Omega-3 enriched Granola bar: Formulation and Evaluation under different Storage Conditions

K. Sarika^{1*}, K. Jayathilakan², R. G. K. Lekshmi¹, E. R. Priya¹. S. S. Greeshma¹ and A. Rajkumar² ¹ ICAR-Central Institute of Fisheries Technology (CIFT), P. O. Matsyapuri, Cochin - 682 029, India ² Defence Food Research Laboratory (DFRL), Siddarthanagar, Mysore - 570 011, India

Abstract

A functional protein rich fish based granola bar was developed and effect of various storage conditions on the quality and stability were evaluated. Granola bar was fortified with omega-3 fish oil, which was emulsified with milk protein and lyophilized to get fish oil microcapsules. Ingredients and process optimization were carried out based on the physical and sensory evaluation. Granola bar was fortified with omega-3 (2%) and extra supplemented with highly digestible dehydrated fish protein (5%) providing higher calorific value of 395-400 kCal 100 g⁻¹. Bars were convenient, ready-to-eat, contributing about 17.25% protein, 82 mg Eicosapentaenoic acid (EPA) + Docosahexaenoic acid (DHA) 100 g⁻¹ for meeting the Recommended Dietary Allowance (RDA). The physico-chemical stability of the bars packed in laminated (Paper-Al foil-LDPE) films were analysed under 30±2°C, 37°C and -18°C in order to simulate different storage conditions. The low moisture content and water activity established microbial safety and maximum extension of shelf life. Oxidative stability was little affected in fortified bars stored at 37°C. Textural analysis showed the development of hard or tough texture in protein bars during storage. The study revealed that fortification of granola bars with fish oil and fish protein enhanced the nutritive value without significantly affecting the safety and stability.

Keywords: Granola bar, functional food, omega-3, nutrition

Received 17 November 2018; Revised 05 April 2019; Accepted 08 April 2019

*E-mail: sarikacift@gmail.com

Introduction

The world food market is focusing more on functional and nutraceutical foods, which provide not only nutritive values but also health benefits to humans by supplementing nutrients or by curing/ preventing diseases. Now these food products are rapidly growing in the market and scientific research also greatly proliferating in this area. Food fortification is a convenient and efficient means, which allows one to have an assortment of foods fortified with different micro and macro nutrients. Supplementation of omega-3 through intake of fortified foods will meet the body's metabolic needs better than a dietary supplement or pills (Maki et al., 2003). Fish oil, an excellent dietary source of omega-3 fatty acids like - EPA and DHA, is beneficial for the healthy functioning of the heart, brain and nervous system. But fish oil has a strong odour and unless it is protected, oil will easily get oxidized. So a possible method of protecting the highly unsaturated oil is by microencapsulation techniques. Microencapsulation is a process by which oil is transformed into a solid ingredient, where the small droplet of oil is surrounded by protein/carbohydrate coating, which helps in retarding oxidation (Ahn et al., 2008). Now microencapsulation technique has drawn considerable attention in the food industry. Similarly, fortification of fish protein in the convenient foods offers high quality digestible protein with fewer calories than similar sized portion of meat. Fish protein having a high biological value, readily broken down and absorbed in the body, can balance the low lysine and sulphur-containing amino-acids (methionine & cysteine) in cereals based diet.

The changing lifestyle adds to the requirement of nutritious energy supplements along with a pleasing food portability method. The nutritious energy bars have gained more importance and popularity in the global market during recent years has provoked the food manufacturers to develop food bars that provide nutrition and convenience (Izzo & Niness, 2001). The food bars are snacks of good sensory and nutritional characteristics due to their high carbohydrates, proteins, lipids and mineral content. Cereal bars are ready-to-eat convenient products occupying larger space in the consumer market which not only satisfy the hunger, but prove as a quality source of nutrients and a convenient means of replacement of a meal (Catherine & Johnston, 2012). These ready-to-eat foods are often susceptible to lipid oxidation, irrespective of lipid content, acquire considerable flavour and odour, and thereby reduce sensory perception, nutritional quality and safety of foods during storage (Esterbaur, 1993; Forss, 1995; Kubow, 1992). The easiness of carrying in hand made the product subjected to wide range of climatic zones like extremely cold to slightly elevated temperatures. So in the present study, a granola bar was formulated with cereals bases fortified with fish proteins and omega-3 and the shelf stability under different storage temperatures (30±2°C, 37°C and -18°C) were evaluated.

