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Estimating the microbial attributes and proximal composition of zn-aided bar

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Abstract

To overcome the malnutrition of micronutrients, specifically in children is the most influential topic now a days. Many interventions has been considered till now but oral dietary interventions has indispensable role to cope micronutrient deficiencies like Zn, which is essential for the rapid growth of adolescent boys. This study assimilated the potential of natural sources to mitigate Zn deficiency among zinc deficient boys through fortified food bars. By following different ratios of natural zinc fortificants i.e. pumpkin and sesame seed, treatments were prepared with oats, milk powder, sugar and butter. After preparation, bars were examined regarding estimation of zinc, proximate and microbial analysis at an equal interval of 15 days for sixty days.T_0 is placebo, T_1 is synthetic fortified bar and T_2 is natural fortified bar. Natural zinc fortificants i.e. pumpkin and sesame seeds have 7.86 and 7.14 zinc. Excluding moisture, all elements of proximate composition were non-significant among storage interval of 2 months. In all treatments moisture ranged from 8.23 to 4.56 %, fat ranged from 17.99 to 27.9 %, protein ranged from 7.20 to 16.52, ash ranged from 2.05 to 3.85 % and Zn content ranged from 0.49 to 5.14 %. In 2 months, storage study the zinc followed a non-significantly decreasing trend in all treatments. Hence, it was concluded that T_7 had the maximum ash content and sensory scores among all treatments, if investment employed it could bring a boon to therapeutic food product manufacturers.

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Introduction

It has been estimated that approximately 19 % of the entire populace (1,200 million individuals) are suffering from multi-nutrient insufficiencies (Hettiarachchi *et al.,* 2006). Undoubtedly, Zn plays an imperative role in health wellbeing and illnesses among all other trace essentials i.e. Mb, Mn, Se, Cr, Cur, I, Fe and F. Zn is recognized as the utmost plenteous intracellular metal (King *et al.*, 2003). There is 18.6 % of *Zn* deficiency with an analogous proportion among boys and girls in Pakistan. A slightly higher prevalence was noticed in rural (19.5%) as compared to urban children (17.1%) (Regulations and Coordination, Government of Pakistan, N. W. M. of N. H. S. 2018). Most of the important body mechanisms such as immunity, vision, cell reproduction, taste discernment, cognition and growth are controlled and managed by Zn. In this regard, supplementation of Zn is proved to be beneficial in innumerable ailments (Brown *et al.*, 2002). There's an existence of almost 02– 04 g of Zn in human body (Wapnir, 2000).Zn is vital trace element essential for over 300 enzymes that required Zn for their proper functioning like nucleic acid, protein, and membrane metabolism (Khalid *et al.*, 2014).

According to the Food and Nutrition Board, recent recommended daily allowance (RDA) of Zn was derived by utilizing diverse assumptions and methodologies. Commendations for females was 8 mg and for men was 11 mg (Barr *et al.*, 2002).The careful mixing of additional nutrients to certain food in order to upsurge the consumption of added nutrients and also to avert or correct an established shortage of nutrients is termed as food fortification (Dary, 2006).Dietary intervention is a principal approach to combat against nutritional complications in a population (Kawade, 2012). Dietary modification\ diversification, supplementation, bio-fortification and fortification are the leading intervention approaches. The purpose of this research project is to provide a best solution to fight against Zn deficiency.

Oats (*Avenasativa*L.) have high nutritional value due to which it became a source of attention regarding to research and commercial viewpoint (Liukkonen*et al.*, 2003). Oats are enriched with vitamin E (Tocopherol) and due to this it is also considered as a best anti-oxidant (Peterson *et al.*, 2005). Pumpkin (*Cucurbita pepo L.)* seeds contained macro elements and micro elements like Mg and Zn. Due to presence of these elements, it is used as a treasured food supplement (Stevenson, 2007). The seeds can also be supplemented in bread and different products of bakery (Kanwal *et al.*, 2015). Sesame seed (*Sesamumindicum*L.) are also great source of Zn and also has phytosterol which helpful in lowering cholesterol in blood (Kim and Park, 2008). Sesame seeds help in digestion, stimulates blood circulation and benefits the nervous system.

Research methodology

11 treatments of Food bars (fortified with an alternative ratio of indigenous Zn fortificants were developed to finally select 01 indigenous treatment regarding to a suitable sensory evaluation and a successful storage together with 01 placebo and 01 synthetically fortified treatment to continue with an efficacy study of treatments (T_0, T_1 and T_2) in volunteers.

Procurement of raw materials

Oats, Pumpkin seeds, Sesame seeds, dried milk powder, Zinc Sulfate Heptahydrate ($ZnSO_4.7H_2O$), sugar, butter, chemicals for analyses and other items were procured from market. A good quality of sesame seeds and pumpkin seeds were procured from the local market of Sargodha-Pakistan. The bars were filled in Bioriented Polypropylene (BOP) baggage and stored at ambient temperature and sanitized place. Pre-cleaned and well labeled steel containers with a proper seal cap was utilized to avoid adulteration while dried milk powder, sugar, butter and oats were procured from Hyper star store, Lahore-Pakistan. Zinc Sulfate Heptahydrate ($ZnSO_4 \cdot 7H_2O$) [Catalog No. Z0251 – SIGMA] from [Sigma-Aldrich, U.S.A.] and additional food grade chemical ingredients for analyses were procured from Shahid Scientific Store, Faisalabad, Punjab-Pakistan.

Preparation of raw-materials

The seeds of Pumpkin and Sesame were grinded and then paste was made. Coded bags of Bioriented Polypropylene (BOP) were utilized to store and preserve the prepared raw materials. Till advance processing, it was kept safely into pliable jar at room temperature.

Preparation of treatments

A specific amount of ingredients was used to avoid bitterness and complete sensory possessions. The quantity of seeds of Pumpkin was 09 – 15 g and Sesame was 20 – 30 g. In treatment T_0 and T_1, butter, sugar and dry milk powder was used. In treatment (T_1), a synthetic fortificant ($ZnSo_4$) was added as mentioned in Table.1. Natural Zn was added in treatments (T_2 – T_{10}) of food bars that were prepared by a method explained by Nadeem *et al.* (2012). Prepared ingredients were blended thoroughly to make dough, than alienated into balls of medium size and then these balls were enfolded into sheets. Bars of 10 – 10.5 cm length and 04 – 4.5 cm width were sliced. Each bar weighed 45 – 46 g. Bars were then packed and stored in refrigerator.

Analysis of proximate composition

The proximate analyses of raw materials were carried out just at 0 day. But, the proximate analyses of all treatments of Food bars were done at starting point and reiterate at an equal interval of 15 days for sixty (60) days.

Moisture content: Moisture content of the Food bars was determined by utilizing a hot air oven (Model: ED 115, Binder, Germany, Modal No. 44 – 15 A as mentioned in AACC (2000).

Crude protein: Determination of nitrogen values was done through Kjeltec apparatus (Model No. 4061412, S1, Behr Labor Technik, GMBH, and Germany) by method no. 46 – 10 as mentioned in AACC (2000).

Crude fat: Soxhlet apparatus (Model No. 0503011, Extraction Unit, and Barcelona, Spain) was utilized to determine the crude fat content of Food bars with Method No. 30 – 10 as given in AACC (2000).

Crude fiber: For the determination of crude fiber content of Food bars, Labconco Fiber (Labconco Corporation Kansas, USA) was utilized by Method No. 32 – 10 given in AACC (2000).

Ash content : All treatments of Food bars were analysed for ash by Method No. 08 – 01 as given in AACC (2000).

