Indian J Dairy Sci 78(2): 114-120
https://doi.org/10.33785/IJDS.2025.v78i02.002

RESEARCH ARTICLE

Flavoured sterilized milk enriched with alpha linolenic acid: physico-chemical properties and evaluation of storage stability

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Received: 01 August 2023 / Accepted: 25 January 2024 / Published online: 23 April 2025

Abstract: Milk is often referred as a complete food, but it lacks certain essential fatty acids like omega-3 fatty acids. Omega-3 fatty acids have been associated with reduced risk of several health diseases. Milk is being widely consumed throughout the masses; it would serve as an ideal vehicle for omega-3 fatty acid fortification. Flaxseed oil is one of the richest vegetarian sources of omega-3 fatty acids. However, due to high susceptibility to oxidation, the use of flaxseed oil is limited in food and dairy products. But this problem can be overcome by encapsulating flaxseed oil where a protective coat is formed on oil droplets. In the present study microencapsulated flaxseed oil was used to fortify milk. The selected microcapsule was used to fortify milk with the aim of providing 25 and 50% RDA of alpha linolenic acid in the milk. Based upon better sensory acceptability, 25% RDA level was selected for further study. Upon addition of flaxseed microcapsules, the sterilized flavoured milk samples were analysed for physico-chemical, sensory and compositional parameters. The samples were also evaluated for storage stability for 28 days. The pH and viscosity of the fortified flavoured milk samples differ significantly ($p < 0.05$) from the control. There was no yeast and mould, coliform count in the samples during storage. Also, the total bacterial count was within the permissible limits for fortified sterilized milk. During storage, the seven, fourteen and twenty-one days of storage depicted non-significant difference ($p > 0.05$) in the flavour score of fortified and control sterilised milk samples. But on 28th day of storage there is significant ($p < 0.05$) difference in sensory score of taste and mouthfeel; however, it was still in the acceptable range. The moisture, fat, protein, ash and total carbohydrates for fortified sterilized milk were 87.30, 3.42, 3.57, 0.85, 4.85%, respectively. Therefore, it can be concluded that the milk can be successfully fortified with alpha linolenic acid fatty acids using the modified starch and soy protein isolate based flaxseed oil microencapsulated powder showed excellent storage stability. The flavoured milk thus developed would provide 0.612 g of ALA in one serving.

Keywords: Fortified milk, Omega-3 fatty acids, Alpha linolenic acid, Storage stability, Sensory acceptability

Introduction

Milk is very nutritious and perhaps requisite food for human being. Milk is fundamental contributor to improve food security and nutrition throughout the world. Predominantly in developing countries, it may serve as a promising food source in reducing malnutrition. However, milk is devoid of omega-3 (ω-3) fatty acids and alpha linolenic acids (ALA), which are considered to be functional ingredients owing to several physiological health benefits. Thus, ALA and Omega-3 fatty acids serve as vital ingredient in developing the nutraceutical and functional food. The Indian Council of Medical Research (ICMR 2010) recommends 1.6 g/ day of ALA and 250 mg of EPA plus DHA per day. This can be achieved through fortification of food products. The rich sources of omega-3 fatty acids include fish oil, flaxseed oil, algal oil, canola oil etc.

Although fish is the greatest contributor of ω-3 fatty acids, but the Indian diets do not include enough oily fish to meet dietary recommendations of ω-3 fatty acids. Moreover, addition of fish oil preparations (in the form of emulsions or spray dried powder) in food is literally impossible for vegetarians due to their religious beliefs and practices. In such a situation, it is very difficult to meet recommended intakes of ω-3 for vegetarians. It has been reported that ω-6: ω-3 ratio in current Indian urban and Western diets is 38-50:1 and 20:1, respectively (Singh et al. 2011; Simopoulos 2011) which appears to be very high as compared to the recommended ratio, i.e. 5:1 (FAO/WHO 2010).

