an oven maintained at 75 °C for 1 h with continuous shaking at regular intervals of 10 to 15 min. The methylation was completed in an hour, as was evident from the change of two phase system into one phase system. At the time of analysis, ester tubes were broken from one end and sample was injected into the GLC column without any further treatment. α -Linolenic acid content in fat sample was analyzed in packed glass column using GLC-2010 plus (Shimadzu, Japan) equipped with flame ionization detector and temperature control modules. Injection temperature of 230 °C and detector temperature of 240 °C were used. Glass column (5 % DEGS) having 2 m length, 3.10 mm inner dia and operated in direct mode with 30 mL/min flow rate was used. Initial temperature of glass column was kept 50 °C, which was increased to 190 °C at the rate of 6 °C/min with 2 min holding time. After that, again the temperature was increased to 200 °C at the rate of 1 °C/min.

Table 1 Composition of fortificant (MFOP), control *dahi* and ω -3 fortified *dahi*

Parameter	MFOP [#] (Fortificant)	Control <i>dahi</i>	ω -3 fortified <i>dahi</i>		
Moisture (%)	3.76 ± 0.27	88.43 ± 0.21	88.60 ± 0.15		
Total fats (%)	35.30 ± 0.84	3.04 ± 0.08	3.03 ± 0.08		
• Milk fat (%)	-	3.04 ± 0.08	Level of fortification		
			1 %	2 %	3 %
			2.65 ± 0.31	2.30 ± 0.25	1.95 ± 0.17
• Flaxseed oil (%)	35.30 ± 0.84	-	0.35 ± 0.16	0.70 ± 0.21	1.05 ± 0.19
Solids-not-fat (%)	-	8.50 ± 0.10	8.63 ± 0.31		
-Protein (%)		3.53 ± 0.06	3.62 ± 0.07		
• Casein	30.76 ± 0.89*				
• -Whey proteins	25.70 ± 0.45**				
-Lactose (%)	35.26 ± 0.58	4.86 ± 0.14	5.09 ± 0.10		

[#] MFOP: Microencapsulated Flaxseed Oil Powder, *Protein content in case of FO/NaCas powder, **Protein content in case of FO/WPC, FO-AO/WPC and FO/WPCΔ formulations

Statistical analyses

All measurements were performed in triplicate and reported as the mean $\pm$ standard deviation (SD). One-way analysis of variance (ANOVA) with Tukey's Multiple Comparison Test and Two-way ANOVA with Bonferroni post-tests were performed to observe the significant effect of fortification on physico-chemical characteristics of *dahi*. All statistical analyses were performed at 95 % confidence interval with GraphPad Prism software (version 5.01 for Windows, San Diego California, USA).

Results and discussion

Optimization of fortification level of MFOP in *dahi*

Initially, MFOP fortification level was optimized in *dahi* on the basis of sensory scores (9 point hedonic scale). Results showed that there was no significant ($p < 0.05$) difference among the scores for colour & appearance and sourness of control and MFOP fortified *dahi* (Table 2), irrespective of the fortification level and type of fortificant; indicating that the type as well as level of MFOP preparations did not significantly affect the colour and sourness of *dahi* samples. In case of body and texture, no significant ($p < 0.05$) difference was observed between control and FO/NaCas added *dahi*, irrespective of the fortification level. On the contrary, *dahi* samples fortified with WPC coated MFOP preparations showed significantly ($p < 0.05$) lower scores for body and texture as compared to control. Data also indicated that scores for body and texture decreased significantly with increase in the level of WPC encapsulated MFOP preparations from 2 to 3 %. Lower scores in *dahi* fortified with FO/WPC, FO-AO/WPC and FO/WPC Δ preparations could be attributed to lower gelling properties of whey proteins as compared to caseins. There was no significant ($p < 0.05$) difference among the flavor scores of control and 1 & 2 % fortified *dahi* irrespective of the type of MFOP formulation. However, *dahi* samples fortified at 3 % level showed significantly lower flavor scores as compared to control. The lower flavor scores in 3 % fortified *dahi* could be due to the presence of higher amount of flaxseed oil in the product. Lower acceptability in terms of flavor can also be associated with the release of slightly higher amount of encapsulated flaxseed oil during pasteurization or fermentation process. Overall, it was concluded that omega-3 *dahi* fortified at 1 and 2 % level showed similar sensory profile as control. Therefore, MFOP preparations at 2 % level were selected for the development and physico-chemical analysis of omega-3 fortified *dahi*.

