Development of omega-3 rich energy bar with flaxseed

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Revised: 20 April 2011 / Accepted: 25 May 2011 / Published online: 18 June 2011

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Abstract Energy bar sample were prepared with different levels of flaxseed (0–20%) in addition to cereals and pulses with varying levels of sweeteners (45, 50, and 55%) to deliver a nutritious food to the consumer. The developed bars were evaluated for textural, colour, nutritional quality, sensory attributes and total microbial load. Different levels of flaxseed and sweeteners significantly affected the hue and chroma values of the energy bar. In general the level of flaxseed in energy bar did not affect the hardness but it was decreased with increasing level of sweeteners except in control sample. The total calories obtained from the energy bar showed significant increase with the increasing levels of flaxseed, the maximum (397.95 kcal) being for bars with 20% flaxseed and 45% sweeteners. This energy bar sample also showed the maximum protein (12.41%), crude fat (11.86%), ash (1.65%), iron (3.77 mg/100 g), crude fiber (2.18%) and omega-3 as alpha-linolenic acid (22.50%, fatty acid basis) content. The overall mean sensory score for overall acceptability for samples with 10% flaxseed and 55% sweeteners and 15% flaxseed and 45% sweeteners were at par but the omega-3 and other nutrients in the later sample was higher than the former sample, hence, 15% flaxseed and 45% sweeteners along with other ingredients may be considered for production of acceptable quality omega-3 fatty acid rich energy bar at commercial scale, which also stored well at refrigerated condition.

Keywords Flaxseed · Energy bar · Omega-3 fatty acid · Free fatty acid · Sensory evaluation

Consumers demand and desire the health foods, which are portable, convenient and proportioned as well (Sloan 2005). Often, many options aren't available that are minimally processed, rich in nutrients and tastes good. Energy bars, a food product that fits these criteria, continue to increase in sales according to the ACNielsen Market Track (Burn 2007). Due to growing consumer demand for healthy, natural and convenient foods, attempts are being made for dehulling of flaxseed (Barnwal et al. 2010; Oomah and Mazza, 1998; Zhang et al. 2009) to improve the nutritional value of snack foods by modifying their nutritive composition (Bhaskaran and Hardley 2002; Gray et al. 2003).

The use of flaxseed as a dietary supplement is increasing in parallel with the research on its multitudinous effects on human health (Tarpila et al. 2005) and designer foods for poultry feeding (Sujatha and Narahari 2010). Detoxified flaxseed using a simple technique for removal of toxic substance can be used to enrich omega-3 in the chapatti made from wheat flour has been reported (Sahu et al. 2009). Flaxseed contains functional components such as dietary fibre, oil, protein and phenolic compounds, which are responsible for a number of health benefits. Flaxseed has a unique fatty acid profile. It is high in polyunsaturated fatty acids and low in saturated fatty acids. Linoleic acid, an omega-6 fatty acid, constitutes about 16% of total fatty acids whereas α -linolenic acid constitutes about 57%, the highest of any seed oil (Ramcharitar et al. 2005). The flaxseed protein has been found to be effective in lowering plasma cholesterol and triacylglycerides (Bhathena et al. 2002). Flaxseed fibre, both soluble and insoluble is considered to reduce the blood glucose and cholesterol levels (Shen et al. 1998). Moreover, flaxseed is one of the

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best source of lignan which has the ability to bind estrogen receptors in the body and act as anti-carcinogenic agent and helps to avoid prostate, breast and endometrial cancers. Flaxseed supplementation improves lipid profiles but has no effect on biomarkers of bone metabolism in postmenopausal women (Lucas et al. 2002). Hundred gram of flaxseed provides 100% of the recommended daily allowance (RDA) for manganese and potassium, 57–65% of the RDA of phosphorus and iron, and 13–35% for zinc, calcium and copper while its recommended daily intake is 25–50 g (Anonymous 1994).

Keeping in view the overall health benefits of flaxseed and the demand of consumers, the objective of this study was to develop a nutritious energy bar utilizing flaxseed in the formulation along with cereal (white oats), pulses (roasted bengal gram) and legumes (soy protein) to deliver a nutritious health product. Nutritional composition, colour, texture profile analysis, sensory evaluation and microbial load of the energy bar samples were determined to evaluate the acceptability of the product.