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Flaxseed (*Linum usitatissimum*) oil, also known as linseed oil, is the rich source of ω-3 fatty acids, having 50-60% á-linolenic acid (ω-3, C18:3). Flaxseed oil comprises the essential fatty acid; alpha-linolenic acid (ALA) which the body converts into eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). Due to its highly polyunsaturated nature, flaxseed oil is highly susceptible to oxidation and leads to production of off-flavours and toxic peroxides during heating, processing and handling of the products. So, working stabilization of the flaxseed oil for its food applications is a challenging job. Microencapsulation is the most commonly used technique for the stabilization of active compounds. Spray drying is a common method of encapsulation in the food industry (Phisut 2012; Renata et al. 2014). In an effort to stabilize flaxseed oil, the authors have developed flaxseed oil microcapsules (Tambade et al. 2020) using modified starch and soy protein isolate as matrix materials. The developed capsules are stable in nature to be utilized in dairy products.

Lin et al. (2014) fortified dairy products with soy lecithin-stabilized emulsions containing 50 % algal oil, 6 % soy lecithin, and 44 % Milli-Q water. Efforts have been made to develop omega-3 fatty acid fortified baked products, processed cheese, fruit yoghurt, market milk (Goyal et al. 2017; Veena and Nath 2017) but limited work has been done on the utilization of flaxseed oil as a useful functional ingredient in sterilized flavoured milk which is consumed by the larger segment of the population. Secondly, although some of the studies have been carried out for encapsulation of flaxseed oil but concerted efforts are lacking for the development of omega-3 fatty acids fortified dairy products utilizing flaxseed oil, which have good potential as a functional ingredient in dairy products. As milk and milk products are widely consumed by all age group of people and by various standards of livings they play a vital role in nutritional security. So, flavoured milk can serve as an ideal vehicle for fortification of alpha-linolenic acids due to higher palatability among different age groups. Thus, with this backdrop, the present investigation is really required for development of omega-3 fatty acid fortified flavoured sterilized milk. Thus, the present study was planned to study the effect of microencapsulated flaxseed oil in sterilized flavoured milk upon storage.

Materials and methods

Materials

We received cold pressed flaxseed oil as a gift from AAK Kamani Pvt. Ltd., Andheri, Mumbai (Maharashtra, India). Soy protein isolate (SPI) and modified starch (NC-46) used for microencapsulation were procured from Shridurga Sales Corporation, Bangalore, India and Ingredion India Private Ltd, Thane, Maharashtra, India, respectively. In this study, all other chemicals and reagents were of AR grade. Toned milk was obtained from the experimental dairy plant of SRS, ICAR-NDRI. Sugar was procured from local market.

Preparation of flaxseed oil microcapsules

Flaxseed oil microcapsules were prepared using the methodology of Tambade et al. (2020). Firstly, the emulsion was prepared by mixing the calculated amount of 25% flaxseed oil (on Total solids basis), 5% soy protein isolate and 20 % modified starch using hand blender (Phillips, India) for approximately 5 minutes and then prepared solution was homogenised with high shear mixer (IKA T-18, Germany) at 18000 rpm for 5 minutes. The prepared emulsion was preheated in waterbath to 40ÚC for microencapsulation in order to decrease the viscosity for proper atomisation in drying chamber of spray dryer (Technosearch Instrument, Thane, Mumbai, India). For spray drying, inlet and outlet hot air temperatures were maintained at 180±5ÚC and 85±5ÚC, respectively, while, flow rate was maintained from 40-60 mL/min. The microcapsules were stored in aluminium laminates for further study.