Material and Methods

The ingredients used in the granola bars preparation were rolled oats (Avena sativa), wheat (Triticum aestivum), corn flour, corn flakes, skimmed milk powder (Nandini brand), flattened rice and dried fruits like almond (Prunus dulcis), cashew (Anacardium occidentale), peanuts (Arachis hypogaea), raisins, honey (Dabur brand), salt, date syrup (Lion dates brand), sugar, cardamom, vanilla essence (Bush brand) and red grape juice procured from market. Dried fish oil microcapsules were added for fortification of omega-3 in granola bar. Microencapsulation of fish oil was done using freeze drying technique according to Keogh et al. (2001). The emulsion was prepared by adding milk powder contained both protein and lactose (23%) to deionized water (65.5%) having pH 7 and kept at 50 to 60°C temperature. After cooling the solution to 20-25°C emulsion was made with fish oil (11.5%) under constant homogenizing, and then centrifuged @ 11000 RPM. The total solid content of the emulsion was calculated as 34.5%. The emulsion microstructure was captured using an optical microscope (Leica ICC50 HD) equipped with microscope digital camera with an objective magnification of 40X. An image of the emulsion was acquired using digital image processing software (Image-pro plusTm, version 6). The emulsion stability was measured as percentage of separation, which is calculated based on the ratio of height of cream layer to initial emulsion height.

Percentage of separation = (Upper phase height/Emulsion initial height) *100

The emulsion was dried by using freeze drying technique for 24 h to make dried fish oil microcapsules. The microencapsulated powder was then used as an ingredient in optimization of fish based granola bars at different levels, to enrich with omega-3.

In addition, freeze dried fish protein powder from Rohu (*Labeo rohitha*) was used as major protein ingredient in granola bar preparation. Cultured Rohu fish, procured from the local fish market of Mysore was used for the preparation of fish protein powder. All the chemicals used for the analysis were of analytical grade and obtained from M/s. Merck (India) and Hi-Media, Mumbai.

Granola bars are snack foods prepared from oats, wheat, puffed rice, corn, nuts and almonds usually baked till crispy and mixture is heated with continuous stirring to maintain a loose but cereal type consistency. Ingredients were standardized based on the physical and sensory properties (Table 1). The ingredients like oats and wheat flour were mixed and roasted at 140°C for 3 min in a hot pan to get-the roasted aroma. Good quality rice flakes, corn flakes and corn flour were then mixed and roasted to have a proper puffing and crispiness. These ingredients contributed to the base formulation. Dried fruits like raisins and dates were slightly warmed and added to make the bar rich in calories. Almond and cashew nuts were roasted at 220°C for 3 min, peanuts for 2-3 min before adding into the hot binder mixture.

The binder formulation was prepared by adding sufficient quantity of sugar (18-20%), glycerin, honey and red grape juice in to the boiling water (35%) and thoroughly mixed. The temperature of the solution was intermittently checked for standardizing the stickiness. Once the temperature reached 100°C, the mixture was maintained at that temperature for further 3-4 min to get thick consistent syrup. The pre-weighed roasted ingredients were added to the hot binder mixture with a through mixing under low flame and consequently supplemented with freeze dried fish protein and fish oil microcapsules to fortify the bar with essential amino acids and fatty acids especially omega-3

Table 1. Optimized ingredients for the formulation of Fish based Granola bar (100 g)

Ingredients	Quantity (100 g)
Base Mixture	50-55 g
Oats	10
Wheat	5.0
Flattened rice	7.5
Corn flakes	5.0
Corn flour	2.5
Milk powder	2.5
Almonds	5.0
Cashew	2.5
Peanuts	2.5
Raisins	5.0
Dates	2.5
Binder	30-35 g
Fish oil microcapsules	2 g
Fish protein powder	5 g
Water	10 ml

The hot mix was then immediately transferred into a stainless steel rectangular mould (40 cm x 30 cm) having 50 small rectangular sections (7.5cm x 2.5 cm x 1.5 cm), evenly filled and pressed hard with a small handled steel presser to obtain bars of uniform shape and size. Samples in the mould were kept in chilled condition for 20 min to get proper setting. Bars were demoulded and individually packed in PFP laminate. Two lot of samples, control-T1 and fortified samples-T2 were prepared and kept under different storage conditions like room temperature-RT: 28±2°C [T1-RT & T2-RT], elevated temperature: 37°C [T1-37 & T2-37] and sub-zero temperature: -18°C [T1-18 & T2-18] for simulating different environment. The stored bars were analysed for nutritional composition, sensory, physico- chemical and microbial quality at a regular interval of 10 days for a period of one month. All samples were taken in triplicates for the analysis.

Fatty acid composition of fish oil and the fish oil microcapsules was determined in a Varian gas chromatograph (Varian CP-3800, USA). Extraction of lipid from the samples was done following Folch method (Folch et al., 1957) and transesterification was performed according to the method of AOAC (2000). Retention time and peak areas of each fatty

acids were recorded and expressed as percentage out of total area of all identified peaks.