Nitrogen free extract (NFE): Determination of nitrogen free extract (NFE) was done according to expression given below

Nitrogen free extract (NFE) % = 100 – [crude protein (%) + crude fat (%) + crude fiber (%) + total ash (%)]

Gross energy of treatments

A gross biochemical energy that is measured after a whole incineration of a food in bomb calorimeter. Oxygen Bomb Calorimeter (Model No. 1341, Parr Instrument Company, Werke IKA) was used as reported by Krishna and Rajhan, (1981).

Determination of Zinc

Method No. 985.35 investigated all the treatments of Food bars for the determination of Zn as mentioned in AOAC (AOAC, 1997).

Microbiology of treatments

After a completion of recommended time, those plates having between 30 and 300 colonies were counted and multiplied by dilution factor. Arithmetic mean was taken as Total Plate Count per gram. Mold/ Yeast count of all the treatments of food bars was determined by utilizing the procedure and designated in bacteriological analytical manual (1992).

Results and discussion

Chemical analyses of ingredients

To estimate the potential outcomes of fortificant and base ingredients of food bars in providing energy and zinc content and proximate composition of raw materials was done.

There was a significant variation in zinc (Zn) content and proximate composition of all the ingredients. An augmented level of moisture was noticed in ingredients. Maximum content of moisture was present in oats and butter. Ash and fiber content were higher i.e. 6.18 ± 0.31 and 14.26 ± 1. In sesame (*SesamumindicumL.*) powder. The content of protein was higher in seeds of pumpkin (Cucurbita pepo L.) i.e. 30.30 ± 0.31 % while ash was 4.72 ± 0.49 %. In butter the content of fat was recorded maximum i.e. 81.16 ± 0.35 %. Maximum NFE 65.23 ± 1.11% was present in flour of roasted oats as exposed in Table 2. Content of zinc in sesame powder and seeds of pumpkin was 7.14 ± 0.0 and 7.86 ± 0.3 6 mg/ 100 g correspondingly. Values of elemental investigation for zinc (Zn) in raw material are shown in Table 3.

Table 1. Treatment Plan of Zn Fortified Food bars (100 g).

Treatments (g)	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}
$ZnSO_4.7H_2O$	0.0176	-	-	-	-	-	-	-	-	-	-
PS		-	9	9	9	12	12	12	15	15	15
SS		-	20	25	30	20	25	30	20	25	30

Minimally 50 % RDA – Zn of adolescent males (04 mg/ day) should be met in intervention as per FAO/ WHO Food Fortification Guidelines

SS = Sesame Seeds; PS = Pumpkin Seeds

To (Dried milk powder, Butter and Sugar)

T_1 (Dried milk powder, Butter, Sugar and $ZnSO_4.7H_2O$),

$T_2 - T_{10}$ (Fixed ingredients (Oats flour, Butter, Dried milk powder and Sugar), variable ingredients used according to treatment plan (PS and SS) and dried milk powder was added to adjust consistency).

Gohari*et al.* (2011) studied the nutritional composition of pumpkin seeds and it was reported that, seeds of pumpkin comprise 23.19 % (NFE), 6.34 % (ash/ minerals), 3.49 % (fiber), 41.59 % (fat), 25.40 % (protein) and 5.20 % (moisture), correspondingly. Karanja*et al.* (2014) inspected pumpkin seeds powder of various areas in Kenya and stated that, pumpkin seeds powder comprises fiber (18.89), protein (27.39), fat (41.37), moisture (5.62), ash (3.83) and carbohydrate (9.37) g/ 100 g. Zebib*et al.*, (2015) studied the physico-chemical characteristics of three different varieties of sesame: T-5, Bawnji 8 and Adi. There was a noteworthy difference among all varieties of Sesame. Differences includes proximate composition, mineral contents, physical properties and antioxidant compositions.

The moisture, crude protein, ash, fat, fiber, total carbohydrate, Ca, Zn and Fe were in following amount: 3.17 % to 3.96 %, 22.58 % to 24.27 %, 4.46 % to 6.19 %, 50.88 % to 52.67 %, 5.60 % to 6.26 %, 8.3 % to 11.69 %, 1172.08 mg/100 g to 1225.71 mg/100 g, 4.23 mg/100g to 4.45 mg/100g and 10.2 mg/100 g to 10.75 mg/100g correspondingly. Kajal*et al.* (2012) proved that milk powder weighing 100 g comprises 5.48 g of ash, 27.83 g of fat, 37.31 g of lactose, 26.04 g of protein and 3.37 of moisture. Youssef *et al.* (2016) reported a proximate composition of diverse varieties of oats. They showed that, moisture, crude protein, fat, fiber NFE and ash ranged from 10.47 – 9.96, 13.62 – 11.61, 8.92 – 7.23, 5.87 – 3.53, 75.62 – 69.43 and 2.15 – 2.00 %.

Chemical Analyses of Proximate Composition of Food bar Treatments during Storage

Moisture content

Initially, the moisture content in all treatments fluctuated from 8.23 ± 0.213 – 4.56 ± 0.3 %. Whereas, moisture content was decreased significantly in all treatments of food bars during the storage study of 60 days (02 months).

Treatment (T_{10}) has a maximum moisture content that ranged from 8.23 ± 0.213 – 8.25 ± 0.068 %. Other treatments (T_2 and T_3) the moisture content is decreased from 7.08 ± 0.107 – 6.80 ± 0.0152 and 7.19

± 0.087 – 6.90 ± 0.095 %. Whereas, treatment (T_1) has the minimum moisture content that was decreased from 4.56 ± 0.059 – 4.11 ± 0.019. Outcomes are mentioned below in Table 4.

Treatments (T_0 and T_1) had different ratio of ingredientslike milk powder, sugar and butter, therefore moisture content in these treatments (T_0 and T_1) was slightly lower whereas, augmented slightly higher content was noticed in all the remaining treatments because of an addition of ordinary fortificants (pumpkin and sesame seeds) along with flour of oats which improved the palatability of food bars.

Table 2. Mean proximate composition of raw materials.

Raw material	Moisture	Fat	Fiber	Protein	Ash	NFE
Oats	10.54±0.37^B	7.17±0.31^E	3.48±0.25^C	11.56±0.38^D	2.16±0.35^D	65.23±1.11^B
Pumpkin seeds	4.62±0.66^C	44.13±0.26^C	6.09±0.36^B	30.30±0.31^A	4.72±0.49^C	9.57±0.41^E
Sesame seeds	3.55±0.57^D	47.46±0.25^B	14.26±1.17^A	16.98±0.23^C	6.18±0.31^A	12.23±0.37^D
Sugar	2.02±0.20^E	0.00±0.00^F	0.00±0.00^D	0.00±0.00^F	1.13±0.56^E	96.62±1.20^A
Butter	15.67±0.40^A	81.16±0.35^A	0.00±0.00^D	0.84±0.51^E	0.00±0.00^F	2.14±0.32^F
Milk powder	3.47±0.41^D	27.73±0.45^D	0.00±0.00^D	26.49±0.40^B	5.47±0.35^B	36.74±1.27^C

Means sharing similar letter in a column are statistically non-significant (P>0.05).

Safdar*et al.* (2014) worked on of guava leather packed in polyethylene showed an insignificant reduction in moisture during storage period of 240 days and showed same pattern.Azmat*et al.*(2017) also described the same trend of moisture in storage study of apple sucrose bar.Bhatt and Jha (2015) prepared and evaluated food bars to detect an effect of moisture content. It was revealed that, there is no need of additional moisture-fixing chemicals to enhance the moisture content as the process of natural evaporation exist in food bars.Munir *et al.* (2016) worked on nut bar and observed the same inclined trend of moisture content in whole storage time.

Table 3. Mean Zn content in raw materials.