Physico-chemical characteristics of MFOP fortified *dahi*

Effect of MFOP fortification and storage time on the titratable acidity (% lactic acid) of dahi

It is evident from the Fig. 1a that acidity was in the range of 0.63–0.68 % for MFOP fortified samples and control *dahi* on zero day. The acidity of fortified *dahi* samples was slightly higher than the control but the difference was non-significant ($p < 0.05$) up to 12 days of storage. However, after 12 days onwards, acidity of fortified *dahi* was high and the difference between control and test samples was significant ($p < 0.05$) and followed the same trend till the end of the storage. This higher acidity in MFOP fortified *dahi* may be due to the presence of lactose in the MFOP preparations. Andino (2011) reported slightly higher acidity in omega-3 fortified yogurt prepared by adding microencapsulated salmon fish oil till 4 weeks of storage. However, Veena (2014) reported a non-significant increase in acidity of *dahi* fortified with flaxseed oil emulsion till eight days of storage.

Effect of MFOP fortification and storage time on syneresis (%) of dahi

Syneresis is defined as gel shrinkage that occurs concomitantly with liquid/whey expulsion and relates to the inability of the gel network to entrap all of the liquid phase. The syneresis in *dahi*/yogurt occurs when the casein particles rearrange themselves in the gel network leading to the spontaneous expulsion of whey without the application of any external force (Serra et al. 2009). In the present study, the effect of storage and MFOP fortification on syneresis of *dahi* samples is shown in Fig. 1b. Syneresis was almost similar (6.7–6.8 %) and statistically non-significant ($p < 0.05$) in control and FO/NaCas fortified *dahi* sample on zero day. However, *dahi* samples fortified with FO/WPC, FO-AO/WPC and FO/WPC Δ preparations showed significantly higher ($p < 0.05$) syneresis as compared to control, which ranged from 7.4 to 7.6 % on the same day. It was observed that syneresis increased significantly ($p < 0.05$) in all the samples throughout the storage period. However, no significant ($p < 0.05$) difference was observed between syneresis of control (10.0 %) and *dahi* fortified with FO/NaCas powder (10.2 %) at the end of storage. In case of *dahi* samples fortified with FO/WPC, FO-AO/WPC and FO/WPC Δ preparations, syneresis increased significantly ($p < 0.05$) up to the 12.4–12.7 % at the end of 15 days of storage.

Generally, syneresis depends upon the total solids content, fat%, and the nature of proteins present in the product. In all samples, total solids were same, but the type of protein was different. Higher syneresis in *dahi* samples added with FO/WPC, FO-AO/WPC and FO/WPC Δ preparations could be attributed to high whey protein to casein ratio, which induced

Table 2 Effect of MFOP fortification levels on colour & appearance, sourness, body & texture and flavour scores of *dahi*