The proximate composition (moisture, total protein, total fat and total ash content) were estimated according to AOAC (2003). The crude carbohydrate and the gross energy values were calculated by (Bhat & Sridhar, 2008) using the following equations:

Total crude carbohydrate (%) = 100– [Moisture (%) + crude protein (%) + crude lipid (%) + total ash (%)] Gross energy kCal 100 g⁻¹ = (protein x 4 kCal g⁻¹) + (fat x 9 kCal g⁻¹) + (carbohydrate x 4 kCal g⁻¹)

The pH of sample was determined by homogenising the test sample in distilled water (1: 5 W/V) and measuring using glass electrode digital pH meter (Cyberscan pH tutor, Eutech instruments). The measurement of water activity was carried out using Novasina Lab Master-aw, (Novasina AG, CH-8853 Lachen, Switzerland) set at 21°C using Novalog software. The peroxide and free fatty acid value of the samples was estimated as per AOAC (2000). TBARS (thio Barbituric acid reactive substances) values in samples were determined as per Taraldgis method (1960).

The colour of samples was measured by using Lab scan XE – spectro-colorimeter (Hunter Associates Laboratory Inc. Reston VA, USA) using Hunter & Harold (1987) method. The shear strength of the bar was measured with Warner Bratzler Blade set of Texture Analyser, (TA Plus, Lloyd Instruments, Hampshire, UK) (Fig. 8) using a single hardness test. The samples were cut horizontally using blade probe to get an imitation of cutting and biting. From graph, parameters like maximum load and work done to rupture were measured and calculated using Nexigen software.

Aerobic plate count was carried out by pour plate technique according to the standard methods (APHA, 1992). Enumeration of coliforms was done by plating 1 ml aliquot in a molten agar VRBA and Yeast and mould in molten PDA acidified with 10% tartaric acid and incubated at 37°C for 24 h and 30°C for 72-120 h respectively.

Hedonic sensory evaluation of the fish based granola bar was carried out by serving the bars to a panel of 10 judges. Snacks were evaluated for different sensory attributes like colour, texture, taste, aroma and overall acceptability. Attributes were scored on a 9-point hedonic scale grading from 9 (like extremely) to 1 (highly disliked samples)

Statistical analyses of data were carried out using statistical package for social science software (SPSS VERSION 16.0, Chicago IL. USA). Analysis of variance (ANOVA) was done to analyze the data and the significant difference among the treatments and days were determined by Duncan's Multiple Range Test (DMRT). The level of significance was set up at pd \leq 0.05. All the analyses was carried out in triplicates and expressed as mean \pm SE.

Results and Discussion

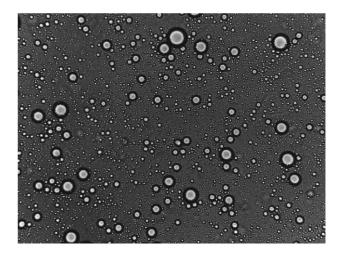
Fish oil microencapsulation was effectively done with a higher encapsulation efficiency (74%) using freeze drying technique. The ability of emulsion to resist changes in physicochemical properties over time is often referred as the emulsion stability (Mc Clements, 2005). The encapsulant used was skimmed milk powder for fish oil in the ratio of 2:1. (skimmed milk powder: fish oil, on w/w basis). The emulsion stability, expressed as the percentage of separation was found to be 2.38%, indicating a larger contribution of unseparated fraction (97.62%). The emulsion microphotograph, (P1) showed the oil droplets were without any coalescence or aggregation and seemed to be separated from each other. The absence of coalescence or aggregation can be attributed as one probable reason for the higher emulsion stability.

The fish oil microcapsules prepared were analysed for the fatty acid composition before incorporation in the granola bar. The Table 2 showed the fatty acid profile of the fish oil and the fish oil microcapsules. Encapsulation process with freeze drying technique did not significantly affect the fatty acid composition in fish oil microcapsules. Even though freeze drying techniques had a negative impact on lipid oxidation, degradation of major fatty acids was not detected, which is beneficial in the fortification of omega 3 fatty acids. The fatty acid profile of fish oil was mainly contributed by 18 fatty acids of which saturated fatty acids 17-20%, monounsaturated 42% and poly unsaturated 25% of the total fatty acids. The EPA and DHA contributed to 8.71 and 11.56% in the oil and 8.43 and 10.75% in the oil microcapsules. The results showed a higher retention of the major fatty acids during encapsulation with freeze drying technique.