Raw Material	Zn Content (mg/ 100g)
Oats	3.42 ± 0.30^C
Pumpkinsedes	7.86 ± 0.36^A
Sesamesedes	7.14 ± 0^B
Sugar	0 ± 0^D
Butter	0 ± 0^D
Milk powder	0 ± 0^D

Means with different letters in each column differs highly significantly at P<0.01

Means ± SD, SD = Standard deviation.

Fat content

In treatments (T_7 and T_{10}) fat content was highest and reduced from 28.88 ± 1.28 – 28.62 ± 1.27 % and 27.9 ± 1.33 – 27.89 ± 1.3 %. Fat content was minimal in treatments (T_0 and T_1) as 17.99 ± 0.98 and 18.32 ± 0.93 % and inclined to 18.08 ± 1.14 and 18.05 ± 1.13% all through the storage period of 02 months (60 days). There was a decrease in fat content of treatment (T_9) from 25.72 ± 1.35 to 25.68 ± 1.29 %. There was a significant impact of fat content among all treatments because of a difference in ratio of ingredients added in treatment composition. Seeds have good amount of fat and no seeds were used in the production of treatments (T_0 and T_1). While, pumpkin seeds and sesame seeds as a natural Zn fortificant and energy providing ingredient was utilized in the production of treatment (T_2 – T_{10}) as exposed in Table 5. Outcomes of this study were authenticated by similar results of further researchers.

Table 4. Treatments and storage Influence on moisture content (%) of food bars.

Treatments	Days					Means ±SD
	0	15	30	45	60	
T_0	4.60±0.031^{h}	4.26±0.071^{hi}	4.21±0.113^{hi}	4.23±0.099^{hi}	3.84±0.040^{i}	4.23±0.070^{F}
T_1	4.56±0.059^{h}	4.29±0.027^{hi}	4.15±0.081^{hi}	4.10±0.067^{hi}	4.11±0.019^{hi}	4.24±0.051^{F}
T_2	7.08±0.107^{b-e}	6.99±0.061^{b-e}	6.87±0.164^{b-e}	6.82±0.087^{c-f}	6.80±0.152^{c-f}	6.91±0.054^{D}
T_3	7.19±0.087^{b-e}	7.09±0.113^{b-e}	7.04±0.085^{b-e}	7.03±0.204^{b-e}	6.90±0.095^{b-e}	7.05±0.053^{CD}
T_4	7.50±0.167^{b}	7.46±0.073^{bc}	7.38±0.139^{bcd}	7.30±0.191^{b-e}	7.27±0.195^{b-e}	7.38±0.065^{B}
T_5	6.16±0.094^{fg}	6.80±0.140^{c-f}	6.68±0.097^{ef}	6.72±0.225^{def}	5.91±0.107^{g}	6.45±0.108^{E}
T_6	7.33±0.155^{b-e}	7.25±0.171^{b-e}	7.18±0.095^{b-e}	7.15±0.107^{b-e}	7.11±0.061^{b-e}	7.20±0.051^{BC}
T_7	7.37±0.169^{bcd}	7.27±0.097^{b-e}	7.22±0.098^{b-e}	7.10±0.101^{b-e}	7.12±0.074^{b-e}	7.22±0.050^{BC}
T_8	7.06±0.044^{b-e}	6.71±0.018^{def}	6.94±0.152^{b-e}	6.68±0.025^{ef}	6.68±0.030^{ef}	6.81±0.050^{D}
T_9	7.10±0.048^{b-e}	6.99±0.065^{b-e}	6.84±0.182^{b-e}	6.85±0.153^{b-e}	6.81±0.081^{c-f}	6.92±0.054^{D}
T_{10}	8.23±0.213^{a}	8.28±0.049^{a}	8.28±0.091^{a}	8.27±0.096^{a}	8.25±0.068^{a}	8.26±0.045^{A}
Means ±SD	6.74±0.200^{A}	6.67±0.213^{AB}	6.62±0.218^{AB}	6.57±0.216^{BC}	6.44±0.227^{C}	

Means sharing similar letter in row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction Means and capital letters are used for overall Means.

Means ± SD, SD = Standard deviation

T_0 (100 g) = Placebo food bars = Milk powder, butter, sugar = (Zn = 0 mg)

T_1 (100 g) = Synthetic Zn fortified food bars = Milk powder, butter, sugar, $ZnSo_4.7\ H_2O$ = (Zn = 4mg)

T1-T10 (100g) = Natural Zn fortified food bars = Milk powder, butter, sugar, $ZnSo_4.7\ H_2O$, pumpkin seeds, sesame seeds and oats = (Zn = 3.7-5.5mg).

Ahmad *et al.* (2017) worked on granola bar enhanced with chickpea, nuts, raisins revealed the parallel effects i.e. all the treatments were significant to each other. Jan *et al.* (2016) worked on nutribar having semolina as base ingredient and supplemented with fenugreek, makkhna and dry ginger, for boosting nutrition of lactating women. Investigation showed that, the fat content following a slightly decreasing trend among treatments. Nadeem *et al.* (2018) and Rehman*et al.* (2012) depicted a non-significant presented a there was a non-significant difference of fat content during a storage period among all treatments bar. Another dense nutri bar showed the identical non-significant effect of fat (Estevez *et al.*, 2000).

Table 5. Treatments and storage influence on fat content (%) of food bars.

Treatments	Days					Means ±SD
	0	15	30	45	60	
T_0	17.99±0.98^{f}	18.16±1.06^{f}	18.28±1.4^{f}	18.16±1.12^{f}	18.08±1.14^{f}	18.14±0^{F}
T_1	18.32±0.93^{f}	18.19±1.14^{f}	18.15±1.34^{f}	18.22±1.18^{f}	18.05±1.13^{f}	18.19±1.12^{F}
T_2	25.26±1.16^{a-e}	24.96±1.25^{a-e}	25±1.26^{a-e}	24.58±0.05^{c-e}	24.95±1.19^{a-e}	24.95±1.04^{DE}
T_3	25.96±1.16^{a-e}	25.93±1.21^{a-e}	26±1.33^{a-e}	26.04±1.15^{a-e}	25.98±1.3^{a-e}	25.98±1.02^{CD}
T_4	25.37±1.34^{a-e}	25.41±1.25^{a-e}	25.35±1.14^{a-e}	25.38±1.3^{a-e}	25.16±0.98^{a-e}	25.34±1.04^{DE}
T_5	24.85±1.09^{a-e}	24.67±1.38^{a-e}	24.65±1.32^{a-e}	24.3±1.04^{e}	24.56±1.21^{c-e}	24.6±1.04^{DE}
T_6	27.28±1.36^{a-e}	27.2±1.22^{a-e}	27.03±0.94^{a-e}	27.08±1.29^{a-e}	27.1±1.27^{a-e}	27.14±1.12^{BC}
T_7	28.88±1.28^{a}	28.86±1.37^{ab}	28.7±1.39^{a-c}	28.76±1.31^{a-c}	28.62±1.27^{a-d}	28.76±1.14^{A}
T_8	24.38±1.45^{e}	24.68±1.1^{a-e}	24.43±1.41^{de}	24.42±1.41^{de}	24.33±1.33^{e}	24.45±1.14^{E}
T_9	25.72±1.35^{a-e}	25.84±1.47^{a-e}	26.09±1.1^{a-e}	25.77±1.47^{a-e}	25.68±1.29^{a-e}	25.82±0.98^{C-E}
T_{10}	27.9±1.33a^{-e}	27.97±1.35^{a-e}	27.98±1.45^{a-e}	28.12±1.16^{a-e}	27.89±1.3^{a-e}	27.97±0.95^{AB}
Means ±SD	24.72±3.55^{A}	24.72±3.53^{A}	24.7±3.53^{A}	24.62±0^{A}	24.58±3.56^{A}	

Means sharing similar letter in row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction Means and capital letters are used for overall Means.