MFOP fortified <i>dahi</i>	Level of fortification (%)					
	Colour and appearance (Scores out of 10)			Sourness (Scores out of 10)		
	1	2	3	1	2	3
Control	8.00 ± 0.82 ^a	8.00 ± 0.81 ^a	8.33 ± 0.47 ^a	8.33 ± 0.41 ^a	8.00 ± 0.83 ^a	8.10 ± 0.43 ^a
FO/WPC	8.33 ± 0.94 ^a	8.60 ± 0.39 ^a	8.00 ± 0.83 ^a	7.33 ± 0.45 ^a	7.33 ± 0.45 ^a	7.67 ± 0.94 ^a
FO-AO/WPC	8.67 ± 0.74 ^a	8.30 ± 1.41 ^a	8.00 ± 0.85 ^a	8.00 ± 0.29 ^a	8.67 ± 0.47 ^a	7.67 ± 0.46 ^a
FO/WPCΔ	8.33 ± 0.47 ^a	8.33 ± 0.47 ^a	8.67 ± 0.45 ^a	8.00 ± 0.80 ^a	8.33 ± 0.47 ^a	7.33 ± 0.23 ^a
FO/NaCas	8.33 ± 0.94 ^a	8.00 ± 0.82 ^a	8.67 ± 0.43 ^a	7.67 ± 0.94 ^a	7.67 ± 0.94 ^a	8.00 ± 0.82 ^a
MFOP fortified <i>dahi</i>	Level of fortification (%)			Flavour (Scores out of 45)		
	Body and texture (Scores out of 30)			1	2	3
Control	27.00 ± 0.82 ^a	26.00 ± 1.63 ^a	26.67 ± 1.70 ^a	36.33 ± 1.25 ^a	36.00 ± 1.63 ^a	35.67 ± 0.42 ^a
FO/WPC	25.67 ± 0.41 ^b	23.33 ± 0.94 ^b	21.67 ± 1.70 ^b	35.00 ± 0.82 ^a	36.33 ± 1.70 ^a	27.00 ± 2.16 ^b
FO-AO/WPC	24.67 ± 0.94 ^b	22.67 ± 0.42 ^b	20.00 ± 0.82 ^b	35.67 ± 1.23 ^a	35.67 ± 0.43 ^a	24.33 ± 1.25 ^b
FO/WPCΔ	25.00 ± 0.82 ^b	23.33 ± 1.26 ^b	22.67 ± 1.25 ^b	36.67 ± 0.46 ^a	36.33 ± 1.70 ^a	26.00 ± 0.82 ^b
FO/NaCas	26.40 ± 0.89 ^a	26.30 ± 0.82 ^a	25.33 ± 1.25 ^a	35.67 ± 1.29 ^a	36.00 ± 2.16 ^a	27.33 ± 1.26 ^b

FO/WPC: *Dahi* fortified with flaxseed oil powder encapsulated with WPC; FO-AO/WPC: Antioxidant added flaxseed oil powder encapsulated with WPC; FO/WPCΔ: Flaxseed oil powder encapsulated with heat denatured WPC; FO/NaCas: Flaxseed oil powder encapsulated with sodium caseinate. Values are mean ± S.E. ($n = 9$). Values with different superscripts letters are significantly different within the column at $p < 0.05$

the shrinkage of the gel, and subsequently increased whey separation (Lucey 2004; Farnsworth et al. 2006). However, Andino (2011) reported no significant increase in syneresis of control and yoghurt fortified with fish oil powder (prepared with gum arabic and maltodextrin) during storage of 4 weeks.

Effect of MFOP fortification and storage time on texture profile of *dahi*

Textural characteristics of set dairy products such as *dahi* and yogurt can be described in terms of firmness, stickiness (adhesiveness), work of shear and work of adhesion. Firmness,

stickiness, hardness, etc. depends upon the composition, type and amount of fat, nature of proteins and total solids present in the product. In the present study, though the total solids content were kept similar in all the samples, but the nature of the protein varied for example whey proteins, denatured whey proteins and sodium caseinate in the form of flaxseed oil microcapsules. The effect of MFOP fortification and storage time on the firmness of fortified *dahi* samples is shown in Fig. 2a. Data showed that firmness ranged from 2.09–2.14 N for control and 0.88–1.7 N for fortified *dahi* samples during the storage of two weeks. Results indicated that firmness was the highest in control, while lowest in the samples fortified

Fig 1 Effect of MFOP fortification and storage on titratable acidity (% lactic acid) (left) and syneresis (right) of *dahi* stored at low temperature (4–7 °C). FO/WPC: *Dahi* fortified with flaxseed oil powder encapsulated with WPC; FO-AO/WPC: Antioxidant added flaxseed oil

powder encapsulated with WPC; FO/WPCΔ: Flaxseed oil powder with heat denatured WPC; FO/NaCas: Flaxseed oil powder with sodium caseinate. Values are mean ± S.E. ($n = 3$)