The fish oil microcapsule was added in different concentrations of 0.5, 0.6, 1, 1.5, 2, 4, 6 and 10% in the granola bar as an omega-3 supplement and an optimum concentration of 2% was giving good sensory acceptance. A higher concentration of oil microcapsules created off flavor to the product and was not acceptable by the sensory panels. Cardamom was added along with fish oil powder, in order to mask the fish oil off flavor at higher concentrations. Kolanowski et al. (2007) reported that the presence of oxygen strongly decreases the sensory quality of fish oil-fortiûed instant foods during storage in air-permeable conditions but the presence of a flavouring substance allows higher level of ûsh oil addition to instant foods due to masking of undesirable off-flavour.

Granola bar was enriched with highly digestible fish protein at 5-10%. An incorporation of 5% level was optimised based on sensory acceptability. The level of incorporation in the present study was little lower when compared to a similar study on the fortification of corn snack with 3, 5, 7 and 9% fish protein powder from saithe (*Pollachius virens*) surumi by Shaviklo et al. (2011). Authors reported that snack containing 9% fish protein powder had significantly lower likings for sensory characteristics than other 3 prototypes and snack containing 7% was selected as acceptance.

Based on sensory acceptability the final composition of granola bar was formulated with 5% fish protein, 2% fish oil in 32.5% cereal base mixture (oats, wheat, flattened rice, corn flour and corn flakes) and 17.5% dried fruits (peanuts, cashew, almonds, dates and



P1: Microstructure of fish oil emulsion before spray drying

raisins.) along with binder mix. A control granola bar was prepared without fortification of fish oil and protein and the nutritional composition of control (T1) and fortified bar (T2) summarized Table 2.

Table 3 shows the nutritional composition of the developed granola bars. The granola bars (T1 and T2) were observed to have low moisture content of 6.5%, which helps in extending shelf life of the product. The total ash content didn't show a significant variation with samples and observed as 2.3% in T1 and 2.08% in the T2. A significant variation was observed in the protein content of two granola bars. The granola bars having a protein content of 14.5 and 17.27% in T1 and T2 respectively contributed from the protein ingredients like nuts, almonds, cashew and dates and also supplemented from fish protein in T2. The crude fat was observed to be 4.63%, 5.89% in T1 and T2 samples respectively. The addition of 2% fish oil microcapsules, contributed to the slight increase in total fat content. The carbohydrate was calculated as difference of other components from 100. The calorific value was calculated using the factors: protein 17 kJ/g (4 kcal g^{-1}), fat 37 kJ g^{-1} (9 kcal g^{-1}), carbohydrate 17 kJ g (4 kcal g^{-1}) (FAO/WHO/ONU, 2004) for granola bars (Roberfroid, 1999). The T1 and T2 contributed 386.71 kCal 100 g⁻¹ and 394.81 kCal 100 g⁻¹ respectively. So fortification with fish oil and protein could slightly enhances the calorific values than control bars. Thus the developed bars can be categorized as nutrient enriched low calorie nutritional bars. Aigster et al. (2011) reported nutritional composition values in similar resistant starch supplemented granola and cereal bars, where moisture in the range of 5.74-7.14%, ash 1.95-2.25%, carbohydrate 62.2-66.4%, with a change in lipid (21.5-22.4%) and protein content (5.87-6.38%).

The fortification of bar did not significantly affect the acidity of the product, which was measured in terms of pH value. An initial pH value of 5.47 and 5.63 was observed in T1 and T2 samples, which showed significant changes during storage. During the ambient RT condition, pH started to increase and after 30 days of storage it was observed to be 5.66 (T1) and 5.71 (T2). Whereas samples stored at extreme conditions like 37°C and -18°C a decrease was observed in pH values and the decrease was more pronounced at 37°C. The changes in the pH values are directly related to maturation stages of the added ingredients like dried fruits in granola bars. Da Silva et al. (2016) observed a significant decrease in pH in the snack bars added with jeriva fruit flour 20% (6.78) compared to control bars (6.92) and such decrease can result from the low pH of jeriva flour (4.96).

Water activity measurements helps in predicting the mechanical properties, stability and shelf life of foods. Table 4 shows the change in water activity during different conditions of storage. All a_w values were found to be below 0.5, which indicated that formulated bars were categorized under low moisture foods, where a_w <0.6. This low a_w values add to the extended shelf life of the product even without refrigeration. The possibility of mycotoxin

Table 2. The fatty acid composition of fish oil and fish oil microcapsules.