Preparation of control and fortified sterilized milks

Three samples were used for the study. The control was prepared using the toned milk (3.0% fat and 8.5% SNF). The second sample, i.e. fortified sterilised milk was prepared by the addition of flaxseed oil microcapsule to meet at least 25% RDA in 240 mL milk. For preparation of the third sample, namely sterilised flavoured fortified milk, milk was standardised to 3.0% fat and 8.5% SNF, sugar was added @8% and then preheated (65°C) for homogenisation. After homogenisation, the IFF banana flavour was added @ 0.05% and calculated amount of microcapsules were added (at 40°C) and mixed properly. Bottles were filled with some headspace and corked. All the three samples were sterilised (In bottle sterilisation at 121°C for 15 min) followed by cooling to room temperature slowly. The sterilised milk samples were then stored (25°C) for storage study and further analysis.

Physico-chemical properties of fortified flavoured sterilized milk

Titratable acidity

Titratable acidity of control and fortified milk was determined as per IS: SP: 18, Part XI (1981).

Free fatty acids (FFA) content

An extraction titration method devised by Deeth et al. (1975) was followed for the determination of FFA content of milk samples.

pH

pH of milk samples was determined by potentiometric method using digital pH meter (Eutech, India). The pH meter was first calibrated using standard buffers of pH 4.0 and 9.2 and standardized using pH buffer of 7.0 at 25.0 ± 0.1°C.

Viscosity

Viscosity of fortified sterilized milk was measured by the capillary viscometer (Ostwald viscometer) following the methodology of Roy and Sen (1994).

Sensory evaluation of fortified flavoured sterilized milk

Sensory evaluation was done by a semi-trained panel of ten judges of different age groups and gender from Dairy Processing section of SRS-NDRI, Bangalore, India. Omega-3 fortified milk was evaluated by the panellists using the nine-point Hedonic scale for colour and appearance, mouthfeel, taste and flavour and overall acceptability in comparison to control milk. Milk was served at 10-15°C temperature for sensory evaluation.

Microbiological evaluation of fortified flavoured sterilized milk

Microbiological analysis of fortified milk was performed on 0, 7, 14, 21 and 28 days of storage. Standard plate count was enumerated using nutrient agar. Plating of serially diluted fortified milk samples were done and plates were incubated at 37°C for 24-48 hours. Coliform count was enumerated using Violet Red Bile agar and plates were incubated at 37°C for 24-48 hours and Yeast and mold count was enumerated using PDA agar and plates were incubated at 30°C for 48-72 hours. Spore count was enumerated using spore count agar and plates were incubated at 30°C for 24-48 hours. Prior to spore count, sterilised milk was incubated at 63°C for 24 hours.

Proximate composition of fortified flavoured sterilized milk

Total solids (TS), fat, ash and nitrogen content of milk samples were determined by the methods as described in IS: SP-18 (1981). Total carbohydrate content was determined by difference method as per IS: SP-18 (1981). Nitrogen content in milk samples was estimated by Kjeldahl method as given in eq (1):

$$Nitrogen\ \% = \frac{14.07 \times (Vs - Vb) \times Normality\,of\ sulphuric\ acid \times 100}{Wt.of\ sample}$$

(1)

Where,

V_s = mL 0.1 N H_2SO_4 titrant used for test portion

V_b = mL 0.1 N H_2SO_4 titrant used for blank

Protein content % in milk= Nitrogen content (%) × 6.38

Alpha linolenic acid (ALA) content of fortified flavoured sterilized milk

The alpha linolenic acid content was determined using the method used by Pandule et al. (2021). Firstly, the fat was extracted and then Fatty acid methyl esters (FAME) were prepared. The internal standard- tridecanoic acid (Sigma Aldrich, India) (200 mL, 25 mg mL^{-1}) was added in the extracted fat before preparation of FAME. The FAME were then subjected to gas chromatography-mass spectrometry (GCMS) for quantification of alpha linolenic acid. The FAME were separated using the DB5 MS (30 m × 0.25 mm × 0.25 mm) capillary column with helium as a carrier gas at a ûow rate of 1 mL min^{-1}. The injector temperature was 230 °C, and column temperature was programmed as follows: 50 °C for initial 1 min, subsequent increase to 220 °C at the rate of 3 °C min^{-1} and maintained it for 1 min. The interface temperature for GC-MS (Agilent Technologies, Santa Clara, CA) was 220 °C. Identification of fatty acids was done based on data from mass spectral libraries (NIST 47, NIST 147 and Wiley 175), literature data and by comparison of retention times with Supalco-37 FAME standards (GLC-85 and FAME Mix GLC-90). Results were expressed as g/100mL ALA content.