Fig. 2 Effect of MFOP fortification and storage time on firmness (N) [A, left] and stickiness (N) [B, right] of *dahi* stored at low temperature (4–7 °C). FO/WPC: *Dahi* fortified with flaxseed oil with WPC; FO-AO/WPC: Antioxidant added flaxseed oil and WPC; FO/WPCΔ: Flaxseed oil with

heat denatured WPC; FO/NaCas: Flaxseed oil with sodium caseinate. Values are mean ± S.E. ($n = 3$). Values with different superscripts letters are significantly different within the group at $p < 0.05$. [Minus (–) sign in stickiness indicates only the direction of probe of texture analyzer]

with FO/WPC, FO-AO/WPC and FO/WPCΔ preparations. However, FO/NaCas-fortified *dahi* showed the firmness (1.55–1.70 N) in between of the control and WPC-encapsulated MFOP preparations. Comparatively higher firmness of FO/NaCas-fortified *dahi* among all fortified samples could be attributed to the better gelling properties of casein micelles. Gelation of milk proteins can be directly correlated with the hydration capacity. The ability of proteins to hydrate and entrap water without syneresis is different for caseins and whey proteins. Hydration values for native whey proteins range from 0.3 to 0.6 g H₂O per g protein. However, casein micelles, can bind up to 4 g H₂O per g protein, mainly due to the enormous amount of void space within the casein micelles' structure and binding of water to the hydrophilic surface of the micelles (Singh and Flanagan 2006). It indicates that caseinates would provide better gelling properties and thus firmness in set *dahi* than that of whey proteins. Similar results have been reported by several researchers, who observed that at similar protein levels, yoghurts enriched with sodium caseinate showed higher firmness compared with

yoghurts enriched with WPC (Modler et al. 1983; Guinee et al. 1995). Similarly, Akalin et al. (2012) studied textural characteristics of probiotic yogurts fortified with sodium calcium caseinate (SCaCN) and WPC; and reported that fortification with SCaCN increased the firmness more than WPC in probiotic yogurts during storage ($P < 0.05$). Damin et al. (2009) also showed that yogurt made with milk supplemented with sodium caseinate resulted in significant increase in firmness. However, Jelen et al. (1987) observed no significant difference in firmness of yoghurt gels made from milk with various casein to whey protein ratios. There was no significant ($p < 0.05$) change in firmness of control and MFOP fortified *dahi* (except FO/NaCas fortified sample) till the end of storage. In case of *dahi* fortified with FO/NaCas preparation, a slight but significant decrease ($p > 0.05$) was observed in firmness after 14 days of storage. It can be suggested that the addition of different MFOP preparations reduced the firmness of *dahi* samples significantly ($p > 0.05$). Veena (2014) developed omega-3 *dahi* supplemented with flaxseed oil emulsion and reported a significant difference between the

Fig. 3 Effect of MFOP fortification and storage time on peroxide value (PV) of *dahi* stored at low temperature (4–7 °C). FO/WPC: *Dahi* fortified with flaxseed oil powder encapsulated with WPC; FO-AO/WPC: Antioxidant added flaxseed oil powder encapsulated with WPC; FO/WPCΔ: Flaxseed oil powder encapsulated with heat denatured WPC; FO/NaCas: Flaxseed oil powder encapsulated with sodium caseinate. Values are mean ± S.E. ($n = 3$). *** indicates the significant difference than the control within the group at $p < 0.001$

Table 3 Effect of storage on α -linolenic acid (% ALA) content (of total fatty acids) in FO/WPC fortified *dahi*

Type of <i>dahi</i>	Period of storage (Days)					
	0	3	6	9	12	15
Control	1.92 $\pm$ 0.1 ^{aA}	1.85 $\pm$ 0.2 ^{aA}	1.81 $\pm$ 0.2 ^{aA}	1.79 $\pm$ 0.4 ^{aA}	1.72 $\pm$ 0.1 ^{aA}	1.68 $\pm$ 0.1 ^{aA}
FO/WPC fortified <i>dahi</i>	10.62 $\pm$ 0.2 ^{bA}	10.36 $\pm$ 0.4 ^{bA}	9.75 $\pm$ 0.1 ^{bB}	9.30 $\pm$ 0.2 ^{bB}	8.87 $\pm$ 0.5 ^{bC}	8.40 $\pm$ 0.1 ^{bD}