Materials and methods

Raw materials Flaxseed (*Linum usitatissimum* var. Garima) was procured from CSAUA&T, Kanpur in the year 2009. The seeds were cleaned, graded and stored in pet jars till further use. Instant white oats (Bagrrys India Ltd, New Delhi), Soya protein isolates (Nutralite, Amway India Enterprises, New Delhi), honey (Markfed Canneries, Jalandhar), corn syrup (Daesang Corporation, South Korea), roasted bengal gram, puffed rice, nuts (equal amount of almond, cashew nuts and raisins) were purchased from the local market. The dry ingredients were roasted for 2 min in a pan, coarsely ground in a grinder (MX-103, Maharaja Appliances Limited, New Delhi) and passed through a sieve (No.14 ASTM) to get ingredients of uniform particle size for preparation of energy bar.

Preparation of energy bar Cold mixing of the ground ingredients (Tables 1 and 2) with sweeteners (honey and corn syrup) was accomplished in the mixer (SP-800 Mixer, SPAR Mixer, Taiwan). The mixture was taken out into tray measuring 20×12×2 cm and sheeting was done with the help of rolling pin. The tray was then kept in the freezer for 1 h, de-moulded and bars weighing approximately 50±2 g were cut out with the help of moulds. Each individual bar were wrapped properly in butter paper and packed individually in required size of LDPE (LDPE) pouches (thickness 0.065 mm) and stored for further quality analysis. In order to reduce the microbial load, if any on the butter paper and pouches before packing the samples, butter paper and pouches were wrapped in brown paper and

kept in hot air oven at 60 °C for 3–4 h and under UV light for 30 min (Mridula et al. 2010). Energy bar samples with 15% flaxseed and 45% sweeteners were considered for shelf life study. These samples were prepared and packed properly and stored in temperature-cum-humidity control cabinet at 25 °C, 65% RH and in refrigerator for 90 days period and evaluated for important quality parameters at 30 days interval.

Physico-chemical analysis Moisture, crude fat, crude protein (using the factor 6.5× N), ash, crude fibre, calcium and iron content in different energy bar samples were determined as per the standard methods of AOAC 2000. Total carbohydrates value was obtained by difference. Total calories were calculated by multiplying protein, carbohydrates and fat content by the factor 4, 4 and 9, respectively.

Omega-3 content as alpha-linolenic acid was determined by gas chromatography (GC). The separation of different components is based on a partition coefficient of various components of the mixture between stationary phase (liquid) and mobile phase which is an inert gas. Fatty acids are made volatile by converting them into methyl/ethyl esters (Appleqvist 1968). The esters were identified and quantified by injecting into GC and comparing with a set of standard esters. 500 mg sample was thoroughly mixed with 1 ml of petroleum ether. To this 1.5 ml sodium ethylate (0.02 M NaOH in 99.5% ethanol) was added and the tube was covered with cap. The content was shaken and kept for 45–50 min at room temperature. 1.5 ml of 8% NaCl (w/v) was subsequently added and contents were mixed well. As

Table 1 Formulation of energy bar using flaxseed (g/100 g)

Flaxseed	Sweeteners	White oats	Puffed rice
0	45	17	17
	50	14.5	14.5
	55	12	12
5	45	14.5	14.5
	50	12	12
	55	9.5	9.5
10	45	12	12
	50	9.5	9.5
	55	7	7
15	45	9.5	9.5
	50	7	7
	55	4.5	4.5
20	45	7	7
	50	4.5	4.5
	55	2	2

Levels of other ingredients: soy isolates 3 g, roasted bengal gram 10 g, and nuts 8 g per 100 g in each sample

Table 2 Nutritional composition of ingredients used in energy bar

Ingredients	Protein, %	Fat, %	Crude fibre, %	Calcium, mg%	Iron, mg%
Soy protein	80	3.0	2.96	70	1.07
Flaxseed	20.9	39.4	8.14	110	6.8
White oat	13.12	7.66	3.21	40	1.48
Puffed rice	8.46	0.76	0.39	22	4.28
Honey	0.34	0.0	0.0	8	0.62
Roasted bengal gram	26.12	5.23	1.39	50	8.69
Nuts	15.77	29.55	1.26	51	5.55

soon as the two layers got separated, upper layer (petroleum ether) was transferred to another tube and allowed to evaporate and finally dissolve in 10 µl of petroleum ether and 2 µl was then injected using micro syringe (Hamilton) on M/s Nucon Engineers AIMIL gas chromatograph (solid state) model 5700 series equipped with flame ionization detector fitted with 6% butane diol succinate (BDS) on chromosorb WAW/DWCS column 6 ft in length and ¼ inch outer diameter. The conditions for the separation were: oven

temperature 250 °C, hydrogen flow 40 ml per minute, nitrogen flow 60 ml per minute, air flow 300–400 ml per minute. Identification of peaks was done by comparison of their retention time with those of standard fatty acyl esters.