Statistical analysis

The data obtained were analyzed using analysis of variance technique for three replicates with the help of SPSS software and statistical significance was set at $p<0.05$. The least significant difference (LSD) test was used to find out significant differences between sample means. Analysis of variance (ANOVA) was used to determine differences among treatment means using the Post Hoc Test (Dunkan). Sensory attribute of milk samples data during storage were analysed using 2-way analysis of variance (ANOVA), with main effects of treatments and day of storage.

Results and Discussion

Selection of level of addition of flaxseed oil microcapsules for fortified flavoured sterilized milk preparation

Milk was fortified with flaxseed oil microcapsules for providing at least 25 and 50% recommended dietary allowance (RDA) of alpha linolenic acid (ALA) in one serving (240mL). The sensory acceptability of fortified samples is represented in Table 1. It is evident from the table data, that there was no significant ($p>0.05$) difference among the scores for colour, mouthfeel of control milk and F25 milk sample, however, the mouthfeel of F50 milk was lower than control milk. Further, the taste and flavour and overall acceptability decreased significantly ($p<0.05$) upon addition of microcapsule. Based upon higher sensory acceptability, 25% RDA level was selected. In order to further increase the acceptability by a wider group of consumers, flavoured fortified milk was also developed and compared for various physico-chemical parameters, sensory acceptability, rancidity and microbiological spoilage during storage.

Compositional analysis of alpha linolenic acid fortified flavoured sterilized milk

The proximate composition of fortified and control sterilized milk were analysed and the results obtained are presented in Table 2.

From the table, it can be interpreted that the control sample and fortified sample were significantly different ($p<0.05$) from each other. The moisture, fat, protein, ash and total carbohydrates for control milk were 88.52, 3.10, 3.47, 0.81, 4.10% respectively. While the fat, protein and total carbohydrates difference in control and fortified milk sample were due to addition flaxseed oil microcapsules made from soy protein isolates and modified starch. The constituents were similar in both milks except for higher carbohydrate content in the flavoured fortified milk (Table 2) owing to the addition of sugar. Further, it is vital to note that 3 g of microcapsules added to 240 mL of milk were sufficient to provide 0.612 g of ALA (Table 2), which accounts to 38.25% of RDA as per ICMR (Indian Council of Medical Research), 2010 guidelines and 27.81 % as per ISSFAL (International Society for the Study of Fatty Acids and Lipids), 2004 guidelines.

Sensory acceptability of fortified flavoured sterilized milk during storage

The changes in the score of colour and appearance are shown Table 3. It is evident from the data that on 0th day, score of plain sterilised milk was slightly higher than the fortified sterilised and fortified flavoured sterilised milk. The sensory scores for colour and appearance were statistically same for first and seventh day of storage and thereafter the score were found to decrease over the period of storage. The colour and appearance score was less for the fortified flavoured sterilised milk because of maillard browning taking place during the heat treatment as addition of sugar was done in to milk. While control and fortified sterilized milk has almost same colour and appearance score over the storage period. Also, the score for mouthfeel of fortified and control milk samples decreased with storage period. It can be seen that the mouthfeel scores followed following trend: fortified flavoured milk > Plain sterilised > fortified sterilised. The mouthfeel score for fortified flavoured sterilised milk was more may be because of smoothness in texture of fortified flavoured milk, while followed by plain sterilised and lastly fortified sterilised, because of addition of microcapsules.

