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Full Length Research

Storage stability of healthy nutrient bar without preservatives as dietary supplement for women at risk of osteoporosis

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ABSTRACT: The objective of this study was to examine the stability of the healthy nutrient bars developed as a supplement for the women approaching menopause, who are at risk of osteoporosis. In this study, convenient, ready-to-eat highly nutritious bars with natural food ingredients of plant origin were developed. These analyzed both nutritionally and microbiologically to check their suitability for supplementation to women at risk of osteoporosis. Bars were packed in metalized polyester films (MPF) under ambient conditions for a period of 90 days to further check their storage stability for a period of three months. The parameters which were determined to check its storage stability were physical, oxidative, sensory and textural profile analysis on monthly basis and microbiological changes on fortnightly basis. Physical characteristics of the bar showed no significant change however chemical parameters like Thiobarbituric acid (TBA) and free fatty acid values increased significantly ($p \leq 0.05$) on storage. Bars remained microbiologically safe and acceptable during entire period of storage under ambient temperature (15-35°C) conditions. After 3 months of storage, the overall acceptability score of bars decreased significantly ($p \leq 0.05$) from 8.25 to 7.42 on a 9 point hedonic scale with an increase in free fatty acid values from 1.15 to 3.36 meqO2/kg fat, TBA value from 0.22 to 0.91 mg malonaldehyde (MA)/ kg and Browning Index showed no significant increase from 0.14 to 0.28 (OD) on three months of storage. Texture profile analysis helped in evaluating the change in hardness gumminess, chewiness and cohesiveness of the bar, which affected the physical appearance and overall acceptability of the product.

Keywords: Bone health, functional foods, oxidation, stability.

INTRODUCTION

Consumer interest in relation to diet and health has increased the demand for information about functional foods. Credible scientific research indicates that there are many clinically proven and potential health benefits obtained from functional food components. These foods, also known as “functional foods,” are thought to provide benefits beyond basic nutrition and may play a role in reducing or minimizing the risk of certain diseases and other health conditions. Examples of these foods include fruits and vegetables, whole grains, fortified foods and beverages and some dietary supplements. Functional characteristics of many traditional foods are being discovered and studied, while new food products are being developed to include beneficial components. By knowing which foods can provide specific health benefits, you can make food and beverage choices that allow you to take greater control of your health. Factors fueling interest in these foods include the rapid advances in science and technology, increasing healthcare costs, changes in food laws affecting label and product claims, an aging population and rising interest in attaining wellness through diet, among others.

Eating well and staying physically active are two essential components of a healthy lifestyle. These are also the pillars of osteoporosis prevention at all stages of life. The nutritional approaches for combating osteoporosis have been stressed mostly upon supplementation of calcium and vitamin D. However, the disease is still increasing among aged population especially women approaching or in menopause. A good nutritional approach towards bone health includes a diet full of vitamins, minerals, high quality protein vitamin D and other phytonutrients.

In the food industry, it is common to mix different ingredients with nutritional and functional properties. Several researchers have evaluated the parameters used by consumers when choosing cereal bars and found that consumers pay special attention to caloric value, type of ingredients and consider taste as most important sensory attribute (Mahanna et al., 2009). It is possible to replace some percentage of cereals in cereal bars with vital ingredients specific to bone health enabling bars to be taken as food supplement for enhancement of bone health. A ready-to eat nutritional supplement which includes ingredients of plant origin and contains all the essential nutrients for bones, besides calcium is a need of the changing lifestyles in current social scenario.

Whatever the product ingredients are, shelf life is a factor of critical importance because it defines the storage period of the product with high initial quality to be safe for consumption. According to Grizotto et al. (2006), food products shelf life study consists of submitting several samples for a series of tests over a long enough period of time so as to establish its limits.

Several changes occur during storage of any food product which includes both physical and chemical parameters. These changes are highly influenced by the type of ingredients present in the food product. Frietas and Moretti (2006) reported several changes during storage of cereal bars made with high protein and vitamin content (Vitamin C and E). They reported that the change in colour, appearance and overall acceptability was negatively affected by oxidation of vitamin C which accelerated the browning process.