Means ± SD, SD = Standard deviation

T_0 (100 g) = Placebo food bars = Milk powder, butter, sugar = (Zn = 0 mg)

T_1 (100 g) = Synthetic Zn fortified food bars = Milk powder, butter, sugar, $ZnSo_4.7\ H_2O$ = (Zn = 4mg)

T_1-T_{10} (100g) = Natural Zn fortified food bars = Milk powder, butter, sugar, $ZnSo_4.7\ H_2O$, pumpkin seeds, sesame seeds and oats = (Zn = 3.7-5.5mg).

Crude protein

Initial protein content in all the treatments of food bars was fluctuating from 7.20 ± 0.0 to 16.52 ± 0.18 %. While it was decreased non-significantly during storage period of 02 months (60 days) and significant among treatments as exposed in Table 6. In treatments (T_0 and T_1), a minimal crude protein content was found that was reduced from 7.20 ± 0.00 to 6.90 ± 0.00 % and 7.23 ± 0.01 to 6.92 ± 0.04 %. While it was observed that, crude protein content maximum over a storage interval of 02 months (60 days) in treatments (T_7 and T_6) as 16.52 ± 0.18 and 16.27 ± 0.18 % which reduced to 16.44 ± 0.19 and 16.17 ± 0.18 %, correspondingly.

Table 6. Treatments and storage influence on protein content (%) of food bars.

Treatments	Days					Means ±SD
	0	15	30	45	60	
T_0	$7.20±0.00^{o}$	$7.22±0.01^{o}$	$7.17±0.01^{o}$	$6.94±0.01^{o}$	$6.90±0.00^{o}$	$7.08±0.04^{G}$
T_1	$7.23±0.01^{o}$	$7.18±0.01^{o}$	$7.16±0.01^{o}$	$6.89±0.01^{o}$	$6.92±0.01^{o}$	$7.08±0.04^{G}$
T_2	$15.53±0.09^{d-j}$	$15.45±0.22^{e-k}$	$15.60±0.09^{b-h}$	$15.55±0.09^{d-i}$	$15.38±0.13^{f-m}$	$15.50±0.05^{C}$
T_3	$15.59±0.19^{c-h}$	$15.39±0.16^{f-m}$	$15.42±0.09^{e-l}$	$15.42±0.19^{e-l}$	$15.37±0.16^{f-m}$	$15.44±0.06^{C}$
T_4	$16.03±0.10^{a-g}$	$15.81±0.19^{a-h}$	$15.83±0.15^{a-h}$	$15.81±0.13^{a-h}$	$15.82±0.17^{a-h}$	$15.86±0.06^{B}$
T_5	$13.04±0.16^{n}$	$13.06±0.13^{n}$	$12.93±0.18^{n}$	$12.97±0.17^{n}$	$12.97±0.27^{n}$	$12.99±0.07^{F}$
T_6	$16.27±0.18^{a-e}$	$16.31±0.13^{a-d}$	$16.32±0.16^{a-d}$	$16.25±0.15^{a-e}$	$16.17±0.18^{a-f}$	$16.26±0.06^{A}$
T_7	$16.52±0.18^{a}$	$16.46±0.19^{a}$	$16.46±0.18^{a}$	$16.41±0.22^{abc}$	$16.44±0.19^{ab}$	$16.46±0.07^{A}$
T_8	$14.73±0.13^{i-m}$	$14.62±0.17^{klm}$	$14.60±0.13l^{m}$	$14.59±0.15l^{m}$	$14.56±0.19^{m}$	$14.62±0.06^{E}$
T_9	$14.68±0.10k^{lm}$	$14.69±0.16^{j-m}$	$14.56±0.16^{m}$	$14.62±0.13^{klm}$	$14.57±0.13^{m}$	$14.62±0.05^{E}$
T_{10}	$15.24±0.12^{g-m}$	$15.20±0.16^{g-m}$	$15.10±0.12^{h-m}$	$15.06±0.18^{h-m}$	$15.04±0.10^{h-m}$	$15.13±0.06^{D}$
Means ±SD	$13.82±0.57^{A}$	$13.76±0.57^{A}$	$13.74±0.57^{A}$	$13.68±0.59^{A}$	$13.65±0.58^{A}$	

Means sharing similar letter in row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction Means and capital letters are used for overall Means.

Means ± SD, SD = Standard deviation

T_0 (100 g) = Placebo food bars = Milk powder, butter, sugar = (Zn = 0 mg)

T_1 (100 g) = Synthetic Zn fortified food bars = Milk powder, butter, sugar, $ZnSo_4.7\ H_2O$ = (Zn = 4mg)

T_2-T_{10} (100g) = Natural Zn fortified food bars = Milk powder, butter, sugar, $ZnSo_4.7\ H_2O$, pumpkin seeds, sesame seeds and oats = (Zn = 3.7-5.5mg).

Silva *et al.* (2012) researched on pumpkin seed bars with different modification with other ingredients like oats. These bars were composite of oats and pumpkin seed flour with different ratios.

Study revealed no significant changes in protein by days but significant in treatments.

Comparable outcomes were found by Gutkoski *et al.* (2006), who already worked on an oat-based cereal barhaving protein ratio of 11.43 g per 100 g. Estevez *et al.* (2000); Agrahari *et al.* (2004) developed and analyzed bar established an increase in the crude protein content.

Fiber content

In all the treatments crude fiber content fluctuated from 0.00 ± 0.003 to 6.66 ± 0.060 %. The crude fiber content trailed a significant intensification in all treatments but a non-significant impact was seen during storage period of 02 months (60 days).

There was no crude fiber content in treatments (T_0 and T_1) because in these treatments, the utilized ingredients were fiber less.

Oats, sesame and pumpkin seeds are the best source of fiber and used to enhance the crude fiber content into naturally Zn-fortified treatments.

Table 7. Treatments and storage influence on crude fiber (%) of food bars.

Treatments	Days					Means ±SD
	0	15	30	45	60	
T_0	0.01±0.003^{k}	0.01±0.003^{k}	0.01±0.003^{k}	0.01±0.003^{k}	0.01±0.003^{k}	0.01±0.001^{H}
T_1	0.00±0.003^{k}	0.00±0.003^{k}	0.00±0.003^{k}	0.00±0.003^{k}	0.00±0.003^{k}	0.00±0.001^{H}
T_2	4.90±0.042^{j}	4.90±0.042^{j}	4.92±0.040^{j}	4.95±0.040^{j}	4.97±0.274^{j}	4.93±0.049^{G}
T_3	5.60±0.051^{hi}	5.60±0.051h^{i}	5.62±0.054^{ghi}	5.64±0.060^{fgh}	5.66±0.067^{e-h}	5.62±0.023^{E}
T_4	5.15±0.071^{j}	5.15±0.071^{j}	5.18±0.073^{j}	5.20±0.077^{ij}	5.22±0.074^{ij}	5.18±0.029^{F}
T_5	6.12±0.066^{bc}d	6.12±0.066^{bcd}	6.14±0.064^{bcd}	6.16±0.061^{bcd}	6.18±0.152^{bc}	6.14±0.034^{C}
T_6	5.74±0.046^{d-h}	5.74±0.046^{d-h}	5.76±0.049^{c-h}	5.79±0.048^{c-h}	5.82±0.055^{c-h}	5.77±0.020^{D}
T_7	6.36±0.028^{ab}	6.36±0.028^{ab}	6.39±0.033^{ab}	6.41±0.038^{ab}	6.43±0.037^{ab}	6.39±0.015^{B}
T_8	5.03±0.013^{j}	5.03±0.013^{j}	5.05±0.013^{j}	5.07±0.045^{j}	5.09±0.013^{j}	5.06±0.010^{FG}
T_9	6.01±0.072^{b-h}	6.01±0.072^{b-h}	6.04±0.076^{b-g}	6.06±0.239^{b-f}	6.07±0.075^{b-e}	6.04±0.048^{C}
T_{10}	6.66±0.060^{a}	6.66±0.060^{a}	6.68±0.065^{a}	6.71±0.068^{a}	6.73±0.060^{a}	6.69±0.025^{A}
Means ±SD	4.69±0.401^{A}	4.69±0.401^{A}	4.71±0.403^{A}	4.73±0.405^{A}	4.74±0.407^{A}	

Means sharing similar letter in row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction Means and capital letters are used for overall Means.