Values are mean $\pm$ S.E. ($n = 3$). Values with different superscripts letters are significantly different within the column at $p < 0.05$

FO/WPC: *Dahi* fortified with flaxseed oil powder encapsulated with WPC. Values with different capital superscripts letters (A, B, C.) are significantly different within the column at $p < 0.05$

firmness of control and sample with lower firmness in fortified *dahi*.

Stickiness is an important physical and sensory property of food and has a critical influence on consumers' preference. An excessive stickiness is normally not desirable for a food product because of the inconvenience of handling and the unpleasant oral experience. In the texture profile diagram, stickiness is the negative peak force obtained during the withdrawal of the

probe, which indicates the structural integrity of the product. The effect of MFOP fortification and storage time on the stickiness of *dahi* is presented in Fig 2b. It is evident from the figure that stickiness was maximum for control (0.65 N) and minimum for *dahi* samples fortified with FO/WPC, FO-AO/WPC and FO/WPC Δ preparations (0.19–0.22 N) at zero day. However, in FO/NaCas fortified *dahi*, stickiness was significantly ($p < 0.05$) lower than that of control, while higher

Fig. 4 Gas-liquid chromatography profile of fat extracted from control and MFOP (FO/WPC) fortified *dahi* stored at low temperature (4–7 °C)

than that of other fortified *dahi* samples. There was no significant ($p < 0.05$) change in stickiness in control samples till 14 days of storage, whereas in MFOP fortified *dahi* samples, stickiness decreased after 7 days of storage and reached to the minimum at the end of the study.

Effect of MFOP fortification and storage time on peroxide value (PV) of dahi

Dairy products fortified with polyunsaturated fatty acids are known to be susceptible to oxidation during processing and storage, resulting in development of rancid and off-flavors. The extent of oxidative deterioration of the control and MFOP fortified *dahi* during storage has been depicted in Fig. 3. It is evident from the figure that PV of control (0.38 meq peroxides/kg oil) and MFOP fortified *dahi* samples (0.39–0.41 meq peroxides/kg oil) were almost similar on zero day and difference was statistically non-significant ($p < 0.05$). PV increased gradually in all the samples during the storage. There was no significant ($p < 0.05$) difference among PV of control and fortified *dahi* samples till nine days of storage. However, after twelve days, all the fortified samples showed significantly ($p < 0.001$) higher PV as compared to control till the end of storage, which indicated that the encapsulating membrane was more stable up to 9 days of storage and later on its integrity was damaged. At the end of storage, *dahi* fortified with FO/NaCas preparation showed significantly higher PV as compared to other fortified samples. Comparatively

higher PV in *dahi* fortified with FO/NaCas could be attributed to higher free fats present in the said MFOP formulation (The data of free fat in different MFOP formulations is described in our previous work: Goyal et al. 2015c). Though PV increased significantly after 9 days till the end of storage, but remained well below to the maximum limit (5 meq peroxides/kg oil) prescribed by Codex Alimentarius Commission (1999).

Effect of storage on α -linolenic acid (ALA) content in MFOP fortified dahi

As omega-3 fatty acids are prone to oxidation, their evaluation in fortified food product is essential during the storage. In the present study, the effect of storage on ALA content in FO/WPC fortified *dahi* was evaluated till 15 days of storage at refrigerated temperature (4–7 °C) and is presented in Table 3. Data showed that % ALA content was 1.92 and 10.62 (of total fatty acids) in control and fortified *dahi* sample, respectively on zero day. There was no significant effect of storage on % ALA content of control till 9 days of storage followed by a decrease from 1.92 to 1.68 after 15 days. However, in case of fortified *dahi*, ALA content continuously decreased during storage and reduced to 8.40 % after 15 days. This decrease in ALA content in fortified *dahi* could be attributed to the oxidation of free or microencapsulated flaxseed oil due to dissolved oxygen in *dahi* matrix (Andino 2011). Approximately 21 % decrease was observed in ALA content of MFOP fortified *dahi* after 15 days of storage. Gas-liquid chromatography profile of fat