Designation		% Fatty acid to total fatty acid		
		Fish oil	Fish oil microcapsules	
C14:0	Myristic	4.08	4.76	
C16:0	Palmitic	10.14	11.51	
C16:1	Palmitoleic	7.49	7.85	
C18:0	Stearic	2.14	2.54	
C18:1	Oleic	15.79	16.14	
C18:2	Linoleic	1.80	1.87	
C18:3	Linolenic	2.83	2.54	
C20:1	cis -11- Eicosenoic	9.58	8.97	
C20:5	Eicosapentanoic	8.71	8.43	
C22:1	Erucic	9.05	8.41	
C22:6	Docosahexaenoic	11.56	10.75	

was also minimal in case of water activity below 0.6. Cereal bars are generally formulated to have moisture between 10 and 15% (w/w) and a,, value less than 0.65 (Loveday et al., 2009). Also, the bars are designed to maintain intermediate aw values between 0.4 and 0.6 and can be stored at room temperature without significant microbial growth. Even though all values were below 0.5, the water activity values were slightly increased during initial storage phase. The low a_w made the products more susceptible to physical transformations such as lipid oxidation and non-enzymatic browning (Maillard reactions) during further storage phase. Aigster, in 2011 reported a similar increase in water activity of the granola cereal bars with time. And the increase in water activity from 0.22 to 0.45 reported during storage might be due to retrogradation of starch molecules with release of water instead of moisture migration through the packaging material into the product.

As fish oil microcapsules was incorporated in mixed food composition, oxidative stability is an important factor determining the shelf life of the product. Higher unsaturation often susceptible to oxidative stability and subsequently affects the flavor and colour of the product. Oxidative stability was measured in terms of PV (peroxide value) and TBARS (Thiobarbituric acid reactive substance) and hydrolytic rancidity was expressed as FFA (free fatty acid value).

The trend in the peroxide value (Fig. 1) showed a significant ($p \le 0.05$) increase in peroxide value of all samples irrespective of conditions of storage. The T2 samples with fish oil microcapsules were shown slightly higher PV values. During one month storage bars stored under different conditions, PV values were found to be below the (10 meqv. Kg⁻¹ sample)

Table 3. The nutritional composition of the control and fortified granola bars.

Nutritional Composition (g 100 g ⁻¹)	T1	T2
Moisture	6.5±0.56	6.54±0.5
Ash	2.3±.0.01	2.08±0.03
Protein	14.5±0.5	17.27±0.25
Fat	4.63±0.07	5.89±0.11
Carbohydrate	71.76±0.73	68.18±0.46
CALORIES (K cal 100 g ⁻¹)	386.71	394.81

Values are indicated as mean ± standard deviation

maximum acceptable limit. Higher temperature favours the lipid oxidation, and the subsequent changes can be observed at 37°C. Similar trend was observed by Padmashee et al. (2012) in composite cereal bar stored at 37°C and room temperature, with higher peroxidation value in 37°C stored samples.

Changes in the FFA value is shown in Fig. 2. The free fatty acid follows a similar trend of increase with PV during storage. Higher FFA values were observed in samples stored at 37°C. An initial lower FFA values were reported (1.07 and 0.56%), which agrees with the findings of Jeyarani et al. (1997) who found 0.98% free fatty acids in cereal-pulse based sweet-bars. Hydrolytic oxidation starts with storage, and it was more pronounced at higher temperature.

The primary oxidation follows with secondary oxidation, which is represented as TBARS value (Fig. 3). TBARS value increased significantly during storage and the increase in the value corresponds to increasing PV and FFA. There was significant difference between two samples, and the oxidation

Table 4. Changes in the water activity values of granola bars at different conditions of storage

Treatments	0 th Day	10 th Day	20 th Day	30 th Day
T1-RT	0.36 ± 0.08^{ab}	0.37 ± 0.06^{d}	0.43 ± 0.08^{d}	0.45 ± 0.06^{c}
T2-RT	0.34 ± 0.05^{a}	0.35 ± 0.04^{bc}	0.42 ± 0.02^{d}	0.45 ± 0.02^{b}
T1-37	0.36 ± 0.04^{ab}	0.35 ± 0.02^{b}	0.37 ± 0.03^{b}	$0.44 \pm 0.04^{\rm bc}$
T2-37	0.34 ± 0.02^{ab}	0.36 ± 0.03^{c}	$0.39 \pm 0.03^{\circ}$	0.43 ± 0.02^{bc}
T1-18	0.37 ± 0.02^{b}	$0.40 \pm 0.08^{\rm e}$	0.38 ± 0.02^{c}	0.43 ± 0.03^{b}
T2-18	0.36 ± 0.04^{ab}	0.33 ± 0.03^{a}	0.36 ± 0.02^{a}	0.40 ± 0.06^{a}

Values are indicated as mean ± standard deviation. Superscripts indicates the changes in values with treatments

was found to be more in samples kept at 37°C. High temperature initiates the free radical mechanism and lipid oxidation in the samples. Since the fortification of fish oil microcapsules increased lipid content, bars were susceptible to have a high rate of oxidation. But the reported TBARS values of samples stored under different conditions were found to be below 0.5 mg Malondialdehyde (MDA) Kg⁻¹ sample, very low and highly desirable even after 30 days of storage. A similar oxidation trend was observed in cereal bars, stored in different packaging material (Padmashee et al., 2012).