Free fatty acids (on whole sample basis) in fresh and stored energy bar, was determined as per Thapar et al. (1988) method. Microbial load i.e. viable bacterial count and yeast and mould (fungi) were determined by standard pour plating method (Cruickshank et al. 1975).

Table 3 Textural profile analysis of omega-3 rich energy bar

Energy Bar samples		Hardness (N)	Cohesiveness	Springiness (mm)	Chewiness (N/mm)	Gumminess (N)
Flaxseed (%)	Sweeteners (%)					
0	45	9.2 ^{defg}	0.333 ^a	0.511 ^{ab}	1.5 ^{cd}	3.1 ^{efg}
	50	14.3 ^{cd}	0.337 ^a	0.467 ^{bc}	2.2 ^{bc}	4.6 ^{cde}
	55	22.2 ^b	0.279 ^{bc}	0.349 ^{ef}	2.1 ^{bc}	6.2 ^{bc}
5	45	13.8 ^{cd}	0.347 ^a	0.480 ^{bc}	2.3 ^{bc}	4.8 ^{cde}
	50	12.6 ^{cdef}	0.291 ^b	0.410 ^{cde}	1.5 ^{cd}	3.7 ^{deh}
	55	6.0 ^{efgh}	0.258 ^{cde}	0.366 ^{def}	0.565 ^{ef}	1.5 ^{fg}
10	45	32.8 ^a	0.262 ^{cd}	0.411 ^{cde}	3.6 ^a	8.6 ^a
	50	14.4 ^{cd}	0.245 ^{def}	0.331 ^f	1.1 ^{de}	3.4 ^{def}
	55	4.4 ^{gh}	0.234 ^{efgh}	0.360 ^{def}	0.371 ^{ef}	1.0 ^h
15	45	19.6 ^{bc}	0.273 ^{bc}	0.428 ^{cd}	2.3 ^{bc}	5.4 ^{bcd}
	50	13.3 ^{cde}	0.233 ^{efgh}	0.323 ^f	1.0 ^{de}	3.1 ^{efg}
	55	1.9 ^{gh}	0.210 ^h	0.390 ^{def}	0.161 ^f	0.413 ^h
20	45	29.7 ^a	0.239 ^{defg}	0.371 ^{def}	2.7 ^b	7.2 ^{ab}
	50	5.4 ^{fgh}	0.217 ^{gh}	0.324 ^f	0.381 ^{ef}	1.2 ^{gh}
	55	0.95 ^h	0.221 ^{fgh}	0.581 ^a	0.119 ^f	0.21 ^h
F Value						
F		3.2 ^{NS}	58.3 ^{***}	4.2 [*]	4.7 [*]	4.1 [*]
S		36.2 ^{**}	39.1 ^{***}	8.4 ^{**}	49.4 ^{***}	39.9 ^{***}
F×S		12.3 ^{**}	3.8 [*]	9.7 ^{**}	7.7 ^{**}	10.4 ^{**}
CD(0.05)						
F		4.3	0.015	0.044	0.477	1.1
S		3.3	0.011	0.034	0.369	0.891
F×S		7.4	0.026	0.076	0.826	1.9

F Flaxseed; S Sweeteners; NS non significant

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; $n = 5$

Texture profile analysis Texture measurements of energy bar samples were performed at room temperature using the texture analyzer (TA-Hdi), (Stable Micro systems, UK). Prior to analysis, samples were allowed to equilibrate to room temperature. Texture profile analysis (Bourne, 1978) was performed using five pieces of each sample ($2.5 \times 2.5 \times 2$ cm), which were compressed twice to 50% of the original height with a cylinder probe P/75. A time of 2 s was allowed to elapse between two compression cycles. The cross-head moved at a constant speed of 1 mm/s. From the resulting curve, hardness, cohesiveness, springiness, chewiness, and gumminess were determined. Hardness was the resistance at maximum compression during the first compression; the ratio (dimensionless) of positive force during the second to that of the first compression cycle (downward strokes only) was cohesiveness; springiness (mm) was the ability of sample to recover its original form after the deforming force was removed; the work needed to chew a solid sample to a steady state of swallowing (springiness $\times$ gumminess) was considered as chewiness (N/mm), gumminess (N) was the force needed to disintegrate a semisolid sample to a steady state of swallowing (hardness $\times$ cohesiveness).