Means ± SD, SD = Standard deviation

T_0 (100 g) = Placebo food bars = Milk powder, butter, sugar = (Zn = 0 mg)

T_1 (100 g) = Synthetic Zn fortified food bars = Milk powder, butter, sugar, $ZnSo_4.7\ H_2O$ = (Zn = 4mg)

T1-T10 (100g) = Natural Zn fortified food bars = Milk powder, butter, sugar, $ZnSo_4.7\ H_2O$, pumpkin seeds, sesame seeds and oats = (Zn = 3.7-5.5mg).

Table 8. Treatments and storage Influence on ash content (%) of food bars.

Treatments	Days					Means ±SD
	0	15	30	45	60	
T_0	2.05±0.007^{o}	2.05±0.007^{o}	2.06±0.007^{o}	2.07±0.009^{o}	2.07±0.011^{o}	2.06±0.004^{I}
T_1	2.07±0.011^{o}	2.07±0.011^{o}	2.08±0.012^{o}	2.09±0.013^{o}	2.10±0.014^{o}	2.08±0.006^{I}
T_2	3.55±0.023^{gh}	3.55±0.023^{gh}	3.57±0.024^{fgh}	3.58±0.024^{fgh}	3.59±0.025^{e-h}	3.57±0.010^{D}
T_3	3.04±0.016^{n}	3.04±0.016^{n}	3.05±0.012^{mn}	3.06±0.015^{mn}	3.08±0.012l^{mn}	3.06±0.007^{H}
T_4	3.60±0.027^{d-h}	3.60±0.027^{d-h}	3.62±0.026^{c-g}	3.63±0.029^{c-g}	3.64±0.024^{c-g}	3.62±0.011^{C}
T_5	3.24±0.018^{jk}	3.24±0.018^{jk}	3.25±0.021^{ijk}	3.27±0.020^{ijk}	3.27±0.020^{ijk}	3.25±0.008^{F}
T_6	3.68±0.022^{c-f}	3.68±0.022^{c-f}	3.71±0.021^{cde}	3.72±0.019^{cd}	3.74±0.022^{bc}	3.71±0.010^{B}
T_7	3.85±0.027^{ab}	3.85±0.027^{ab}	3.86±0.028^{a}	3.87±0.027^{a}	3.89±0.024^{a}	3.86±0.011^{A}
T_8	3.15±0.021^{k-n}	3.15±0.021^{k-n}	3.16±0.021^{k-n}	3.17±0.021^{klm}	3.19±0.021^{kl}	3.16±0.009^{G}
T_9	3.33±0.016^{ij}	3.33±0.016^{ij}	3.34±0.014^{ij}	3.35±0.017^{ij}	3.37±0.015^{i}	3.34±0.007^{E}
T_{10}	3.53±0.018^{gh}	3.53±0.018^{gh}	3.54±0.020^{gh}	3.49±0.051^{h}	3.57±0.020^{fgh}	3.53±0.013^{D}
Means ±SD	3.19±0.103^{A}	3.19±0.103^{A}	3.20±0.103^{A}	3.21±0.103^{A}	3.23±0.104^{A}	

Means sharing similar letter in row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction Means and capital letters are used for overall Means.

Means ± SD, SD = Standard deviation

T_0 (100 g) = Placebo food bars = Milk powder, butter, sugar = (Zn = 0 mg)

T_1 (100 g) = Synthetic Zn fortified food bars = Milk powder, butter, sugar, $ZnSo_4.7\ H_2O$ = (Zn = 4mg)

T1-T10 (100g) = Natural Zn fortified food bars = Milk powder, butter, sugar, $ZnSo_4.7\ H_2O$, pumpkin seeds, sesame seeds and oats = (Zn = 3.7-5.5mg).

Except treatments (T_0 and T_1),a minimum crude fiber content was present in treatments (T_2 and T_4) that was augmented from 4.90 ± 0.042 to 4.97 ± 0.274 % and 5.15 ± 0.071 to 5.22 ± 0.074 %. While crude fiber content was supreme in treatments (T_{10} and T_7) i.e. 6.66 ± 0.060 and 6.36 ± 0.028 % that was augmented over a storage period of 02 months (60 days) to 6.73 ± 0.060 and 6.43 ± 0.037 %, correspondingly as exposed in Table 7.

Table 9. Treatments and storage Influence on NFE content (%) of food bars.

Treatments	Days					Means ±SD
	0	15	30	45	60	
T$_0$	68.26±1.207^{a}	68.26±1.207^{a}	68.32±1.215^{a}	68.39±1.217^{a}	68.46±1.202^{a}	68.34±0.458^{A}
T$_1$	68.41±0.950^{a}	68.41±0.950^{a}	68.47±0.963^{a}	68.54±0.977^{a}	68.62±0.958^{a}	68.49±0.363^{A}
T$_2$	44.14±0.918^{bc}	44.14±0.918^{bc}	44.18±0.915^{bc}	44.21±0.919^{bc}	44.29±0.916^{bc}	44.19±0.347^{BC}
T$_3$	42.73±1.051^{bc}	42.73±1.051^{bcd}	42.79±1.061^{bcd}	42.83±1.081^{bcd}	42.90±1.086^{bcd}	42.80±0.403^{C}
T$_4$	42.48±0.994^{bc}	42.48±0.994^{bcd}	42.52±1.002^{bcd}	42.55±1.001^{bcd}	42.58±1.012^{bcd}	42.52±0.378^{C}
T$_5$	46.27±1.008b	46.27±1.008^{b}	46.32±0.996^{b}	46.36±1.005^{b}	46.42±1.013^{b}	46.33±0.380^{B}
T$_6$	40.10±0.992^{bd}	40.10±0.992^{bcd}	40.15±0.995^{bcd}	40.18±0.997^{bcd}	40.24±1.001^{bcd}	40.15±0.376^{D}
T$_7$	37.37±1.380^{d}	37.37±1.380^{d}	37.40±1.392^{d}	37.44±1.384^{d}	37.48±1.386^{d}	37.41±0.523^{E}
T$_8$	45.72±1.430^{b}	45.72±1.430^{b}	45.75±1.426^{b}	45.81±1.444^{b}	45.86±1.433^{b}	45.77±0.542^{B}
T$_9$	43.09±0.912^{bd}	43.09±0.912^{bcd}	43.13±0.924^{bcd}	43.16±0.930^{bcd}	42.53±0.664^{bcd}	43.00±0.336^{C}
T$_{10}$	38.31±1.244^{cd}	38.31±1.244^{cd}	38.34±1.250^{cd}	38.37±1.265^{cd}	38.40±1.275^{cd}	38.35±0.475^{DE}
Means ±SD	46.99±1.860^{A}	46.99±1.860^{A}	47.03±1.862^{A}	47.08±1.864^{A}	47.07±1.870^{A}	

Means sharing similar letter in row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction Means and capital letters are used for overall Means.

