



RESEARCH PAPER

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Preparation and nutritional evaluation of protein enriched composite cereal bar

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Key words: Cereal bar, protein enriched, proximate, phytochemical, antioxidant activity.

<http://dx.doi.org/10.12692/ijb/13.5.278-292>

Article published on November 18, 2018

Abstract

Protein enriched cereal bar is made in order to keep in mind today's life style, as people are more fond of junk food. Food consumption is just one of the multiple factors which have an impact on the nutritional status of the overall population. A primary contributor to obesity is the increase in unhealthy eating habits. Ready to eat Protein bar is made to combat unhealthiest junk food. Nutritional composition of protein enriched cereal bar is pronounced to be a good source of protein and minerals. A protein enriched composite cereal bar composed in combination of healthful ingredients, all these ingredients are readily available and affordable. The storage stability of protein enriched composite cereal bar will include complete proximate analysis (crude protein, crude fiber, crude fat, ash content) and their antioxidant capacity (Vitamin C, total phenolic content, total flavonoid content, radical scavenger activity, DPPH) due to their bioactive compounds.

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Introduction

(Dhumal *et al.*, 2014) Ready to use, fast cooking and instant foods have become very common largely due to today's fast life style and the demand for swift serving. Basically this study aim is to put together different beneficial ingredients in appropriate quantity to get better texture, mouth feel and impart the organoleptic qualities through the use of appropriate process and technology at a cost that is acceptable for consumer and for readily available high protein food in market for people who can't afford high protein food especially in Pakistan.

(Rawat and Darappa, 2015) Bars are ideal in terms of nutrient accessibility because of compactness, palatability, and ease. Bars are comparatively free from microbial spoilage, have longer shelf life because of low moisture content. Such ready to eat products are easy to utilize functional nutrients. All these reasons further motivated the development of ready to eat food industry. Cereal bars are the good source of energy, rich in fiber, protein, vitamins, antioxidants an instant food that is highly nutritious. The bars are often fortified using a wide range of proteins, fibers or energy-rich ingredients and all the bars focused on two factors one is health and convenience available in market.

Food consumption is one of the key factors which can impact the nutritional status of the overall population. The purpose of preparing nutritional bar is to satisfy the daily energy requirement of a person and to evaluate its nutritional value and effects. This protein enriched cereal bar is composed of nine topnotch nutritious ingredients, honey, almonds, oats, sesame seeds, chia seeds, puffed millet, black seeds, organic coconut oil, and brown sugar, which are non GMO and gluten free. All these ingredients are readily accessible and affordable. The main objectives of this study are to

- Develop ready to eat protein enriched rich composite cereal bar with balanced nutrients.
- Assess chemical parameters and sensory attributes during storage.
- Develop cost effective bar for local market.

Material and methods

The research was conducted in the Food Analysis Laboratory in Department of Food Technology, Balochistan Agriculture College and NARC Islamabad from January 2017 to April 2017.

Preliminary Protein bar was prepared with three different ratios of oat and millet such as 40:60, 75:25 and 50:50. Bar with ratio of 40:60 was selected by a panel of 6 judges for organoleptic evaluation during storage. The selected ratio of protein bar were further evaluated for their proximate analysis and phytochemical analysis during storage at room temperature on fortnightly basis.

Preparatory operations and product development

Collection of Ingredients

Protein enriched composite cereal bar was prepared by the healthfull ingredients that was millet (120 gm), oat (150 gm), black cumin seeds (16 gm), sesame seed (20 gm), peanut (125 gm), almond (142 gm) and syrup was prepared with brown sugar (72 gm) and butter (108 gm) respectively. All these ingredients were purchased from reputed superstore of Rawalpindi/Islamabad.

Puffing of millet

Millet was first converted into powder form by help of grinder machine (BRUAN, Saudi Arabia) and then it was roasted in a fry pan at low heat for 7 to 10 min.

Preparation of binder

Syrup was used as a binding agent for enriched bars. Syrup was prepared as: butter (108 gm) was added in nonstick fry pan (Tefal, Saudia Arabia) and heated at low flame. After melting, brown sugar (72 gm) was added and mixed thoroughly untill the solution (called as syrup) start thickening.