Effect of storage on sensory scores of taste and flavour and overall acceptability of fortified and control sterilized milk are given in Table 3. The taste and flavour scores were maximum on 0 day for all the three samples. Acceptability of fortified milk on zero day was comparable to the control milk while sterilised flavoured milk had slightly higher taste and flavour scores than the fortified milk. Upon storage for seven, fourteen and twenty-one days there were non-significant ($p>0.05$) differences in the taste and flavour score of fortified and control sterilised milk samples. But on 28th day of storage, there were significant ($p<0.05$) differences in the sensory scores for taste and flavour of fortified and control samples.

The fortified flavoured sterilised milk was liked the most by the sensory panellist as compared to control and fortified sterilised milk samples. The overall acceptability scores on the 0th day for plain sterilised and fortified sterilised milk were 8.05 and 7.88

Table 1 Effect of level of microcapsules on sensory acceptability of fortified flavoured sterilized milk

Sensory Parameters	control	F25	F50
Colour and appearance	8.78 ± 0.25^{a}	8.72 ± 0.56^{a}	8.81 ± 0.12^{a}
Mouthfeel	8.61 ± 0.65^{a}	8.58 ± 0.78^{a}	8.39 ± 0.77^{b}
Taste and Flavour	8.72 ± 0.23^{a}	8.47 ± 0.46^{ab}	7.39 ± 0.82^{b}
Overall Acceptability	8.67 ± 50^{a}	7.78 ± 0.72^{b}	7.28 ± 0.24^{c}

F25- sample having microcapsules providing 25% RDA of ALA, F50- sample having microcapsules providing 50% RDA of ALA, Results are expressed as Mean±SD, n=10; Means with different small letters superscript (a,b,c) within row differ significantly ($p<0.05$) among the samples

Table 2 Proximate composition of fortified flavoured sterilized milk

Constituents (%)	Control	Sterilized Milk	
		Plain fortified sterilized milk	Flavoured fortified sterilized milk
Moisture content	88.52 ± 0.13^{a}	87.30 ± 0.21^{b}	79.56 ± 0.47^{c}
Fat	3.10 ± 0.03^{b}	3.42 ± 0.02^{a}	3.37 ± 0.02^{a}
Protein	3.47 ± 0.05^{b}	3.57 ± 0.03^{a}	3.57 ± 0.03^{ab}
Ash	0.81 ± 0.13^{c}	0.85 ± 0.21^{b}	0.99 ± 0.47^{a}
Total Carbohydrates	4.10 ± 0.11^{c}	4.85 ± 0.19^{b}	12.51 ± 0.44^{a}
ALA, g/100mL	---	0.255	0.255

Results are expressed as Mean±SD, n=3; Means with different small letters superscript (a,b,c) within row differ significantly ($p<0.05$) among the samples respectively, while on 28th day, it was 7.11 and 7.94. Whereas score for the fortified flavoured sterilised was 8.61 on first day while, at end of storage it was 7.55 (Table 3). The higher overall acceptability may be due to the addition of sugar and artificial flavour in fortified flavoured sterilised milk.

Viscosity of fortified flavoured sterilized milk during storage

Viscosity is the most important physical change taking place during the storage of market milk. The extent depends on temperature and time of storage and history of heat treatment (Usarek et al. 1997). It may be seen from the results (Table 4) that the viscosity of the fortified milk was significantly ($p<0.05$) higher than the control sample throughout the storage period. The viscosity of both control and fortified milk increased significantly ($p<0.05$) with increasing storage period. Cano-ruiz and Richter (1998) reported that the apparent viscosity of the samples increased as milk solids non-fat increased. The highest viscosity was found in fortified flavoured sterilised milk (5.290 mPas), followed by fortified sterilised milk (2.833 mPa-s) and sterilised milk (2.012 mPa-s) while, at the end of storage it was 6.009, 3.796 and 2.509 mPa-s in fortified flavoured, fortified, plain sterilised milk, respectively. This observation indicated that the interaction of added sugar, milk proteins and milk fat caused a significant increase in the viscosity of the fortified milk. Similar findings were also reported by Veena and Nath (2017) for increase in the viscosity for fortified milk. A higher volume fraction of milk solids would result in greater viscosity. Thus, higher fat and total solids content in fortified milk might have contributed for a higher viscosity. Further, Rauh et al. (2014) attributed the rise in viscosity to proteolysis, resulting in gel formation during the storage of UHT milk due to protein interactions and imbibition of water leading network of bonds.