Means ± SD, SD = Standard deviation

T$_0$ (100 g) = Placebo food bars = Milk powder, butter, sugar = (Zn = 0 mg)

T$_1$ (100 g) = Synthetic Zn fortified food bars = Milk powder, butter, sugar, $ZnSo_4.7\ H_2O$ = (Zn = 4mg)

T1-T10 (100g) = Natural Zn fortified food bars = Milk powder, butter, sugar, $ZnSo_4.7\ H_2O$, pumpkin seeds, sesame seeds and oats = (Zn = 3.7-5.5mg.

Sotiles *et al.* (2017) prepared and evaluated the oat bars with pumpkin seeds and proved an augmentation in crude fiber content by an addition of green banana flour. Similarly, Damsceno *et al.* (2016) made and evaluated cereal bars and added pineapple peel flour that augmented the crude fiber content fiber in bars. Outcomes were confirmed by the study showed by Maurer *et al.* (2005) in which a cumulative trend in crude fiber content of granola bars was noticeable when supplemented with red and black beans.

Table 10. Treatments and storage influence on gross energy content (Kcals) of food bars.

Treatments	Days					Means ±SD
	0	15	30	45	60	
T$_0$	449.56±4.01^{bcd}	449.65±3.47^{bcd}	449.39±2.86^{bcd}	448.98±2.81^{bcd}	443.35±4.86^{cd}	448.18±1.54^{D}
T$_1$	451.82±3.41^{a-d}	450.16±3.11^{bcd}	448.34±3.32^{bcd}	447.43±3.42^{bcd}	439.99±6.34^{d}	447.55±1.89^{D}
T$_2$	456.87±3.15^{a-d}	454.01±5.09^{a-d}	451.83±3.42^{a-d}	448.75±4.58^{bcd}	446.57±4.19^{bcd}	451.60±1.85^{CD}
T$_3$	458.06±3.10^{a-d}	456.97±2.89^{a-d}	457.14±4.25^{a-d}	457.40±2.70^{a-d}	474.41±3.16^{a}	460.80±2.20^{AB}
T$_4$	453.87±3.95^{a-d}	453.53±2.87^{a-d}	452.18±3.16^{a-d}	451.51±3.17^{a-d}	466.36±2.63^{abc}	455.49±1.90^{BCD}
T$_5$	450.85±3.03^{a-d}	448.80±3.54^{bcd}	447.19±3.29^{bcd}	447.11±5.21^{bcd}	445.59±5.77^{bcd}	447.91±1.70^{D}
T$_6$	462.76±2.87^{a-d}	460.81±3.39^{a-d}	458.08±3.71^{a-d}	458.24±2.70^{a-d}	455.52±6.07^{a-d}	459.08±1.63^{ABC}
T$_7$	467.61±4.99^{ab}	467.08±4.86^{ab}	464.63±2.90^{abc}	464.16±3.52^{abc}	461.32±6.75^{a-d}	464.96±1.91^{A}
T$_8$	454.01±5.34^{a-d}	452.34±3.87^{a-d}	451.80±4.80^{a-d}	450.01±3.20^{bcd}	447.34±6.32^{bcd}	451.10±1.92^{CD}
T$_9$	454.34±3.62^{a-d}	455.12±3.96^{a-d}	455.26±3.09^{a-d}	453.55±2.35^{a-d}	450.58±3.70^{bcd}	453.77±1.36^{BCD}
T$_{10}$	458.29±4.95^{a-d}	457.74±4.53^{a-d}	457.22±3.77^{a-d}	456.61±5.44^{a-d}	455.47±2.74^{a-d}	457.07±1.68^{ABC}
Means ±SD	456.18±1.34^{A}	455.11±1.32^{A}	453.91±1.24^{A}	453.07±1.30^{A}	453.32±2.17^{A}	

Means sharing similar letter in row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction Means and capital letters are used for overall Means.

Means ± SD, SD = Standard deviation

T$_0$ (100 g) = Placebo food bars = Milk powder, butter, sugar = (Zn = 0 mg)

T$_1$ (100 g) = Synthetic Zn fortified food bars = Milk powder, butter, sugar, $ZnSo_4.7\ H_2O$ = (Zn = 4mg)

T1-T10 (100g) = Natural Zn fortified food bars = Milk powder, butter, sugar, $ZnSo_4.7\ H_2O$, pumpkin seeds, sesame seeds and oats = (Zn = 3.7-5.5mg)

Ash content

Primarily, in all the treatments ash content fluctuated from 2.05 ± 0.007 to 3.85 ± 0.027 %. The ash content trailed a significant intensification in all treatments but a non-significant impact was seen during storage period of 60 days. Ash content was minimum in treatments (T_0 and T_1) that augmented from 2.05 ± 0.007 to 2.07 ± 0.013 % and 2.07 ± 0.011 to 2.10 ± 0.014 %. While, ash content was maximum in treatments (T_7 and T_6) as 3.85 ± 0.018 and 3.68 ± 0.022 % that augmented over a storage period of 02 months (60 days) to 3.89 ± 0.02 and 3.74 ± 0.022 %, correspondingly as exposed in Table 8. Because of an addition of natural dried fortificants oat flour, pumpkin and sesame seeds, ash content may had been high.

Table 11. Treatments and storage Influence on TPC (cfu/g) of food bars.

Treatments	Days					Means ±SD
	0	15	30	45	60	
T_0	1.134±0.019^{xyz}	1.253±0.014^{uv}	1.502±0.008^{pqr}	1.704±0.005^{i-l}	2.032±0.002^{abc}	1.525±0.086^{CD}
T_1	1.143±0.021^{wxy}	1.260±0.014^{uv}	1.447±0.009^{rs}	1.712±0.005^{i-l}	1.969±0.003^{bcd}	1.506±0.081^{CDE}
T_2	1.037±0.026^{a}	1.325±0.008^{tu}	1.641±0.006^{k-n}	1.793±0.004^{f-i}	1.999±0.003^{bc}	1.559±0.091^{B}
T_3	1.051±0.025^{za}	1.156±0.019^{wxy}	1.544±0.007^{opq}	1.722±0.005^{h-k}	1.956±0.003^{cd}	1.486±0.091^{E}
T_4	0.851±0.052^{b}	1.160±0.018^{wxy}	1.587±0.007^{m-p}	1.823±0.004^{efg}	2.049±0.002^{ab}	1.494±0.117^{DE}
T_5	1.152±0.019^{wxy}	1.289±0.013^{tuv}	1.676±0.005^{j-m}	1.895±0.003^{de}	2.124±0.002^{a}	1.627±0.097^{A}
T_6	0.520±0.043^{c}	0.834±0.039^{b}	1.476±0.008^{qr}	1.674±0.005^{j-m}	2.050±0.002^{ab}	1.311±0.150^{F}
T_7	1.017±0.027^{a}	1.255±0.015^{uv}	1.626±0.006^{l-o}	1.746±0.005^{g-j}	2.046±0.002^{abc}	1.538±0.097^{BC}
T_8	1.141±0.020^{w-z}	1.357±0.011st	1.664±0.006^{j-m}	1.882±0.003^{def}	2.113±0.002^{a}	1.631±0.094^{A}
T_9	1.232±0.018^{vw}	1.376±0.011st	1.697±0.005^{jkl}	1.897±0.003^{de}	2.014±0.003^{bc}	1.643±0.080^{A}
T_{10}	1.079±0.023^{yza}	1.215±0.016^{vwx}	1.568±0.007^{nop}	1.807±0.004^{e-h}	1.966±0.003^{bcd}	1.527±0.090^{BC}
Means ±SD	1.032±0.034^{E}	1.225±0.025^{D}	1.584±0.014^{C}	1.787±0.014^{B}	2.029±0.009^{A}	

Means sharing similar letter in row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction Means and capital letters are used for overall Means.