Preparation of bar

In a mixing bowl puffed millet was mix with grinded oats, crushed peanut and almonds, and with seasonings sesame seeds, black cumin seeds, then syrup was added slowly in a bowl contains dry ingredients and mixed well after that spread the

whole mixture in a tray and let it stand until it hardens, then cutted in a bar shape slices with help of slicer. The prepared bars were stored for 45 days at ambient temperature (15-34°C) in high density polyethylene bags and the samples were tested for physiochemical and sensory attributes on fortnightly basis (Padmashree *et al.*, 2012).

Proximate analysis of prepared bar at ambient temperature

Moisture Content

The moisture content of each sample was determined by drying the sample in an oven at a temperature of 105°C up to constant weight according to AACC (2000) Method No. 44-19. Weigh the empty crucible dish then add 10gm sample in it and place crucible dish contained sample in desiccator and set oven at 105°C, as required temperature retain place sample containing crucible in oven for 16 hours after then weighed the sample and applied following formula to calculate moisture content.

$$\text{Moisture (\%)} = \frac{W_1 - W_2 \times 100}{W_1}$$

W₁ = weight of sample before drying W₂ = weight of sample after drying.

Crude protein

Crude protein content of each protein bar sample was determined by Kjeldhal's method according to AACC (2000) Method No. 46-10. Weigh approximately 1 gm of sample containing protein and placing a sample in digestion flask along with 20 ml of concentrated H₂SO₄ then add 5gm. of digestion mixture of potassium sulphate, copper sulphate 7%, and iron sulphate 3%. Bringing the digestion mixture to boiling at 150°C about 2 to 3 hrs. then after few hours green color appeared that showed digestion was complete. Then digested mixture dilute with 100 ml of distilled water volume makeup. Then took 10 ml of diluted solution and distillate it with 40% of NaOH and boric acid 3% having one drop of brome methyl red as indicator and solution color changes into pink color. After distillation solution is titrated with 0.1 N H₂SO₄ now note the reading as pink color again

shows then note the reading.

$$\text{Nitrogen (\%)} = \frac{A \times B \times 0.0014 \times 100}{C \times W}$$

A = Volume of acid used in sample titration

B = Volume of sample solution

C = Volume of diluted solution used for distillation

W = Weight of sample.

Crude fiber

The defatted and dried samples were taken for the determination of crude fiber content is to be digested with NaOH solution. As mentioned by AACC (2000) method No. 32-10.01. Weigh the 2gm of sample on weighing machine. Add the 2 gm of protein enriched bar sample in a beaker with 200 ml of H₂SO₄ (1.25%) for digesting the sample by heating for half an hour.

After heating the mixture was filtered and residue collected and add NaOH (1.25%) in residue and again heating of sample perform for 20 minutes after then filter again and collect residue in already weighed crucible and place for 90 minutes at 135°C in muffle furnace till the formation of white ash. Crude fiber percentage was calculated according to the formula given below:

$$\text{Crude fiber (\%)} = \frac{\text{Weight of dried fiber} - \text{Weight of residual ash} \times 100}{\text{Weight of sample}}$$

Crude fat

The fat percentage was determined by using solvent in Soxhlet apparatus according to method described in AACC (2000) method No.30-10. Briefly add 3 gm. of protein enriched bar sample in thimble and the covered the mouth of thimble with help of cotton wool. After that thimble was place in extraction chamber already weighed conical flask attached to soxhlet apparatus containing 250 ml solvent N-hexane as it is effective solvent for extraction. After attaching the condenser with soxhlet apparatus, flask heated at 60°C to 70°C. Continuously running water passes through the condenser to avoid evaporation. After complete extraction by passing solvent two to three times through sample .solvent was evaporated

and weight of flask contains solvent and extract was calculated. The % crude fat was determined by the following equation:

$$\text{Fat (\%)} = \frac{\text{Weight before extraction} - \text{Weight after extraction} \times 100}{\text{Weight of sample}}$$

Ash content

The ash content of each sample was determined by incinerating the dried sample in Muffle furnace as described in AACC (2000) method No. 08-01.3 gm of moisture free sample were taken in crucible i.e. already weighed .then crucible was directly place in muffle furnace at temperature of 600°C for 2 to 3 hrs, until grayish white residue obtained. The crucible was then place in desiccator to keep sample protected from environmental moisture. Following formula was applied to calculate the total ash content:

$$\text{Ash (\%)} = \frac{\text{Weight of crucible with ash} - \text{Weight of empty crucible} \times 100}{\text{Weight of sample}}$$