Acidity and pH of fortified flavoured sterilized milk during storage

The chemical properties viz. acidity and pH of control and fortified milk samples during storage are shown in Table 4. Lactic acid is the principal acid produced due to which titratable acidity of milk rises. Increase in free fatty acids is also responsible for increasing the total titratable acidity of milk (Swartzel 1983). From the results, it can be seen that titratable acidity increased significantly ($p< 0.05$) with storage in both control and fortified sample. Titratable acidity of control and fortified sterilised and flavoured fortified sterilised milk increased from 0.159 to 0.339% LA, 0.168 to 0.369% LA and 0.168 to 0.375% LA, respectively, at

Table 3 Sensory attributes of control and fortified flavoured sterilized milk samples during storage (at 25^{o}C)

Colour and appearance	Days	Sample		
		PS	FS	FFS
	0	8.27 ± 0.26^{aA}	8.16 ± 0.25^{aA}	8.16 ± 0.25^{aA}
	07	8.05 ± 0.63^{aA}	8.05 ± 0.30^{aA}	8.12 ± 0.20^{aA}
	14	7.66 ± 0.75^{aAB}	7.55 ± 0.68^{aAB}	8.05 ± 0.05^{aAB}
	21	8.00 ± 0.43^{aB}	7.88 ± 0.22^{aB}	8.11 ± 0.22^{aB}
	28	7.20 ± 0.26^{aC}	7.22 ± 0.26^{aC}	7.22 ± 0.36^{aC}
Mouth feel	0	7.94 ± 0.30^{bA}	7.88 ± 0.33^{bA}	8.23 ± 0.27^{aA}
	07	7.94 ± 0.39^{bA}	7.94 ± 0.30^{bA}	8.33 ± 0.25^{aA}
	14	7.55 ± 0.58^{bAB}	7.27 ± 0.66^{bAB}	8.16 ± 0.50^{aAB}
	21	7.75 ± 0.35^{bB}	7.83 ± 0.25^{bB}	7.88 ± 0.33^{aB}
	28	7.33 ± 0.35^{bC}	7.33 ± 0.35^{bC}	7.44 ± 0.46^{aC}
Taste and Flavour	0	7.94 ± 0.30^{cA}	7.77 ± 0.26^{bA}	8.61 ± 0.48^{aA}
	07	7.56 ± 0.62^{cB}	7.33 ± 0.66^{bB}	8.55 ± 0.30^{aA}
	14	7.44 ± 0.39^{cAB}	6.88 ± 0.65^{bAB}	8.05 ± 0.52^{aAB}
	21	6.34 ± 0.33^{cAB}	7.22 ± 0.61^{bAB}	7.77 ± 0.66^{aAB}
	28	7.31 ± 0.45^{cC}	7.16 ± 0.35^{bC}	7.72 ± 0.44^{aC}
Overall acceptability	0	8.05 ± 0.30^{bA}	7.88 ± 0.33^{bA}	8.61 ± 0.41^{aA}
	07	7.55 ± 0.60^{bA}	7.84 ± 0.32^{bA}	8.61 ± 0.22^{aA}
	14	7.44 ± 0.46^{bB}	7.16 ± 0.55^{bB}	8.11 ± 0.48^{aB}
	21	7.27 ± 0.06^{bB}	7.38 ± 0.54^{bB}	7.83 ± 0.61^{aB}
	28	7.11 ± 0.33^{bC}	7.94 ± 0.39^{bC}	7.55 ± 0.39^{aC}