The objective of the current study was to check the stability of healthy nutrient bars on storage to add value to the food supplement which has been developed to adjust to the modern trends in food habits. The shelf life stability was determined for a period of ninety days. The parameters which were determined to check its storage stability were physical, oxidative, sensory and textural profile analysis on monthly basis and microbiological changes on fortnightly basis. All the samples for the study were prepared in a single lot and packed in metalized polyester films (MPF) (0.14mm) and stored under ambient conditions.

MATERIAL AND METHODS

The healthy nutrient bars were made from cereal mix of oats brown rice and corn flakes, defatted soy flour, dried fruit solids, flax seeds, herbs, very low fat and honey for binding. All the raw materials were procured from the local market of Ludhiana. Weighed amounts of all the ingredients for preparing a formulation was prepared beforehand and separately packed and stored in airtight containers under cold storage. Weighed amounts of all the dry ingredients were taken and mixed together, except dried fruit solids. It was followed by addition of honey and date pulp-which was prepared beforehand. The formulated ingredient mixes were first filled in the tin moulds with dimensions 11 cm(L), 3cm (B) and 1.5 cm (H), slightly pressed to get a shape of bars, covered with tin foil and baked at standardized temperature of 125±5°C for 25±5 minutes. After baking, moulds were cooled; bars removed and packed immediately in metalized polyester films (MPF) (0.14 mm) and stored at ambient temperature for study.

Preparation of nutrition bars for storage

Development of formulations

A total of twenty four prototype formulations (Fs) were developed using all the major food groups viz. Cereal Mix (CM) of brown rice (*Oryza sativa*), corn flakes and oats (*Avena sativa*), soy flour (SF), fruit mix (FM) of dates (*Phoenix dactylifera*), dried figs (*Ficus carica*), golden seedless raisins (*Vitis Vinifera*), flax seeds (FS) (*Linum Usitatissimum*), honey (H), fat (F) from rice bran oil (*Gamma oryzanol*) and herbs (Hs) namely *Taraxicum officinale* and *Ocimum basilicum*. All these ingredients are rich in bone specific nutrients like calcium, protein, vitamin K and other phytochemicals. All the nutrition bars developed were prepared in a single attempt to yield enough bars for studying their parameters on storage. Each bar weighing about 50±2 g at the time of packing and was marked with date of packing on each packet. All the bars were stored at same room temperature of range 15 to 35°C.

Physical parameters

Simple physical parameters like loss of weight and shape were examined to check the effect of storage on physical appearance.

Oxidative analysis

Lipid oxidation was monitored by the analyses of the oxidative capacity, through Thiobarbituric acid Value (TBA), free fatty-acid value, and browning index. All the tests were performed at 0 (t_0), 30 (t_1), 60 (t_2), and 90 (t_3)

days of storage. Lipid oxidation was determined by the TBA test according to Tarledgis et al. (1960) and free fatty-acid value as per AOCS (1973). The TBA test was performed by homogenizing 10 g of sample transferred to a Kjeldahl flask wit 97.5 ml of distilled water and 2.5 ml of 6 N Hcl. The mixture was heated with steam distillation until 200 ml of distillate was collected. Five ml of distillate was added to 5 ml of thiobarbituric reactive agent containing 0.02 M TBA in 90% glacial acetic acid and incubating in boiling water for 35 minutes. After cooling, the absorbance was read at 538 nm. The constant 7.8 and 6.2 were used to calculate the distillation TBA number as recommended by Tarledgis et al. (1960). For measuring free fatty-acid value, titration method was used to determine fatty acid conversion during esterfication. One gram of sample was weighed accurately in the Erlenmeyer flask followed with addition of **Ca**. 50 to 100 ml of neutralized solvent to dissolve the sample. After adding solution of phenolphtalein, titration with adjusted potassium hydroxide solution was carried out while constantly swirling until the color changes consistently. Formula for calculation is as under;