Phytochemical analysis of prepared bar at ambient temperature

Sample preparation

Briefly, 3 gm of protein enriched bar sample was taken and added in three reagent bottles and marked these bottles A, B and C. Then 60 ml of 80% ethanol is added in each bottle, and then place these bottles in sonicator at temperature of 50°C for one hr. After one hr. reagent bottles were taken out from sonicator and wait till temperature fall down. As temperature decreased perform filtration and collects supernatant in round bottom flask, attach this round bottom flask to rotary evaporator at 45°C and temperature of its chiller compartment at 20°C. After few minutes solvent evaporate from sample and only extract left behind in round bottom flask. Repeat this step with all the three samples. Collect these samples in beaker and left in oven at 50 °C for overnight next day you get extract and test them for TPC, Flavonoid content and DPPH content.

Total phenolic content (TPC)

Total phenolic content was determined by using method performed by Soni *et al.* (2014) with some modification. Absorbance was measured at 765 nm

using a spectrophotometer. The results were expressed in terms of µg Gallic acid equivalents (GAE)/mg of dry extract. Now take 0.5 gm of sample from extract that place overnight add 5ml of ethanol in it and dissolve by the help of spatula .take now 0.5 ml of sample with 2.5 Folin–Ciocalteu's reagent (10%) and for neutralizing Na₂CO₃ (7.5%) in test tube and place them in incubator for half an hour. First we run blank i.e. ethanol in spectrophotometer. Now run sample in spectrophotometer at 765 nm.

Total flavonoids content

Total flavonoids content was measured by using Aluminum chloride colorimetric method as described by Solomon *et al.* (2006) and Soni *et al.* (2014). Catechin was used as standard. The results were expressed as catechin equivalents (CE) in µg/mg of dried extract. Take 0.3 ml of sample add sodium nitrite 3ml of 5 % of solution. After 5 minutes of interval add 0.3 ml of aluminum chloride (10%) then after 6 minutes add NaOH 2 ml of 1 M. Then after 5 minutes note the absorbance at 415 nm at spectrophotometer.

DPPH free radical scavenging activity

Antioxidant activity was determined by method reported by Zhang and Hamauzu (2004) with some variations using the 2, 2-diphenyl-1-picryl-hydrazyl (DPPH), the absorbance was being record at 517 nm. Radical scavenging activity was calculated by following formula. Take 0.02 gm. of sample in beaker then add 6 mg of DPPH in it then add 25 ml of methanol dissolve in it, store it in refrigerator then dark purple color solution obtain note down the reading of absorbance at 517 nm DPPH create oxidation.

$$\text{Radical scavenging activity (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}}$$

Sensory evaluation of fresh and stored bars

Panel containing 6 judges was used to evaluate the protein enriched cereal bar for flavor, texture, color, taste and overall acceptability (OAA) measured through hedonic scale as mentioned by Sharma *et al.*, (2002).

Statistical analysis

The mean values significant difference calculated by using one way of analysis variance (ANOVA) and then through the software STATISTIC version 8.1. Statistical difference with p-values under 0.05 was considered significant and comparison of mean values was carried out by applying LSD method (Steel *et al.*, 1997).

Results and discussion

Proximate, phytochemical and sensory parameters of protein enriched cereal bar

The research work was conducted to assess the storage stability of protein enriched composite cereal bar. Prepared protein cereal bar was then evaluated for their proximate and phytochemical analysis for 60 days on fortnightly basis stored at room temperature. Sensory properties were also accessed. The obtained results are discussed as follows.

Table 1. Estimated Protein contents (%) of enriched cereal bar on fortnightly basis stored at room temperature.

S. No.	Days of Storage	Crude Protein (%)
1	AT 0 DAY	14.07a ± 0.53
2	AT 15 th DAY	13.33b ± 0.25
3	AT 30 th DAY	13.16b ± 0.06
4	AT 45 th DAY	12.80bc ± 0.03
5	AT 60 th DAY	12.39c ± 0.43

Table 2. Estimated fat content (%) of protein enriched cereal bar on fortnightly basis stored at room temperature.