Colour determination Colour (L, a and b values) of the samples was determined by using Hunter Colorimeter (model no. 45/0 L, made in U.S.A). 'L' is known as the lightness and extends from 0 (black) to 100 (white). The other two coordinates 'a' and 'b' represents redness (+ a values) to greenness (− a values) and yellowness (+ b values) to blueness (− b values), respectively. h^0 (Hue angle) is the attribute of the colour by means of which the colour is perceived. C^* (chroma) is the attribute of colour used to indicate the degree of departure of the colour from gray of the same lightness. h^0 and C^* are computed by using the following formula.

$$h^0 = \tan^{-1}(b/a) \quad C = [a^2 + b^2]^{1/2},$$

where b = b values, a = a values

Sensory characteristics Sensory characteristics of energy bar samples were evaluated for different sensory attributes by a group of nine panelists. Sensory attributes like appearance and colour, texture, odour, flavour and taste and overall acceptability for all samples were assessed using nine point hedonic scale. Hedonic scale was in the following sequence: like extremely - 9, like very much - 8, like moderately - 7, like slightly - 6, neither like nor dislike - 5, dislike slightly 4, dislike moderately, - 3, dislike very much - 2, dislike extremely - 1 (BIS 1971).

Statistical analysis Data pertaining to three replicates of each parameter except texture ($n=5$) and sensory character-

istics ($n=9$) were analyzed as per two factor analysis of variance using LSD of AgRes software statistical package. Linear regression was computed using Microsoft Excel 2003.

Results and discussion

Textural profile analysis of energy bar samples is presented in Table 3. Hardness of energy bar was found significantly affected with the levels of sweeteners and flaxseed. Results indicated that hardness decreased with increasing levels of sweeteners except in control samples. This may be due to comparatively lower binding of the ingredients in control sample at 45% sweeteners that also exhibited crumbly texture while preparation and resulted in lower hardness than control sample with 55% sweeteners. Cohesiveness, springiness, chewiness and gumminess were affected by the levels of flaxseed and sweeteners in energy bars. In general,

Table 4 Colour quality of omega-3 rich energy bar

Energy bar samples		Colour values				
Flaxseed, %	Sweeteners, %	L	a	b	h^0	C^*
0	45	62.4	7.9	29.2 ^a	74.9	30.3 ^{ab}
	50	61.3	8.8	30.1 ^a	73.7	31.46 ^a
	55	58.6	8.9	29.8 ^a	73.3	31.1 ^a
5	45	58.9	8.5	29.1 ^a	73.7	30.3 ^{ab}
	50	56.3	8.9	27.7 ^b	72.2	29.1 ^{bc}
	55	53.0	8.8	26.5 ^{bc}	71.6	27.9 ^{cd}
10	45	52.4	8.6	25.8 ^{cd}	71.5	27.2 ^{de}
	50	49.8	8.8	24.1 ^{ef}	70.0	25.7 ^{fg}
	55	49.3	9.1	24.5 ^{de}	69.7	26.2 ^{ef}
15	45	50.4	8.7	24.6 ^{de}	70.5	26.1 ^{ef}
	50	63.9	8.1	21.4 ^h	69.2	22.9 ⁱ
	55	45.4	8.6	21.9 ^{gh}	68.4	23.5 ^{hi}
20	45	47.3	8.5	22.9 ^{fg}	69.6	24.4 ^{gh}
	50	44.9	8.6	20.9 ^h	67.7	22.6 ⁱ
	55	48.7	8.7	23.8 ^{ef}	69.8	25.4 ^{fg}
F Value						
F		4.1*	1.6 ^{NS}	126.7**	87.7**	112.9**
S		1.2 ^{NS}	5.4*	11.9**	23.5**	9.4**
F $\times$ S		1.0 ^{NS}	2.4 ^{NS}	5.3*	2.7 ^{NS}	5.2*
CD (0.05)						
F		7.6	0.312	0.815	0.656	0.821
S		5.9	0.242	0.632	0.507	0.636
F $\times$ S		13.1	0.541	1.4	1.1	1.4