$$\text{Acid value [mg KOH/g]} = \frac{\text{V x 56.1 x N}}{\text{E}}$$

$$\text{Free fatty acid [\%]} = \frac{\text{V x MG x N}}{\text{10 x E}}$$

For the measurement of browning index, 4 g of sample was treated with 50 ml of distilled water. It was then shaken vigorously in a mechanical shaker for 2 hours, followed by the addition of 5 ml 45% lead acetate solution. The shaking was presumed for another 5 minutes. Filtered and the absorbance of the supernatant was measured at 420 nm in a spectrophotometer (Model UV 1601, Shimadzu, Japan) (Padmashree, Sharma and Givindaraj, 2013). All the chemical analysis was performed in triplicate.

Sensory analysis

The sensory evaluation was carried out to monitor stability of the product to check its acceptability for the period of storage. Tasting was carried out repeatedly by the same trained 10 voluntaries for the specified period of storage, using 9-point hedonic scale for attributes like overall acceptance, color, appearance, texture and flavour.

Microbiological analysis

Microbial analysis for total mesophilic count and yeast and mould count was checked on fortnightly basis for a period of 90 days and total fecal coliforms and *Bacillus cereus* were checked only initially (t_0) for safety as described in AOAC (1995).

Textural profile analysis

This was carried out to check the hardness, cohesiveness, gumminess, and chewiness of the product initially and changes in these characteristics on storage of the product. Textural profile analysis was done at room temperature using the texture analyzer (TA-Hd*i*), (stable micro system, UK). Texture profile analysis (Bourne, 1978) was performed using five pieces of each sample (2.5 cm × 2.5 cm × 2 cm), which was then compressed twice to 505 of the original height with a cylinder probe P/75. The elapsed time between two compression cycles was 2 s and cross head was moving at constant speed of 1 mm/s resulting in a curve from which the parameters of texture i.e. hardness, cohesiveness, springiness, chewiness and gumminess was determined (Mridula et al., 2011).

Statistical analysis

Statistical analyses were conducted using SPSS software version 16. Tukey's adjusted p-value was used to determine significance ($p \leq 0.05$) after applying ANOVA to check the significant differences on different parameters for the storage period.

RESULTS AND DISCUSSION

The products developed from prototype formulations were first organoleptically evaluated. Four formulations were found statistically comparable on overall acceptability for sensory characteristics and their scores for overall acceptability ranged between7.65 to 8.25 as shown in Table 1. These were further analysed for proximate composition and other nutritional evaluation. On statistically analysing of the overall results, one bar with highest overall acceptability score of 8.25 was selected for supplementation and storage study. Based on RDA for Indians, each bar weighing 50±2 g provided daily requirements of nutrients *viz:* Protein, Calcium, Phosphorus and Magnesium at 17, 43, 59 and 61 percent respectively with appreciable amounts of omega 3, isoflavones, vitamin K and total carotenoids. All the values were estimated after the process of baking, taking into account the loss on processing.

Sensory attribute for healthy nutrient bars were evaluated for appearance, colour, texture, flavour and over all acceptability using 9-point hedonic scale, from like extremely-9 to dislike extremely-1 (Larmond 1970). The changes in sensory attributes during storage period and their effect on overall acceptability is shown in Table 2. From the data, it can be observed that there was some significant ($p \leq 0.05$) decrease in the sensory attributes till 30 days after which a non significant ($p \leq 0.05$) decline in overall acceptability was recorded till the end of storage study. Colour and texture of the bar was bit of a concern and its score decreased from 8.2 to 7.3 for both attributes.

Table 1. Sensory scores of four formulations.