S.No.	Days of Storage	Crude Fat (%)
1	0 DAY	34.7a ± 0.36
2	15 th DAY	33.66b ± 0.28
3	30 th DAY	33.2c ± 0.17
4	45 th DAY	32.33d ± 0.25
5	60 th DAY	31.10e ± 0.10

Protein content of protein enriched composite cereal bar

The analysis of variance (ANOVA) for crude protein component of cereal bar have been given in Appendix

3. The statistical results showed that there is significant variation ($p < 0.05$) in protein content among the storage days.

Table 3. Estimated Moisture content (%) of protein enriched cereal bar on fortnightly basis stored at room temperature.

S. No.	Days of storage	Moisture content (%)
1	0 DAY	8.20b ± 0.1
2	15 th DAY	8.26b ± 0.06
3	30 th DAY	9.16a ± 0.06
4	45 th DAY	9.20a ± 0.26
5	60 th DAY	9.33a ± 0.15

Protein content ranged from 12.39 to 14.07 % during storage and it showed a significant variation. Protein calculated on 15th and 30th day was 13.33% and 13.16%, showed non-significant change in protein

content that shows keeping good quality of product during its storage time period. The finding of protein content are in line with Padmashree *et al.* (2012) and Torres *et al.* (2011) they were of the view that their

prepared cereal bar having protein content range varies from 10 to 18% respectively. An average 11.43gm of crude protein level was determined in oat based cereal bar by Gutkoski *et al.* (2007). Marques *et al.* (2015) made cereal bar with protein estimation of 9.05 %, the variation among the results may be due to

different composition of their recipe. Protein bar that we made provided 16.64 kcal/32gm energy protein per bar. During storage there was a continuous reduction in the percentage of protein with the passage of time.

Table 4. Estimated Fiber content (%) of protein enriched cereal bar on fortnightly basis stored at room temperature.

S.No.	Days of storage	Fiber (%)
1	0 DAY	4.44a ± 0.22
2	15 th DAY	4.36ab ± 0.06
3	30 th DAY	4.33ab ± 0.15
4	45 th DAY	4.23ab ± 0.06
6	60 th DAY	4.20b ± 0.03

Table 5. Estimated Ash content (%) of protein enriched cereal bar on fortnightly basis stored at room temperature.

S.No.	Days of storage	Ash (%)
1	0 DAY	3.41a ± 0.26
2	15 th DAY	3.23ab ± 0.06
3	30 th DAY	3.13b ± 0.06
4	45 th DAY	3.13b ± 0.06
5	60 th DAY	3.01b ± 0.02

In present study different sources of protein were used cereal, sesame seed and nuts in same proportion to evaluate the best formulation that contain maximum amount of protein which fully justify the daily requirement of protein with reference to men and women. Protein content is rich in millet sesame seed and peanut such type of addition not only raises the overall protein content but also provide good proportion of essential amino acids (Gambus *et al.*, 2011). Millets generally contain

significant amounts of essential amino acids particularly the sulphur containing amino acids methionine and cysteine (Amadou *et al.*, 2013). As protein sources are different in sulphur containing amino acid such as methionine, cysteine and cysteine, that's why adding sesame seed and peanut provide an opportunity to develop cereal bar with higher biological values and higher protein content with better amino acid profile in composite cereal bar.

Table 6. Estimated TPC (mg GAE/gm) of protein enriched cereal bar on fortnightly basis stored at room temperature.

S. NO	Days of Storage	TPC (mg GAE/ gm. of extract)
1	0 DAY	3.64a ± 0.05
2	15 th DAY	3.06b ± 0.06
3	30 th DAY	2.43c ± 0.01
4	45 th DAY	2.01d ± 0.01
5	60 th DAY	2.89b ± 0.01

Sesame seed is unique in having total sulfur-containing amino acid components such as methionine and cysteine (Shewry, 2007). Adding peanut in composite cereal bar enhance the quality of

protein content and also best utilization of peanut in Pakistan where average peanut growth is 114700 tons per year. Peanuts contain all the 20 amino acids in variable proportions and is the biggest source of the

protein called arginine. The amino acid profile of the peanut meals showed that they are good ingredient for protein fortification (Yu *et al.*, 2006). The true

protein digestibility of peanuts is comparable with that of animal protein. Components present in peanuts are highly digestible (Arya *et al.*, 2016).