Means ± SD, SD = Standard deviation

T_0 (100 g) = Placebo food bars = Milk powder, butter, sugar = (Zn = 0 mg)

T_1 (100 g) = Synthetic Zn fortified food bars = Milk powder, butter, sugar, $ZnSo_4.7\ H_2O$ = (Zn = 4mg)

T1-T10 (100g) = Natural Zn fortified food bars = Milk powder, butter, sugar, $ZnSo_4.7\ H_2O$, pumpkin seeds, sesame seeds and oats = (Zn = 3.7-5.5mg).

Aigsteret *al.* (2011) also revealed the similar findings. Outcomes of this study were in close resemblance with the results of Munir *et al.* (2016).

They presented a non-significant increase during a storage of 60 days in an ash content of fortified bar prepared with char maghaz and whey powder. Nadeem *et al.* (2018) reported a non-significant difference in ash content was noticed during storage period. Values of this study were highest as compared to the study conducted (Garcia *et al.*, 1998).

NFE content

Through study, it was detected that, there was a non-significant change in NFE of food bars during storage period but it was highly significant in treatments of food bars. In all the treatments, NFE varied from 37.37 ± 1.380 to 68.26 ± 1.207 %. NFE trailed a significant intensification in all treatments during storage period of 02 months (60 days). Minimum NFE was present in treatments (T_7 and T_{10}) that was augmented from 37.37 ± 1.380 to 37.44 ± 1.384 % and 38.31 ± 1.244 to 38.37 ± 1.256 %. While NFE was extreme in treatments (T_3 and T_5) as 42.73 ± 1.051 and 46.27 ± 1.008 % that was improved over a storage period 60 days to 642.83 ± 1.081 and 46.36 ± 1.005 % as portrayed in Table 9.

Garcia *et al.* (1998) found difference in NFE that was fluctuated among 67.37 and 72.11%. Nadeem *et al.* (2018) specified a non-significant intensification of NFE in bars during storage period. It was fluctuated from 86.14 ± 0.07 to 86.15 ± 0.07.Mourão*et al.* (2009) shown resemblance with these studies. Munir *et al.* (2016) stated that, storage had no influences upon NFE protein fortified fruit bar. Mridula *et al.* (2011) revealed flax seed product showed a slightly increased but statistically non-significant NFE.

Gross energy

Through study, it was detected that, there was a non-significant change in gross energy of food bars during storage period but it was highly significant in treatments of food. All the treatments gross energy fluctuated from 467.61 ± 4.99 to 449.56 ± 4.01 Kcal. Gross energy trailed a significant intensification in all treatments during storage period of 02 months (60 days).

Minimum gross energy was present in treatments T_0 that was augmented from 449.56 ± 4.01 to 443.35 ± 4.86 Kcal. While gross energy was extreme in treatments T_7 as 467.61 ± 4.99 to 461.32 ± 15.09 and 383.85 ± 0.65 Kcal, correspondingly. Gross energy in treatment (T_1) was augmented from 334.39 ± 0.56 to 335.03 ± 0.348 Kcal as portrayed in Table 10.

Table 12. Treatments and storage Influence on mold count (cfu/g) of food bars.

Treatments	Days					Means ±SD
	0	15	30	45	60	
T_0	$0.783{\pm}0.047^{y}$	$1.016{\pm}0.025^{r\text{-}u}$	$1.154{\pm}0.018^{opq}$	$1.406{\pm}0.010^{g\text{-}j}$	$1.583{\pm}0.007^{bcd}$	$1.188{\pm}0.076^{F}$
T_1	$0.918{\pm}0.032^{u\text{-}x}$	$1.008{\pm}0.024^{stu}$	$1.234{\pm}0.015^{m\text{-}p}$	$1.438{\pm}0.009^{f\text{-}j}$	$1.611{\pm}0.006^{a\text{-}d}$	$1.242{\pm}0.070^{CDE}$
T_2	$0.737{\pm}0.051^{yz}$	$0.842{\pm}0.043^{v\text{-}y}$	$1.121{\pm}0.020^{p\text{-}s}$	$1.392{\pm}0.010^{g\text{-}k}$	$1.575{\pm}0.007^{b\text{-}e}$	$1.133{\pm}0.086^{G}$
T_3	$0.945{\pm}0.031^{t\text{-}w}$	$1.064{\pm}0.023^{q\text{-}t}$	$1.230{\pm}0.015^{m\text{-}p}$	$1.424{\pm}0.010^{g\text{-}j}$	$1.596{\pm}0.006^{a\text{-}d}$	$1.252{\pm}0.063^{CD}$
T_4	$0.644{\pm}0.015^{z}$	$0.816{\pm}0.045^{w\text{-}y}$	$1.154{\pm}0.019^{opq}$	$1.353{\pm}0.012^{i\text{-}m}$	$1.566{\pm}0.007^{c\text{-}f}$	$1.107{\pm}0.091^{G}$
T_5	$0.737{\pm}0.051^{yz}$	$0.995{\pm}0.027^{stu}$	$1.242{\pm}0.015^{l\text{-}p}$	$1.443{\pm}0.009^{e\text{-}i}$	$1.580{\pm}0.007^{bcd}$	$1.199{\pm}0.082^{EF}$
T_6	$0.795{\pm}0.028^{xy}$	$0.962{\pm}0.030^{tuv}$	$1.305{\pm}0.013^{j\text{-}n}$	$1.487{\pm}0.008^{d\text{-}h}$	$1.606{\pm}0.006^{a\text{-}d}$	$1.231{\pm}0.083^{DEF}$
T_7	$0.918{\pm}0.032^{u\text{-}x}$	$0.934{\pm}0.034^{t\text{-}w}$	$1.317{\pm}0.012^{i\text{-}n}$	$1.496{\pm}0.008^{d\text{-}h}$	$1.615{\pm}0.006^{a\text{-}d}$	$1.256{\pm}0.077^{CD}$
T_8	$0.995{\pm}0.026^{stu}$	$1.143{\pm}0.019^{o\text{-}r}$	$1.266{\pm}0.014^{k\text{-}o}$	$1.503{\pm}0.008^{d\text{-}h}$	$1.681{\pm}0.005^{abc}$	$1.318{\pm}0.066^{B}$
T_9	$1.022{\pm}0.026^{q\text{-}u}$	$1.213{\pm}0.016^{nop}$	$1.374{\pm}0.011^{h\text{-}l}$	$1.510{\pm}0.008^{d\text{-}g}$	$1.706{\pm}0.003^{ab}$	$1.365{\pm}0.063^{A}$
T_{10}	$0.783{\pm}0.047^{y}$	$1.054{\pm}0.023^{q\text{-}t}$	$1.321{\pm}0.012^{i\text{-}n}$	$1.519{\pm}0.008^{d\text{-}g}$	$1.718{\pm}0.005^{a}$	$1.279{\pm}0.089^{BC}$
Means ±SD	$0.843{\pm}0.022^{E}$	$1.004{\pm}0.021^{D}$	$1.247{\pm}0.014^{C}$	$1.452{\pm}0.010^{B}$	$1.621{\pm}0.009^{A}$	

Means sharing similar letter in row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction Means and capital letters are used for overall Means.