S.no	Ingredients	Percentage	Appearance	Color	Texture	Flavor	Overall acceptability
1	CM+SF_FM+Hs+FS+H+F	20+20+30+2+2+20+6	8.3±0.22	8.2±0.23	8.2±0.17	8.3±0.20	8.25
2	CM+SF_FM+Hs+FS+H+F	25+20+25+2+2+20+6	8.0±0.36	8.1±0.35	8.0±0.28	8.1±0.31	8.05
3	CM+SF_FM+Hs+FS+H+F	20+25+25+2+2+20+6	7.5±0.16	7.8±0.30	7.6±0.21	7.7±0.30	7.65
4	CM+SF_FM+Hs+FS+H+F	25+25+20+2+2+20+6	7.8±0.28	7.9±0.22	7.8±0.29	7.7±0.30	7.8
p value		p<0.01	p<0.01	p<0.01	p< 0.01		

Cereal Mix: C M; Soy flour: S F; Fruit Mix: FM; Herbs: Hs; Flax Seeds: FS; Honey: H; Fat: F. (W)* whole form; (G)* Ground form I-VI -No. of trials. Values represent Mean ± SD.

Table 2. Change in sensory attribute of nutrition bar on storage.

Months	Appearance	Color	Texture	Flavor	Overall acceptability
0	8.3±0.22	8.2±0.23	8.2±0.17	8.3±0.20	8.25a
1	7.8±0.17	7.6±0.15	7.5±0.13	7.70±0.12	7.65b
2	7.8±0.17	7.5±0.13	7.5±0.13	7.6±0.15	7.60b
3	7.6±0.15	7.3±0.19	7.3±0.19	7.5±0.13	7.42b

Data are expressed as mean ± standard deviation. Values in the same columns with different superscripts are significant (p≤0.05).

Table 3. Effect of storage on weight and shape.

Days	Weight (g)	Shape (No Change /Change)
0	50^a	*No visible change*
30	49.7^a	*No visible change*
60	49.3^a	*No visible change*
90	48.9^a	*No visible change*

Data are expressed as mean ± standard deviation. Values in the same column with same superscripts are not significant (p ≤ 0.05).

The change in texture occurs due to the presence of defatted soy flour, high in proteins and known for hardening of product on storage due to formation of cross links and secondary structures. Similar changes were observed by Padamshree et al. (2012) in overall acceptability of composite cereal bars which declined from 7.9 to 7 on 9 months of storage and were again confirmed by Yaseen et al. (2012). In the current study, the healthy nutrient bars were shelf stable for 3 months in MP films at temperatures below 25°C but overall acceptability declined from 8.25 to 7.42. The increase in FFA value contributed to the decline in overall acceptability with increased storage time.

Weight and shape of the nutrition bars showed no significant difference on the storage (Table 3) owing to the fact that higher presence of fibrous fruit solids maintained the weight and shape of product. The bar required little force for breaking, and was retaining the softness due to presence of fibrous fruit solids inside. The packing material (MPF) was able to retain the moisture in them and prevented weight loss. The presence of fruit chunks and coarse grains gave rough appearance and crunch to the bars and helped in retaining their shape, besides adding value to their physical characteristics. Loveday et al. *(*2009) also reported little or no variation in weight and shape of its bars and later on it was again verified by Silva et al. (2013) that little variation in weight and appearance of cassava flour based bars was noticed.

Oxidative stress analysis on storage of healthy nutrient bars was monitored by free fatty acid value, Thiobarbituric acid (TBA) value and browning Index (BI) performed at 0 (t_0), 30 (t_1), 60 (t_2), and 90 (t_3) days (Table 4) and represented graphically in Figure 1. After 90 days of storage, it was observed that there was slight but significant (p≤0.05) chemical changes in samples packed in MP films at ambient temperature. After 3 months of storage, FFA value increased significantly (p≤0.05) from 1.15 to 2.47 meqO2/kg fat till first month and then a non significant (p≤0.05) increase was recorded to 3.36 meqO2/kg fat till three months of storage. The TBA value also increased significantly (p≤0.05) from 0.22 to 0.43 mg malonaldehyde (MA)/kg till first month, with non significant increase of 0.91 mg malonaldehyde (MA)/ kg till end of study. Browning index (BI) showed a non significant

Table 4. Effect of storage on oxidative properties of nutrition bar (Free fatty acid value, TBA value and Browning Index.