Table 7. Estimated Flavonoid content (mg QE/g of extract) of protein enriched cereal bar on fortnightly basis stored at room temperature.

S. No.	Days of Storage	Flavonoid (mg QE/g of extract)
1	0 DAY	2.18a ± 0.01
2	15 th DAY	1.66c ± 0.01
3	30 th DAY	1.37d ± 0.01
4	45 th DAY	1.12e ± 0.01
5	60 th DAY	1.85b ± 0.10

Table 8. Estimated DPPH content (µg/ml) of protein enriched cereal bar on fortnightly basis stored at room temperature.

S. No.	Days of storage	Dpph (µg/ml)
1	0 DAY	28.38a ± 0.03
2	15 th DAY	25.13b ± 0.03
3	30 th DAY	19.79d ± 0.15
4	45 th DAY	11.85e ± 0.01
5	60 th DAY	23.40c ± 0.30

Fat content of protein enriched composite cereal bar
The analysis of variance (ANOVA) for crude fat component of cereal bar have been given in Appendix

4. The statistical results showed that there is significant variation ($p < 0.05$) in fat content observed during the storage days.



Fig. 1. Protein bar prepared from the selected ratio.

Fat content of protein enriched composite cereal bar ranged from 31.10 to 34.7% during storage interval. A significantly highest content of fat observed in all samples. The fat content indicated at day 0 and at 15th day of storage, showed significant variation of 34.7 to 33.66 % respectively. The storage interval of 15th and

30th day was shown non-significant variation such as 33.6 to 33.2% respectively. The result of crude fat content in the present study was in line with the finding of Padmashree *et al.*, (2012) such as 24.3%. Addition of sesame seed in recipe increased the oil content of protein bar because fat ratio of sesame

seed is about 50% Moazzami and Kamal-Eldin, (2006). Butter in combination with brown sugar used in recipe of protein bar which results in high fat content, that gives the soft and chewy texture to protein enriched cereal bar. Addition of sesame seed,

peanut and butter may improve the nutritional value of product due to better fatty acid profile of polyunsaturated and monounsaturated fatty acids Kaushal *et al.*, (2005).

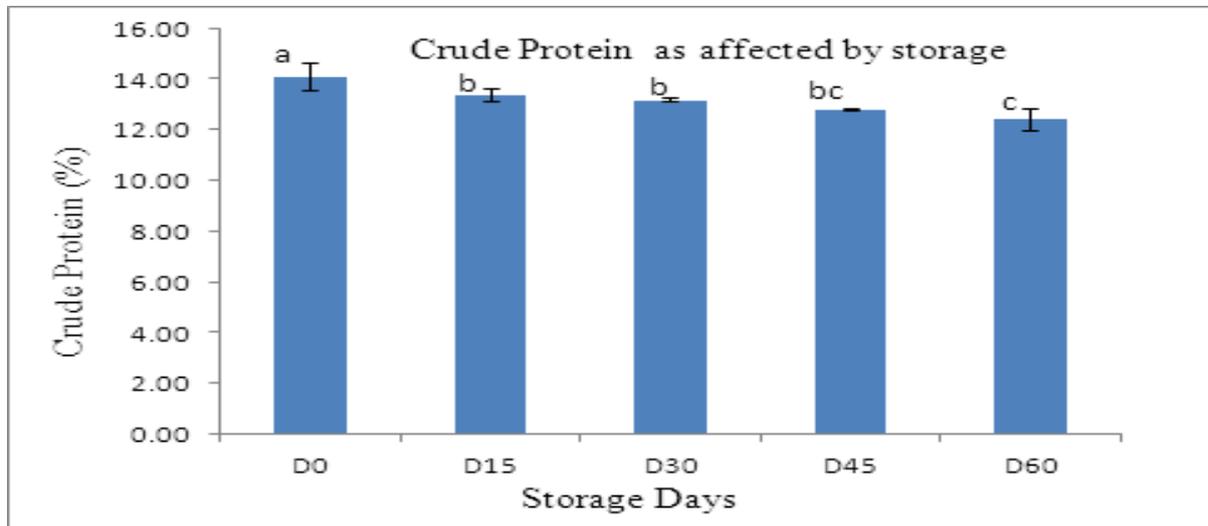


Fig. 2. Graphical representation for the reduction of protein content (%) during storage.