Table 4 Effect of MFOP fortification and storage on colour & appearance, sourness, body & texture and flavor of *dahi*

MFOP fortified <i>dahi</i>	Days of storage					
	Colour and appearance (Scores out of 10)			Sourness (Scores out of 10)		
	0	9	15	0	9	15
Control	8.83 ± 0.24 ^{aA}	8.83 ± 0.24 ^{aA}	8.67 ± 0.25 ^{aA}	8.83 ± 0.24 ^{aA}	7.57 ± 0.33 ^{aB}	6.54 ± 0.43 ^{aC}
FO/WPC	8.33 ± 0.24 ^{aA}	9.00 ± 0.41 ^{aA}	8.67 ± 0.24 ^{aA}	8.17 ± 0.20 ^{aA}	7.53 ± 0.12 ^{aB}	6.10 ± 0.19 ^{aC}
FO-AO/WPC	8.33 ± 0.47 ^{aA}	8.67 ± 0.21 ^{aA}	8.77 ± 0.17 ^{aA}	8.33 ± 0.47 ^{aA}	7.53 ± 0.26 ^{aB}	6.40 ± 0.72 ^{aC}
FO/WPCΔ	8.33 ± 0.42 ^{aA}	8.67 ± 0.47 ^{aA}	8.90 ± 0.22 ^{aA}	8.33 ± 0.43 ^{aA}	7.60 ± 0.29 ^{aB}	6.25 ± 0.38 ^{aC}
FO/NaCas	9.00 ± 0.31 ^{aA}	8.33 ± 0.47 ^{aA}	8.70 ± 0.16 ^{aA}	8.17 ± 0.24 ^{aA}	7.50 ± 0.22 ^{aB}	6.50 ± 0.46 ^{aC}
MFOP fortified <i>dahi</i>	Days of storage					
	Body and texture (Score out of 30)			Flavor (Scores out of 45)		
	0	9	15	0	9	15
Control	27.67 ± 0.47 ^{aA}	23.33 ± 0.36 ^{aB}	22.67 ± 1.25 ^{aC}	36.10 ± 1.04 ^{aA}	32.30 ± 0.47 ^{aB}	31.67 ± 0.47 ^{aC}
FO/WPC	25.33 ± 0.23 ^{aA}	21.00 ± 0.82 ^{aB}	21.00 ± 0.32 ^{aB}	35.57 ± 1.17 ^{aA}	33.33 ± 0.94 ^{aB}	32.37 ± 0.45 ^{aC}
FO-AO/WPC	24.33 ± 0.48 ^{aA}	21.67 ± 0.47 ^{aB}	19.67 ± 0.47 ^{aC}	34.67 ± 1.25 ^{aA}	32.63 ± 0.52 ^{aB}	31.33 ± 1.89 ^{aC}
FO/WPCΔ	24.33 ± 0.88 ^{aA}	22.67 ± 0.29 ^{aB}	20.33 ± 0.47 ^{aC}	34.67 ± 2.05 ^{aA}	32.53 ± 0.41 ^{aB}	32.17 ± 1.43 ^{aB}
FO/NaCas	26.33 ± 1.25 ^{aA}	23.00 ± 1.63 ^{aB}	22.33 ± 0.29 ^{aB}	35.43 ± 1.74 ^{aA}	33.43 ± 1.14 ^{aB}	32.00 ± 1.63 ^{aC}

FO/WPC: *Dahi* fortified with flaxseed oil powder encapsulated with WPC; FO-AO/WPC: Antioxidant added flaxseed oil powder encapsulated with WPC; FO/WPCΔ: Flaxseed oil powder encapsulated with heat denatured WPC; FO/NaCas: Flaxseed oil powder encapsulated with sodium caseinate. Values are mean ± S.E. ($n = 9$). Values with different small superscripts letters (a, b, c) are significantly different within the column at $p < 0.05$. Values with different capital superscripts letters (A, B, C) are significantly different within the column at $p < 0.05$.