S. no.	Test	t(0)	t(1)	t(2)	t (3)
1	Free fatty acid value	1.15±0.14a	2.47±.04b	3.2±0.10b	3.36±0.16b
2	Thiobarbituric acid value (TBA) (OD)	0.22±0.01a	0.43±0.02b	0.76±0.01b	0.91±0.01b
3	Browning Index (OD)	0.14±0.02a	0.15±0.01a	0.23±0.0a	0.28±0.01a

Data are expressed as mean ± standard deviation. Values in the same row with different superscripts are significant (p ≤ 0.05).

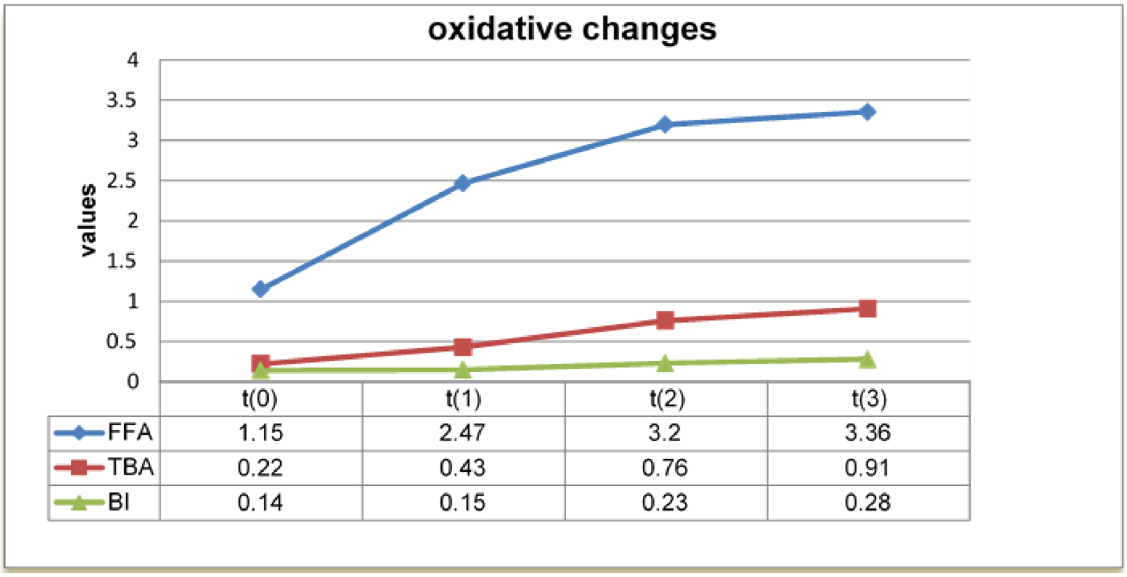

Figure 1. Effect of storage on oxidative properties of nutrition bar (Free fatty acid value (FFA), Thiobarbituric acid value (TBA) and Browning Index (BI).

(p≤0.05) increase from 0.14 to 0.28 (OD) for all the three months of storage. Such oxidative changes on storage occur in almost all foods and had been reported in chapattis also (Khan et al 2011) and changes were more pronounced at higher temperatures. Chemical changes in protein rich composite bars, vacuum packed in MP and stored at ambient temperature followed by MP, PFP and PP packed samples on 9 months of storage with FFA value varying from 1.9 to 4.99 meqO2/kg fat, TBA from 0.19 to 0.41 mg (M/A)/kg sample and BI (OD) from 0.05 to 0.17. Padamshree et al. (2012, 2013) also reported significant increase in FFA, TBA and browning index values of bars on storages in MP films twice in two different studies carried out in 2012 and 2013. The ingredients present in both the bars were different and highly responsible for difference in chemical changes.