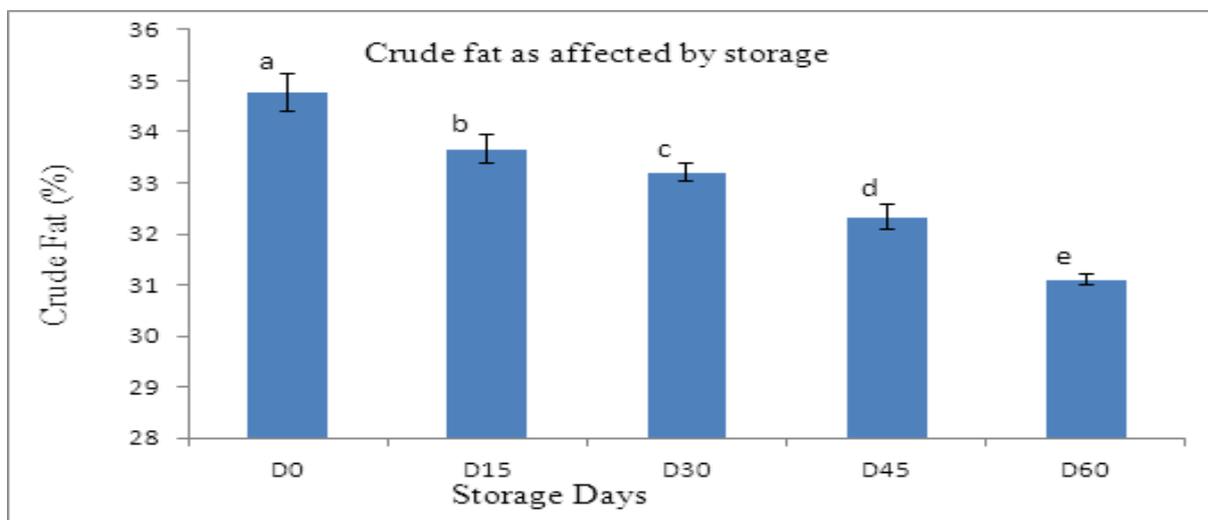


Fig. 3. Graphical representation for the reduction of Fat content (%) during storage.

In fat based enriched food, lipid matrix has one of the major advantages over the food spoilage, create hurdle in multiplication of food borne bacteria's. Vitamin A, E which helps to reduce the degree of peroxidation of the subjected product due to its antioxidant activity, that increases the shelf life at ambient temperature is a valuable innovation. The high monounsaturated fat peanut diets lowered total body cholesterol by 11% and LDL by 14% (Pelkman, 2004). There is strong evidence that nut intake

reduce the risk of coronary heart disease (Matilsky *et al.*, 2009).

Moisture content of protein enriched composite cereal bar

The analysis of variance (ANOVA) for moisture content of cereal bar have been given in Appendix 1 .The statistical results showed that there is significant variation ($p < 0.05$) in moisture content observed during the storage days interval.

In cereal bar moisture content ranged from 8.2 to 9.33 % (Table 3) during storage interval. The lowest moisture content was found 8.2%, significant loss of moisture content during storage interval noticed.

Most of protein bar contain high amount of moisture range such as 10 to 15% Aigster *et al.*, (2011) reported 15.1% moisture that make the protein bar harder by the passage of time.