Colour is an important quality attribute of granola bars which attracts the consumer. The formulation contributes an appealing colour to the product, which was unaffected during fortification. Change in the lightness value is shown in Fig. 4a. The samples stored at room temperature and -18°C showed an increased lightness value, whereas, 37°C stored samples were having decreased lightness. The higher temperature favors the maillard reaction in sugar-protein based foods, which might lower the L* value. The presence of date syrup and red grape juice in bar might favoured the maillard reaction. Yousif et al. (1990), found that colour of the date jelly varied during storage, due to non-enzymatic browning. Similarly, + a* value, (Fig. 4b) shows the redness of the product, which increased during storage especially in fortified room temperature and 37°C stored samples due to the browning reaction, happened to occur more at these conditions. A similar kind of increasing trend was observed for yellowness (b*) value, and is shown in Fig. 4c. Sunwaterhouse et al. (2010) noticed a positive a* and b* values of all the formulated bars, indicating a usual redness and yellowness colour of all the snack bar bases.

The granola bars having an appealing crunchy texture, will attract the consumer. This unique crunchy texture of the bar will often get affect with different environmental conditions. Stored bars were shown texture hardening under different conditions. A sheer force of 50 N required for cutting initially was increased to 250 N during storage period. Simon et al. (2009) reported that the shelf life of a protein bar often limited by the development of a hard or tough texture which become unpalatable for consumers. The development of hard texture during storage may be attributed to thiol–disulphide interchange reactions during storage which lead to protein cross linking

aggregation and network formation. Hard texture development may also be due to the migration of moisture as well as formation of most ordered secondary structure and lower surface hydrophobicity of protein particles (Simon et al., 2009). Besides this, Maillard reactions between reducing sugars and reactive lysine residue play a part in the hardening of a protein bar (Gerard 2002).

The average microbial counts were well below 10 CFU $\rm g^{-1}$ in the initial period, irrespective of the storage conditions. Staphylococcus, *E. coli* and Yeast and mold were absent during the entire period of storage. The countable colonies started appearing after 20 days at room temperature stored and $37^{\rm 9}$ C due to the slow growth and proliferation of mesophilic counts. The low moisture and $\rm a_w$ remained in the granola bar will favour the inhibition of growth and proliferation of vegetative microbes, spoilage fungus, yeast and molds and contributed towards the safety during the entire storage period.

The sensory analysis of the granola bars was done by a panel of 10 members on hedonic scale. The sensory preference of the consumers had changed after storage and the average sensory scores were shown in the Table 5. Initially the control and

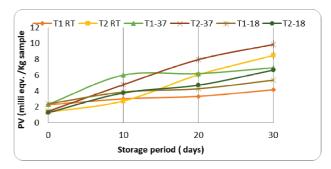


Fig. 1. Changes in peroxide value of granola bars stored at 3 different conditions of storage

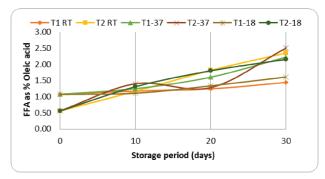


Fig. 2. Changes in Free fatty acid value of granola bars stored at 3 different conditions of storage

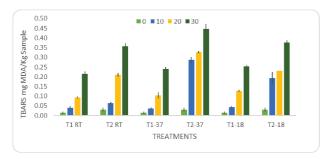


Fig. 3. Changes in TBARS value of granola bars stored at 3 different conditions of storage

fortified granola bars recorded an overall acceptability score of 8 (T1) and 8.3 (T2) on a 9-point Hedonic scale and the addition of oil microcapsules in T2 sample did not affect the aroma and taste preference of the sensory panelists. This showed an initial consumer acceptance towards the fortified bars. But the different storage conditions affect the flavor and overall acceptability of granola bars, especially at high temperature storage. Aroma and taste scores reduced in the fortified bars stored at high temperature due to slight release of fish oil flavor in the granola bars.