The analysis of microbial count of any food is important as it declares the food to be safe for consumption but rare studies were found on this parameter. In the current study the material was analyzed for coliforms (total and fecal) and *Bacillus cereus* only initially, but none was found. For storage period of 90 days bars were screened on fortnightly basis for aerobic mesophilic count (CFU/g) and yeast and mould count (CFU/g) and its data is presented in Table 5. Initially, the aerobic mesophilic count was slightly higher but decreased with storage period, then increased again after 45 days but dropped consistently with storage time from initial value of 8400 (CFU/g) to 119.00 (CFU/g) on 90th day and all these changes were significant (p≤0.05). This drop may be attributed to reduction in available oxygen present in the packaging material. Yeast and mould count got a significant increase (p≤0.05) on first month of storage but then declined significantly (p≤0.05) with further storage time. The changes in microbial count may be due to variation in water activity on storage time; however, the product was stable and safe for consumption till the end of 90 days as total bacterial count was within the normal safe range (1,000,000 CFU) (Garg et al., 2010). The increase in total bacterial count and yeast and mould count (log cfu/g) for omega-3 rich energy bar with flax seeds from 3.4 to 4.6 and 3.45 to 3.62 respectively during 90 days of storage was also reported by Mridula, et al. (2013). This was again verified by da Silva et al. (2013) in her study on functional food supplements; which showed initially a trend of higher aerobic mesophile counts followed by a decrease until the end of the storage period.

The results of texture analysis of healthy nutrient bar on

Table 5. Microbial count detected for bars on fortnightly basis stored in PP film for a period of 90 days.

Days	Aerobic Mesophilic count (CFU/g)	Yeast and mould count (CFU/g)
0	8400.00±176.77a	3.60±0.63d
15	3900.00±353.55b	98.00±2.26b
30	410.00± 21.21cd	181.00±9.89a
45	630.00±0.00cd	78.00±3.53c
60	881.00±12.02c	80.00±0.00c
75	343.00±16.97cd	18.00±3.53d
90	119.00±4.24d	6.00±0.70d

Data are expressed as mean ± standard deviation. Values in the same columns with different superscripts are significant (p≤0.05).

Table 6. Texture profile analysis of bar after every month till three months.

Months	Hardness (N)	Fracturability (N)	Adhesiveness (N)	Springiness (mm)	Cohesiveness	Gumminess (N)	Chewiness (N/mm)	Resilience
Beginning	344.84±12.89[d]	0.09±0.03[a]	0.007±±0.00[a]	0.513±0.02[c]	0.432±0.01[c]	163.8±7.25[d]	69.73±2.74[d]	0.201±0.01[b]
Ist Month	408.66±4.55[c]	0.06±0.01[a]	0.29±±0.00[b]	0.513±0.01[c]	0.519±0.03[b]	223.4±1.37[c]	122.77±3.25[c]	0.303±0.01[b]
2nd Month	542.46±1.58[b]	--	0.59±0.05[c]	0.642±0.03[b]	0.579±0.02[a]	314.01±1.30[b]	201.7±0.43[b]	0.360±0.02[a]
3rd Month	593.81±4.31[a]	--	--	0.793±0.01[a]	0.610±0.01[a]	332.61±0.88[a]	267.05±2.05[a]	0.412±0.00[a]

texture analyzer are given in Table 6. The fresh sample had significantly (p≤0.05) less hardness, which increased further with storage time as observed in other studies (Nadeem et al 2012, Loveday et al 2009). The texture did not show any sign of crumbliness. Fracturability was more at the beginning and had a significant (p≤0.05) decrease with storage time. Cohesiveness showed a non significant increase with time owing to decrease in moisture content on storage similar to the results obtained by Mridula et al. (2013). Adhesiveness was significantly (p≤0.05) less at beginning and increased further on storage. The bar was significantly (p≤0.05) less cohesive when fresh, this increased for the 1st month as hardness also increased and remained stable till the end. The bar also had significant increase (p≤0.05) in gumminess, chewiness as also observed by da Silva et al. (2013) in her bars. Springiness and non significant change in resilience was observed with advanced storage as a result of decrease in moisture content with increasing storage time.

Conclusion

It is evident from the study that healthy nutritious bars can be prepared from the ingredients with functional properties specific to bone health without preservative through baking process. The product remains stable and highly acceptable even after a storage period of 3 months under ambient temperature (15–35°C) conditions. It showed some oxidative changes but that is common to foods on storage. However, the product was safe for consumption and has increasing commercial scope and viability.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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