F Flaxseed; S Sweeteners; NS non significant

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, $n=3$

Table 5 Nutritional composition of omega-3 rich energy bar

Flax Seed (%)	Sweeteners (%)	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Calcium (mg/100 g)	Iron (mg/100 g)	Crude Fibre (%)	Total Carbohydrates (%)	Omega-3 fatty acid (% fatty acid)	Calories (kcal)
0	45	11.7	10.3	4.6	1.3	28.9	3.2	0.9	71.2	12.4	367.4
	50	12.6	9.5	4.4	1.2	28.2	3.1	0.8	71.5	11.5	363.6
	55	13.2	9.1	4.3	1.2	27.4	3.0	0.8	71.4	10.7	360.6
5	45	11.7	10.9	6.4	1.4	28.8	3.4	1.2	68.5	15.0	374.9
	50	12.4	10.2	6.2	1.3	28.1	3.2	1.2	68.8	14.1	371.5
	55	13.1	9.9	6.1	1.1	27.4	3.2	1.1	68.7	13.2	369.1
10	45	11.4	11.3	8.3	1.5	28.8	3.6	1.5	65.9	17.5	383.3
	50	12.2	10.8	8.0	1.4	28.1	3.4	1.5	66.2	16.6	379.6
	55	13.1	10.2	7.9	1.3	27.3	3.3	1.4	66.2	15.7	376.2
15	45	11.4	11.8	9.9	1.5	28.7	3.7	1.9	63.4	20.0	390.5
	50	12.3	11.3	9.9	1.4	27.1	3.6	1.8	63.3	19.1	387.2
	55	13.1	10.8	9.6	1.3	27.3	3.5	1.7	63.5	18.3	383.9
20	45	11.5	12.4	11.9	1.7	28.7	3.8	2.2	60.4	22.5	397.9
	50	12.4	11.9	11.6	1.5	27.9	3.7	2.1	60.5	21.7	393.7
	55	13.1	11.4	11.4	1.4	27.1	3.6	2.1	60.6	20.8	390.5
F Value											
F		2.5 ^{NS}	170.2 ^{***}	9934.54 ^{***}	18.5 ^{**}	0.12 ^{NS}	10.5 ^{**}	162.1 ^{**}	1281.1 ^{**}	23303.9 ^{**}	909.8 ^{**}
S		249.9 ^{***}	101.2 ^{***}	67.6 ^{***}	19.9 ^{**}	10.7 ^{**}	3.2 ^{NS}	6.3 [*]	1.1 ^{NS}	1781.2 ^{**}	120.5 ^{**}
F×S		0.436 ^{NS}	0.42 ^{NS}	1.1 ^{NS}	0.78 ^{NS}	0.001 ^{NS}	0.06 ^{NS}	0.08 ^{NS}	0.20 ^{NS}	0.14 ^{NS}	0.25 ^{NS}
CD(0.05)											
F		0.187	0.20	0.082	0.08	0.84	0.21	0.115	0.345	0.076	1.2
S		0.145	0.15	0.067	0.06	0.65	0.16	0.09	0.27	0.059	0.89
FS		0.323	0.34	0.142	0.14	1.5	0.36	0.20	0.60	0.131	1.9

F Flaxseed; S Sweeteners; NS non significant

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; $n = 3$

cohesiveness reduced with increasing level of sweeteners at the same level of flaxseed, may be due to the higher moisture content at increasing level of sweeteners. The

similar trend was observed for the chewiness except for the control sample. Although textural profile of energy bar was significantly affected with levels of flaxseed and sweet-

Fig. 1 Effect of storage on quality characteristics of energy bar ($n = 3$)

eners, poor correlation may be due to the heterogeneity in the textural properties of samples which may be due to difference in the hardness of ingredients.

Colour data indicated that different levels of flaxseed and sweeteners affected the colour of energy bar significantly (Table 4). *L* values decreased significantly ($p<0.05$) with increasing levels of flaxseed but different levels of sweeteners did not brought significant difference while reverse trend was observed for *a* values, because of the sample darkening due to addition of brown coloured flaxseed. From Table 4, it is clear that hue and chroma varied significantly with the levels of flaxseed and sweeteners due to variation in *a* and *b* values. Although colour of the samples was significantly affected with the levels of flaxseed and sweeteners, energy bar samples were well accepted on sensory evaluation even at 20% level of flaxseed which was in the hedonic scale category of ‘moderately liked’ to ‘very much liked’.