Means ± SD, SD = Standard deviation

T_0 (100 g) = Placebo food bars = Milk powder, butter, sugar = (Zn = 0 mg)

T_1 (100 g) = Synthetic Zn fortified food bars = Milk powder, butter, sugar, $ZnSo_4.7\ H_2O$ = (Zn = 4mg)

T1-T10 (100g) = Natural Zn fortified food bars = Milk powder, butter, sugar, $ZnSo_4.7\ H_2O$, pumpkin seeds, sesame seeds and oats = (Zn = 3.7-5.5mg).

Padmashree (2012) explained cereal bar provides 484 Kcal of energy/100 g bar, which is computed. The pumpkin seeds (12014) have 559 KCal/ 100 g explained by USDA (2014).

Outcomes of this study was in close resemblance with the study conducted by Munir *et al.* (2016). In that study protein, bars were made and it was claimed that, gross energy altered non-significantly through a storage period of 02 months. There is a close resemblance in results of calories value change with the outcomes of (Shaheen *et al.*2013).

Total plate count and mold growth

Through study, it was detected that, there was a highly significant change in total plate count (TPC) and mold growth of food bars during storage period in all the treatments. Initially, the total plate count

(TPC) of all the treatments of food bars fluctuated from 0.520 ± 0.043 to 1.232 ± 0.018 cfu/ g. A significant increase in total plate count (TPC) of all the treatments throughout the storage study of 02 months (60 days) was happened. In treatments (T_6 and T_4), a lowest value of total plate count (TPC) was found which increased from 0.520 ± 0.043 to 2.050 ± 0.002 and 0.851 ± 0.052 to 2.049 ± 0.002 cfu/ g. Treatments (T_9 and T_5) have the highest value of total plate count (TPC) which was increased from 1.232 ± 0.018 to 2.041 ± 0.003 and 1.152 ± 0.019 to 2.124 ± 0.002 cfu/ g as showed in Table 11.

Table 13. Treatments and storage Influence on Zn content (mg) of food bars.

Treatments	Days					Means ±SD
	0	15	30	45	60	
T_0	0.49±0.045^b	0.49±0.045^b	0.49±0.045^b	0.49±0.045^b	0.49±0.045^b	0.49±0.017^E
T_1	4.10±0.131^a	4.10±0.131^a	4.09±0.129^a	4.07±0.126^a	3.99±0.062^a	4.07±0.046^{CD}
T_2	3.76±0.125^a	3.76±0.125^a	3.74±0.126^a	3.73±0.125^a	3.72±0.121^a	3.74±0.047^D
T_3	3.98±0.215^a	3.98±0.215^a	3.96±0.217^a	3.95±0.218^a	3.94±0.221^a	3.96±0.082^D
T_4	3.88±0.310^a	3.88±0.310^a	3.87±0.315^a	3.86±0.317^a	3.84±0.314^a	3.87±0.119^D
T_5	3.98±0.325^a	3.98±0.325^a	3.96±0.324^a	3.95±0.323^a	3.94±0.321^a	3.96±0.122^D
T_6	3.97±0.144^a	3.97±0.144^a	3.96±0.143^a	3.94±0.143^a	3.93±0.144^a	3.95±0.054^D
T_7	4.57±0.287^a	4.57±0.287^a	4.56±0.286^a	4.54±0.284^a	4.52±0.280^a	4.55±0.108B^C
T_8	4.21±0.364^a	4.21±0.364^a	4.20±0.365^a	4.18±0.366^a	4.17±0.363^a	4.20±0.138^{CD}
T_9	4.73±0.245^a	4.73±0.245^a	4.72±0.246^a	4.70±0.243^a	4.68±0.239^a	4.71±0.092^{AB}
T_{10}	5.14±0.272^a	5.14±0.272^a	5.12±0.269^a	5.11±0.261^a	5.10±0.259^a	5.12±0.101^A
Means ±SD	3.89±0.212^A	3.89±0.212^A	3.88±0.211^A	3.87±0.210^A	3.85±0.209^A	

Means sharing similar letter in row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction Means and capital letters are used for overall Means.

Means ± SD, SD = Standard deviation

T_0 (100 g) = Placebo food bars = Milk powder, butter, sugar = (Zn = 0 mg)

T_1 (100 g) = Synthetic Zn fortified food bars = Milk powder, butter, sugar, $ZnSo_4.7\ H_2O$ = (Zn = 4mg)

T1-T10 (100g) = Indigenous Zn fortified food bars = Milk powder, butter, sugar, $ZnSo_4.7\ H_2O$, pumpkin seeds, sesame seeds and oats = (Zn = 3.7-5.5mg).

Outcomes of this study are in close resemblance with the results of study conducted by Ainiet *al.* (2018). They noticed a significant intensification throughout a storage period. Liu *et al.* (2009) explicated the same trend in results. Al-Hooti *et al.* (1997) noticed a significant intensification in total plate count from 1.00 – 2.18 Log 10 cfu / g was observed but within same range.

Initially, the mold growth of all the treatments of food bars fluctuated from 0.644 ± 0.015 to 1.022 ± 0.026 cfu/ g. A significant increase in mold growth of all the treatments throughout the storage study of 02 months (60 days) was happened. In treatments (T_4 and T_5) a lowest value of mold growth was found which increased from 0.644 ± 0.015 to 1.566 ± 0.007 and 0.737 ± 0.051 to 1.580 ± 0.007 cfu/ g. Treatments (T_9 and T_8)) have the highest value of mold growth which was increased from 1.022 ± 0.026 to 1.706 ± 0.003 and 0.995 ± 0.026 to 1.681 ± 0.005 cfu/ g as showed in Table 12.

Silva *et al.* (2012) prepared cassava flour bar supplemented with nuts and dried fruits showed the significant microbial study within safe range in storage period of 6 months. Rehman *et al.* (2012) showed the similarity with the study. Chen *et al.* (2010), described that a significant microbial growth beyond safe range have ability to change in sensory and nutritional properties. Jan *et al.* (2012) found that this value indicates a microbial stability of the bars.

Zn-content analyses during storage

The zinc (Zn) content of all treatments of food bars fluctuated from 0.49 ± 0.045 to 5.14 ± 0.272 mg. A significant decrease in zinc content of all the treatments but a non-significant change occurred throughout the storage study of 60 days was happened. In treatments (T_0 and T_2), a lowest value of zinc content was found which decreased from 0.49 ± 0.045 to 0.47 ± 0.045 and 3.76 ± 0.125 to 3.72 ± 0.121 mg. Treatments (T_{10} and T_9) have the highest value of zinc content which was reduced from 5.14 ± 0.272 to 5.10 ± 0.259 and 4.73 ± 0.254 to 4.68 ± 0.239 mg.

In treatment (T_7) level of zinc content was decreased from 4.57 ± 0.287 to 4.52 ± 0.280 mg. While a reduction from 3.98 ± 0.325 to 3.94 ± 0.321 mg was observed in treatment (T5) as showed in Table 13.

Hemery *et al.* (2018) originated that the levels of zinc (Zn) and iron (Fe) were changed insignificantly throughout the 180 days storage duration that good packing and handling practices might be the key sources. Abdulghani *et al.* (2015) researched on fortified UHT milk. Milk was fortified with zinc, magnesium and iron.

The fortified milks were analyzed. Research concluded that UHT milk supplemented with zinc and magnesium had no significant changed. Pilon *et al.* (2006) stated chemical configuration of vegetables remained constant. All treatments exhibited an insignificant change in zinc content throughout a storage period in slightly processed carrots as well as pepper.

Conclusion

In all treatments, T_7 had the maximum ash content and sensory scores among all treatments as well as it gave the 50 % RDA of zinc thus selected as T_2 (natural fortified bar). Microbial analysis based upon the storage study of 60 days, showed the safe use of the food bar.

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