An omega-3 fortified fish based granola bar was developed and evaluated its shelf life under different storage temperature. The omega-3 was fortified in the bar at 2%, in the form of fish oil microcapsules. The developed bar was also rich in highly digestible fish protein optimized at 5% level of incorporation and provides additional calories. The bars were convenient, ready-to-eat contributing about 17.25% protein, 82 mg EPA + DHA 100 g⁻¹ for meeting the Recommended Dietary Allowance. Since the bars are fortified with high quality protein and omega-3 fatty acids, this high energy food can be taken as supplement to meal and/ or can be recommended for diet concerned people, who prefer low calories nutrient enriched foods. Standardizing the conditions for the development of fortified bar and establishing its shelf life will benefit the consumers to meet their requirements towards functional foods. The quality evaluation comprehends the significant effect of different climatic conditions on the quality and shelf stability of the product. The developed bars had an expected shelf life of more than one month in different storage conditions without affecting the quality. A further study needs to be carried out in optimizing the maximum shelf life under different conditions.

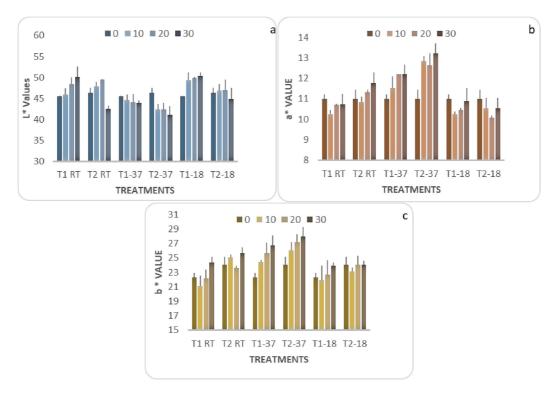


Fig. 4. Changes in colour values as indicated by L*, a* and b* value of Granola Bar stored at 3 different conditions of storage.

Treatments	Taste	Initial Texture	Aroma	Colour	Overall Acceptability
T1-RT	8.10 ± 0.66	8.40± 0.20	7.60± 0.49	8.10 ± 0.66	8.0 ± 0.32
T2-RT	8.04 ± 0.14	8.10 ± 0.66	7.85 ± 0.67	8.60 ± 0.19	8.30 ± 0.25
		Final			
	Taste	Texture	Aroma	Colour	Overall Acceptability
T1-RT	7.18 ± 0.2^{bc}	7.93 ± 0.55^{cd}	7.3 ± 0.75^{abc}	7.3 ± 0.41^{ab}	7.58 ± 0.38^{bc}
T2-RT	6.38 ± 0.3^{a}	8.35 ± 0.38^{d}	6.55 ± 0.36^{a}	$8.03 \pm 0.68^{\rm d}$	7.08 ± 0.08^{ab}
T1-37	6.85 ± 0.21^{ab}	$7.68 \pm 0.34^{\rm bc}$	7.08 ± 0.68^{abc}	7.50 ± 0.41^{bcd}	7.20 ± 0.49^{bc}
T2-37	6.83 ± 0.64^{ab}	7.13 ± 0.22^{a}	6.60 ± 0.81^{ab}	6.83 ± 0.2^{a}	6.63 ± 0.41^{a}
T1-18	7.40 ± 0.37^{c}	7.28 ± 0.33^{ab}	$7.43 \pm 0.38^{\rm bc}$	$7.43 \pm 0.38^{\rm bc}$	7.63 ± 0.30^{cd}
T2-18	$7.63 + 0.41^{\circ}$	$7.35 + 0.38^{ab}$	$7.70 + 0.41^{\circ}$	7.95 ± 0.29^{cd}	7.93 ± 0.26^{d}

Table 5. Change in Sensory scores during initial and final days of storage

Values indicated as mean ± standard deviation. Within the columns values superscripted with different letters (a-e) with mean indicate changes between treatments.

Acknowledgements

Authors express sincere thanks to Director, DFRL, Mysore and Director, CIFT, Cochin for providing necessary facilities and staff of Freeze drying & animal products technology division of DFRL for support during the course of investigation. Authors greatly acknowledge ICAR for granting the financial assistance.

References

- AOAC (2003) Official methods of analysis of AOAC International, 17th edn., revision 2, Vol II (Horwitz, W., Ed). Association of Official Analytical Chemists (AOAC) International, Gaithersburg, MD
- Ahn, J. H., Kim, Y. P., Lee, Y. M., Seo, E. M., Lee, K. W. and Kim, H. S. (2008) Optimization of microencapsulation of seed oil by response surface methodology. Food Chem. 107: 98-105
- Aigster, A., Duncan, S.E., Conforti, F.D. and Barbeau, W.D. (2011) Physicochemical properties and sensory attributes of resistant starch-supplemented granola bars and cereals. LWT Food Science and Technology, 44: 2159-2165
- AOAC (2000) 17th edn., Washington D.C. USA, 2000; 4: 969.3 and 991.39
- APHA (1992) Standard Methods for Examination of Water and Wastewater. 18th edn., American Public Health Association. Washington DC
- Bhat, R. and Sridhar, K. R. (2008) Nutritional quality evaluation of electron beamed-irradiated lotus (*Nelumbo nucifera*) seeds. Food Chem. 107: 174-184