Different levels of flaxseed did not bring any significant difference in the moisture content of energy bar but the increasing level of honey increased the moisture content significantly ($p>0.05$), which is obvious (Table 5). The protein content of different energy bar samples was in the range of 9.12–12.41%, which was increased significantly with increasing level of flaxseed due to higher protein content in flaxseed but found decreased with increasing level sweeteners due to dilution effect of honey. Similar trend was observed for fat, ash, crude fibre and total calories content in energy bar samples. Calcium content was also found reduced with increasing levels of sweeteners. Although increasing levels of sweeteners brought reduction in iron content and slightly increased the total carbohydrates content but it was statistically not significant. As energy bar samples were prepared by cold mixing and sheeting, almost no processing losses would have been occurred in the nutritional quality of energy bar samples. Omega-3 content as alpha-linolenic acid in the flaxseed, used in this study was 44.58% of fatty acids. Omega-3 content in different samples was in the range of 10.68–22.51% of fatty acids, which was found minimum in the control sample with 55% of sweeteners and maximum in samples with 20% of flaxseed with 45% of sweeteners due to presence of highest amount of flaxseed in this sample. During 90 days storage period, the total reduction in omega-3 fatty acid as alpha-linolenic acid was 10.34% and 4.45% in the energy bar samples kept at 25 °C and refrigerator, respectively (Fig. 1). Although storage brought a significant reduction in omega-3 fatty acid, FFA content, and microbial load but Fig. 1 indicated that energy bar sample can be stored even at room temperature for 30 days period without much of perceivable changes in the product quality.

Effect of storage on free fatty acids (FFA) in energy bar samples is presented in (Fig. 1). FFA content of fresh

Table 6 Sensory characteristics of omega-3 rich energy bar

Energy bar samples		Appearance and colour	Texture	Odour	Flavor & Taste	Overall acceptability
Flaxseed (%)	Sweeteners (%)					
0	45	7.5	7.2 ^{de}	7.1	7.2 ^{efg}	7.3 ^{cd}
	50	7.2	7.2 ^{de}	7.1	7.3 ^{ef}	7.3 ^c
	55	7.4	7.5 ^{cd}	7.0	7.1 ^{figh}	7.4 ^c
5	45	7.8	7.7 ^{bc}	7.3	7.9 ^a	7.8 ^b
	50	7.8	7.5 ^c	7.3	7.5 ^{cd}	7.7 ^b
	55	8.1	8.0 ^a	7.4	7.9 ^a	8.2 ^a
10	45	7.3	7.6 ^c	7.4	7.4 ^{de}	7.3 ^{cd}
	50	7.1	7.0 ^{ef}	7.2	6.9 ^{gh}	7.3 ^c
	55	7.6	7.9 ^{ab}	7.7	7.8 ^{abc}	7.9 ^b
15	45	7.2	7.5 ^c	7.4	7.8 ^{ab}	7.7 ^b
	50	7.4	7.5 ^{cd}	7.4	7.3 ^{ef}	7.8 ^b
	55	7.6	7.6 ^c	7.4	7.6 ^{bcd}	7.8 ^b
20	45	7.0	7.0 ^{ef}	7.0	6.9 ^h	7.0 ^{de}
	50	6.6	7.0 ^{ef}	7.1	7.2 ^{ef}	7.2 ^{cd}
	55	6.8	6.9 ^f	7.3	7.1 ^{figh}	6.9 ^e
F Value						
F		6.7 ^{**}	29.6 ^{**}	6.9 ^{**}	28.8 ^{**}	43.0 ^{**}
S		1.4 ^{NS}	18.4 ^{**}	2.6 ^{NS}	11.8 ^{**}	10.7 ^{**}
F×S		0.34 ^{NS}	4.6 [*]	2.0 ^{NS}	6.6 ^{**}	5.0 [*]
CD(0.05)						
F		0.42	0.151	0.171	0.150	0.145
S		0.32	0.117	0.133	0.116	0.112
F×S		0.73	0.261	0.296	0.260	0.250

F Flaxseed; S Sweeteners; NS non significant

* $p<0.05$, ** $p<0.01$, *** $p<0.001$; $n=9$

sample was 0.07% (on whole sample basis), which was slightly increased with increasing storage period. Although storage brought a linear increase in FFA content with increasing storage period but no rancid or off flavour was observed on sensory evaluation. If it is compared on fat basis, the FFA content in energy bar sample, stored in