PS- Plain sterilized milk (Control), FS- fortified sterilized milk, FFS- fortified flavoured sterilized milk, Results are expressed as Mean±SD, n=10; Means with different small letters superscript (a,b,c) within row and capital letters (A,B, C) within the column differ significantly (p<0.05) among the samples

28 days of storage. Similar findings were reported by Goyal et al. (2017) for fortified market milk. According to Cais-Sokoliñska et al. (2002), acidity changes in sterilised milk could be attributed to the course of enzymatic (mainly lipolytic) reactions, which result in the formation of free fatty acids. A similar phenomenon, in which pH decreased and potential acidity increased with the time of UHT milk storage, was described by Biliñska et al. (1998). A certain effect on the acidity of sterilized milk (mainly milk sterilized by the long-time method) can be exerted by small amounts of formic, acetic and other acids and maillard-type reactions (Fink and Kessler 1986). As reported in the literature, acidity of sterilised and UHT milk reflects product freshness and, to a certain extent, the quality of raw milk used for processing and the intensity of heat treatment used during the technological process (Kruk et al. 1995).

It can be inferred from results (Table 4) that the pH of the control and fortified milk decreased significantly ($p<0.05$) with storage period, which corresponds to the increasing acidity. The results are in agreement with Veena and Nath (2017) for milk fortified with omega-3 fatty acids, phytosterols and soluble dietary fibre. Between zero and the 28th day of storage, significant differences ($p<0.05$) in pH value were noticed in both control and fortified milk samples. Fortified milk had the lowest pH value throughout storage period and did not differ significantly ($p>0.05$) from the control sample. The initial pH of the control and fortified sterilised and flavoured fortified sterilised milk was 6.68, 6.67 and 6.65, respectively and at the end of storage (28th day) it was 6.42, 6.36 and 6.33, respectively. Venkatachalm and McMahon (1991) verified a drop in pH during storage of UHT milk and associated it with browning reactions. Andrews et al. (1977) confirmed similar effects and concluded that the level and extent of pH decrease was related to age-gelation. When milk is heated at a temperature above 100^{o}C and subsequently stored, lactose is degraded to acids.

Hydrolytic rancidity of fortified flavoured sterilized milk during storage

The hydrolytic rancidity of control and fortified milk during storage was evaluated by determining FFA content and the results are presented in Table 4. The level of un-esterified fatty acids in milk provides a measure of the extent of lipolysis in milk. The free fatty acids (FFA) content in milk is dependent on the changes in the lipolytic activity of sterilized milk. From the results, it can be seen that FFA content increased significantly ($p<0.05$) with storage in both control and fortified milk samples. Between control and fortified milk samples, significant ($p<0.05$) difference in FFA content was observed. The increase in the FFA content was 0.170 ìEq/ml, 0.315 ìEq/ml and 0.166 ìEq/ml for control, fortified sterilised and fortified flavoured sterilised milk, respectively after 28 days of storage. After 28th day of storage at 25^{o}C, the FFA content in control, fortified milk and fortified flavoured sterilised milk samples was 0.417 ìEq/ml, 0.644 ìEq/ml and 0.517 ìEq/ml, respectively. The results are in agreement with the findings of Deeth et al. (1975) for milk with low levels of lipolysis. They reported FFA content of d” 1.0 ìEq/ml for low lipolysis milk.