- Catherine, M. T. and Johnston, C. M. (2012) Ingestion of Nutrition Bars High in Protein and Carbohydrates Does Not Impact 24 h Energy Intake in Healthy Young Adults. Appetite, 59(3): 778-781. doi:10.1016/ j.appet.2012.08.012
- Da Silva, E.P., Siqueira, H.H., Damiani, C., Vilas Boas, E.V.de-B. (2016) Physicochemical and sensory characteristics of snack bars added of jerivá flour (*Syagrus romanzoffiana*). Food science and Technology. DDOI: http://dx.doi.org/10.1590/1678-457X.08115
- Esterbaur (1993) Cytotoxicity and Genotoxicity of Lipid Oxi- dation Products. Am. J. Clin. Nutr. 57(5): 7795-7855
- Folch, J., Lees, M. and Stanley G.H.S (1957) A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226: 497-509
- Forss, D. A. (1995) Odour and Flavour Compounds from Lipid. Prog. Chem. Fats Other Lipids. 15 (4): 177-258
- Gerard, J. A. (2002) Protein-protein cross linking in food: methods, consequences, application. Trends Food Sci Technol. 13: 391–399. doi: 10.1016/S0924-2244(02)00257-1
- Hunter, R. S. and Harold, R. W. (1987) The measure of appearance. Willey Interscience, New York, Second Edition, 411p
- Izzo, M. and Niness, K. (2001) Formulating nutrition bars with inulin and oligofructose. Cereal Food World. 46: (3) 102-106
- Jeyarani, T., Mahadeviah, B. and Shurpalekar, S.R. (1997) Studies on packaging and storage of cereal-pulse based sweet bars. J. Food Sci. Technol. 34: 245-247

- Keogh, M. K., O'kennedy, B. T., Kelly J., Auty, M. A., Kelly, P. M., Fureby, A. and Haahr, A. M. (2001) Stability to oxidation of spray-dried fish oil powder microencapsulated using milk ingredients. J. Food Sci. 66: 217-224
- Kolanowski, W., Jaworska, D., Laufenberg, G. and Weibrodt, J. (2007) Evaluation of sensory quality of instant foods fortified with omega- 3 PUFA by addition of fish oil powder, Euopean Food Res. Technol. 225(5-6): 715-721
- Kubow, S (1992) Routes of Formation and Toxic Consequences of Lipid Oxidation Products in Foods. Free Radic. Biol. Med. 12(1): 63-81. doi:10.1016/0891-5849(92)90059-P
- Loveday S.M, Hindmarsh J.P, Creamer L.K, Singh, H. (2009) Physicochemical changes in a model protein bar during storage. Food Res. Int. 42: 798-806
- Maki, K.C., Dicklin, M.R., Ingram, K.A., Cyrowski, M. and Umporowicz, D. M. (2003) Bioavailability of eicosapentaenoic and docosahexaenoic n-3 polyunsaturated fattyacids in salmon patties compared with capsules. J. Food Sci. 68: 761-764
- Mc Clements D. J. (2005) Food Emulsions: Principles, Practices, and Techniques. London, New York, Washington, D.C.: CRC Press LLC. 229-300

- Padmashee, A., Sharma, G. K., Srihari, K. A., Bawa, A. S. (2012) Development of shelf stable protein rich composite cereal bar. J. Food Sci. Technol. 49(3): 335-341
- Roberfroid, M. (1999) Caloric value of inulin and oligofructose. J. Nutr. 129(7): 1436-1437
- Shaviklo, G. R., Thorkelsson, G., Sveinsdottir, K. and Rafipour, F. (2011) Chemical properties and sensory quality of ice cream fortified with fish protein. J. Sci. of Food & Agric. 91(7): 1199-1204
- Simon, M. L., Jason, P. H., Lawrence, K. C. and Harjinder, S. (2009) Physicochemical changes in a model protein bar during storage. Food Res. Int. 42: 798-806
- Sun-Waterhouse, D., Teoh, A., Massarotto, C., Wibisono, R and Wadhwa. S. (2010) Comparative analysis of fruit-based functional snack bars. Food Chem. 119: 1369-1379
- Tarladgis, B. M., Watts, M. T., Younathan and Duggan, L. R. (1960) A distillation method for the quantitative determination of malonaldehyde in rancid foods. J. Am. Oil Chem. Soc. 37: 44-48
- Yousif, A.K., Ali, M. A. and Idrees, B. (1990) Processing, evaluation and storability of date jelly. J. Food Sci. Technol. 27: 264-267