Table 7 Effect of storage on sensory characteristics of energy bar ($n=9$)

Storage period, days	Appearance and colour	Texture	Odour	Flavor & Taste	Overall acceptability
25 °C					
0	7.2	7.5	7.4	7.8	7.7
30	7.2	7.4	7.3	7.7	7.6
60	7.2	7.3	7.4	7.5	7.1
90	7.2	7.0	7.0	7.0	7.1
Refrigerator					
0	7.2	7.5	7.4	7.8	7.7
30	7.2	7.5	7.4	7.7	7.7
60	7.2	7.4	7.4	7.7	7.6
90	7.3	7.4	7.4	7.5	7.5

refrigerator for 90 days was lesser (1.45%, on fat basis) than samples stored at 25 °C for the same storage duration than the maximum limit of 2.0% (Nagaraj 2009). This showed that refrigerator storage of energy bar samples should be preferred over 25 °C. The increase in FFA content may be mainly from degradation products of hydroperoxide (Thakur and Arya 1990), which is directly related with RH and moisture content of the products (Sowbhagya and Bhattacharya 1976). Similar type of linearly increased FFA content with increasing storage period was also observed in the fortified bengal gram *sattu* stored at 25 °C (Mridula et al. 2010). The relationship between the FFA content (F) and storage (S) period (days) can be expressed by using the following regression equation.

$$F(25^{\circ}\text{C}) = 0.041S + 0.02; R^2 = 0.9062$$

$$F(\text{refrigerator}) = 0.017S + 0.055; R^2 = 0.9797$$

Total bacterial counts and yeast and mould counts (log cfu/g) increased from 3.4 to 4.6 and 3.45 to 3.62, respectively during 90 days storage (Fig. 1) but were within the acceptable limits of total bacterial counts of 4.7 log cfu/g (Deshpande et al. 2004).

Mean sensory scores, for different levels of flaxseed and sweeteners in energy bar samples, for all the sensory characteristics were more than the minimum acceptable score of 6 (Table 6). The result thus indicated that the energy bar prepared from different levels of flaxseed and sweeteners were accepted by the panelist. Different levels of flaxseed brought significant variation in the mean sensory scores for appearance and colour, and odour of energy bar samples but sensory texture, flavour and taste and overall acceptability were affected by sweeteners too. Highest mean sensory scores i.e. 7.9 and 8.2 for flavour and taste and overall acceptability, respectively were observed for samples with 5% of flaxseed and 55% sweeteners but the mean sensory scores for texture (7.9) and flavour and taste (7.8) of energy bar with 10% of flaxseed and 55% sweeteners was at par with the farmer sample. As per the mean comparison by LSD, overall acceptability scores of energy bar samples with 45 and 50% sweeteners were at par. The overall acceptability score for flavour and taste and overall acceptability samples with 10% flaxseed and 55% sweeteners and 15% flaxseed and 45% sweeteners were at par but the protein, omega-3 fatty acid and other nutritional constituents in the later sample was higher than the former one (Table 4). Hence, 15% flaxseed and 45% sweeteners along with other ingredients may be considered for production of energy bar at commercial scale.

Energy bar samples with 15% flaxseed and 45% sweeteners, which were found good in omega-3 fatty acid and well accepted during sensory evaluation, hence they were

considered for shelf life study. Mean sensory scores for energy bar samples, stored at 25 °C were slightly lower than samples stored in refrigerator but were in the same hedonic scale category i.e. like very much to like moderately (Table 7). This may be due to suitability of lower temperature i.e. refrigeration for storage of energy bar samples, which did not cause much deterioration in physico-chemical and sensory quality of products as compared to 25 °C.

Conclusion

Nutritional quality particularly protein, fat, crude fibre, iron, total calories and omega-3 content increased with increasing flaxseed (5–20%) in energy bar. Keeping in view the omega-3 content and acceptability of energy bar, 15% flaxseed and 45% level of sweeteners along with other important ingredients may be considered for production of acceptable quality omega-3 rich energy bar at commercial scale, which also stored well for 90 days at refrigerated condition.

Acknowledgement Authors express sincere thanks to DST, New Delhi for financial assistance and Director, CIPHET for providing facilities for conducting this study.

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