Table 4 Effect of storage (4-7^{o}C) on physico-chemical properties of fortified flavoured sterilized milk

Parameter	DAY	PS	FS	FFS
Acidity (% Lactic acid)	0	0.159 ± 0.005^{cE}	0.168 ± 0.005^{bE}	0.168 ± 0.005^{aE}
	7	0.180 ± 0.009^{cD}	0.192 ± 0.005^{bD}	0.204 ± 0.014^{aD}
	14	0.219 ± 0.005^{cC}	0.225 ± 0.009^{bC}	0.234 ± 0.009^{aC}
	21	0.285 ± 0.005^{cB}	0.303 ± 0.005^{bB}	0.318 ± 0.005^{aB}
	28	0.339 ± 0.005^{cA}	0.369 ± 0.016^{bA}	0.375 ± 0.010^{aA}
pH	0	6.68 ± 0.01^{aA}	6.67 ± 0.01^{bA}	6.65 ± 0.00^{cA}
	7	6.64 ± 0.00^{aB}	6.64 ± 0.00^{bB}	6.63 ± 0.02^{cB}
	14	6.57 ± 0.01^{aC}	6.55 ± 0.01^{bC}	6.51 ± 0.01^{cC}
	21	6.49 ± 0.01^{aD}	6.45 ± 0.01^{bD}	6.41 ± 0.01^{cD}
	28	6.42 ± 0.01^{aE}	6.36 ± 0.01^{bE}	6.33 ± 0.01^{cE}
Viscosity (m Pas)	0	2.012 ± 0.033^{cD}	2.833 ± 0.042^{bD}	5.290 ± 0.247^{aD}
	7	2.062 ± 0.01^{cC}	2.883 ± 0.028^{bC}	5.793 ± 0.028^{aC}
	14	2.093 ± 0.042^{cBC}	2.978 ± 0.047^{bBC}	5.827 ± 0.074^{aBC}
	21	2.259 ± 0.024^{cB}	3.099 ± 0.053^{bB}	5.938 ± 0.039^{aB}
	28	2.509 ± 0.250^{cA}	3.796 ± 0.070^{bA}	6.009 ± 0.070^{aA}
FFA (µEq/ml)	0	0.247 ± 0.000^{cD}	0.329 ± 0.005^{bE}	0.351 ± 0.004^{aE}
	7	0.272 ± 0.001^{cD}	0.464 ± 0.012^{bD}	0.356 ± 0.007^{aD}
	14	0.329 ± 0.004^{cC}	0.498 ± 0.001^{bC}	0.433 ± 0.004^{aC}
	21	0.360 ± 0.002^{cB}	0.496 ± 0.005^{bB}	0.430 ± 0.000^{aB}
	28	0.417 ± 0.005^{cA}	0.644 ± 0.003^{bA}	0.517 ± 0.003^{aA}

PS- Plain sterilized milk (Control), FS- fortified sterilized milk, FFS- fortified flavoured sterilized milk, Results are expressed as Mean±SD, n=3; Means with different small letters superscript (a,b,c) within row and capital letters (A,B, C) within the column differ significantly ($p<0.05$) among the samples

Microbiological quality of fortified flavoured sterilized milk during storage

The application of heat at high temperatures for a sufficient time renders milk or milk products commercially sterile, thus resulting in products that are safe and microbiological stable at room temperature. The control and fortified sterilised milks were analysed for standard plate count, yeast and mold, coliform count and spore count during the 28 days' storage period and were not detected throughout the storage period. Thus, it could be interpreted that the sterilisation treatment to milk was carried out successfully.

Conclusions

The present study resulted in the development of a sensorially acceptable alpha linolenic acid fortified sterilized milk. It can be inferred that the microencapsulated flaxseed oil powder can be suitably used to fortify milk to provide at least 25% recommended dietary allowance of alpha-linolenic acid in one serving of fortified milk with acceptable physico-chemical characteristics. The developed sterilized milk had acceptable sensory acceptability and was microbiologically safe throughout the storage period of four weeks at 25°C. Further, encapsulated flaxseed oil powder can also be used for the fortification of several other products such as bakery products, confectionery, fruit juices and other dairy and food products.

Acknowledgments

The authors gratefully acknowledge Director, ICAR-National Dairy Research Institute, Karnal, India and Head, SRS, ICAR-NDRI, Bengaluru for financial assistance and AAK Kamani Oil Industry, Mumbai, India for gifting flaxseed oil to conduct the present study.

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