extracted from control milk and fortified *dahi* is shown in Fig. 4. In the said figure, the first chromatogram shows the peak of standard ALA; and other chromatograms shows the complete fatty acids profile of extracted fat from MFOP fortified *dahi*. It is clear that the area of ALA peak (corresponding to the standard ALA peak) of fortified *dahi* sample at 15th day of storage decreased, indicating oxidation of ALA in fortified sample at the end of storage. Our results are in accordance with the results of Andino (2011), who prepared ω -3 yogurt enriched with salmon fish oil powder and reported a significant reduction of 14.52 % in omega-3 contents after 4 weeks of storage. Similarly, Estrada et al. (2011) reported ~5.0 and 27.42 % decrease in ALA and DHA content of microencapsulated salmon oil enriched yogurt. It can be calculated from the data that 15 days stored fortified *dahi* sample had five times higher ALA content than control *dahi* indicating good oxidative stability of α -linolenic acid in MFOP preparation. According to ICMR (2010), 1.6 g of ALA is recommended per day for adults. Assuming the standard serving size of *dahi* as 200 mL, approximate ~31.00 % of the RDA could be met by developed fortified *dahi* even after 15 days of storage.

Effect of MFOP fortification and storage time on the sensory acceptability of dahi

All *dahi* samples were evaluated for sensory attributes such as colour and appearance, body and texture, flavor, sourness and overall acceptability during 15 days of storage. Effect of omega-3 fortification and storage time on the sensory scores of fortified *dahi* is presented in the Table 4. No significant difference ($p < 0.05$) was observed among the scores for colour and appearance of control and fortified *dahi* samples throughout the storage period. Veena (2014) reported no significant ($p < 0.05$) difference between the colour and appearance of control and MFOP fortified *dahi* till 8 days of storage. It is evident from the data that though the scores for flavour and sourness decreased significantly ($p < 0.05$) during storage for control as well as MFOP fortified samples, but on the same day of evaluation, the sensory scores for the said attributes were similar and comparable in all the samples (control and fortified *dahi* samples). It can be inferred from the data that the type of flaxseed oil powder did not significantly affect the sensory scores of fortified *dahi* samples. Although it has been discussed in previous sections that *dahi* samples fortified with flaxseed oil powder (encapsulated by WPC) showed more syneresis and lower firmness (measured by instrument: objective evaluation); but probably due to subjective evaluation, it did not appear in the sensory scores of fortified *dahi*. There was no significant ($p > 0.05$) difference in the scores for body and texture among the control and MFOP fortified *dahi* samples, when evaluated on the same day of analysis. However, data showed that during the storage, scores for body and texture decreased significantly ($p < 0.05$) in control as well as

MFOP fortified *dahi* samples. Veena (2014) reported significant decrease in the scores of body and texture of ALA fortified *dahi* samples during eight days of storage. Our results are also in agreement with the results of various workers (Tarakci and Kucukoner 2003; Salwa et al. 2003), who reported a decrease in score for body and texture of yogurt during storage. Liutkevicius et al. (2010) studied the effect of addition of cold pressed flaxseed oil on the quality of curd. They observed that enrichment of curd with 2 % flaxseed oil resulted in changes in its taste and flavour; which had negative influence on its acceptability.

Conclusions

It can be concluded that market and nutritional value of *dahi* can be enhanced by adding microencapsulated polyunsaturated flaxseed oil powder. In this study, the oxidative stability of microencapsulated flaxseed oil was not compromised and the majority of the physico-chemical characteristics of *dahi* (fortified at 2 % level) remained unaffected. Out of various MFOP preparations, FO/NaCas fortified *dahi* showed physical characteristics comparable to control. This value added functional *dahi* appeals to a wide variety of consumers; therefore it may have the potential to increase sales in the dairy market.

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Compliance with ethical standards

Conflicts of interests No conflicts of interests are to declare.

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