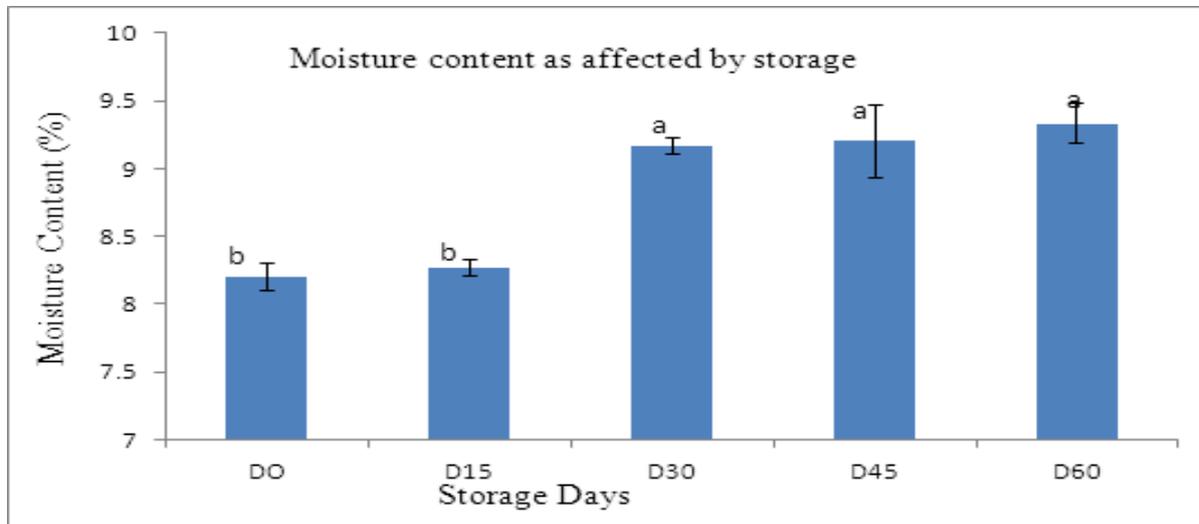


Fig. 4. Graphical representation for the increment of Moisture content (%) during storage.

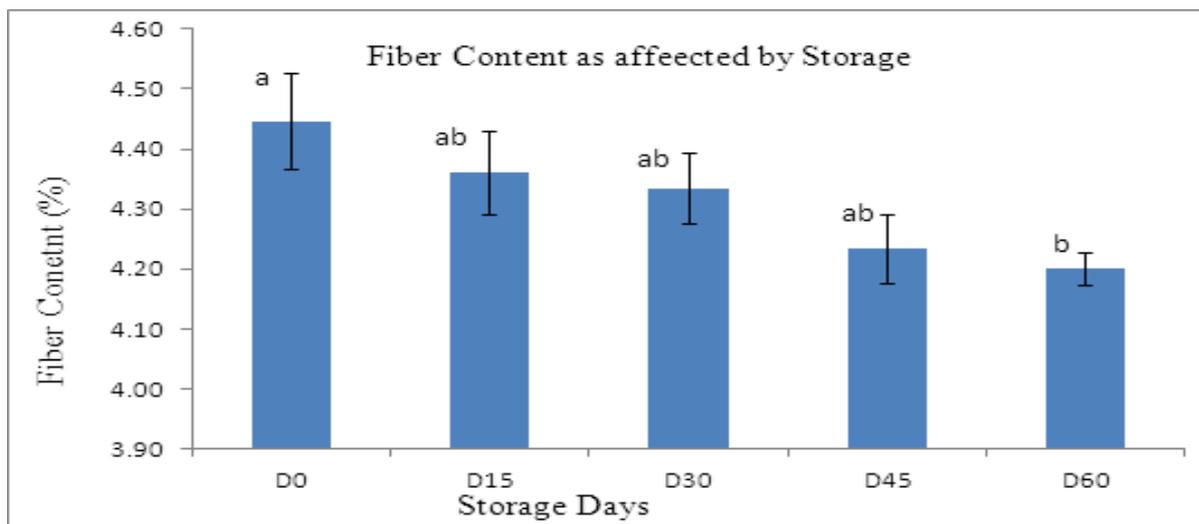


Fig. 5. Graphical representation for the reduction of fiber content (%) during storage.

The development of a hard texture reduces the shelf life of protein bar. Hardening caused from a substance called thiol–disulfide because of its exchange reactions during storage Zhou *et al.*, (2008).

This was supposed the main reason of protein cross linking, aggregation, and network formation, which give rise to hard texture of protein bar. Other reason may of bar hardening during storage was may be more ordered protein secondary structure and lower

surface hydrophobicity of protein particles so different reasons were studied (Loveday *et al.*, 2009). The high amount of moisture content would help the growth of microbes and reduces the shelf life of product. No water was used in recipe composition, nutritional quality of various products determined by the moisture content (Inyang and Abgo, 1995).

The deterioration of quality was also less at lower moisture content which can be credited to retarded respiration and activity of microorganisms (Butt *et*

al., 2004). Generally, moisture content of 9% or lower restricts infestation. (Hoseney, 1994). Higher lipolytic and proteolytic activities are related to higher moisture content, which further lead to loss in

nutrients (protein and fat) and production of more free fatty acid resulting in inferior sensory characteristics (Ball, 1960).

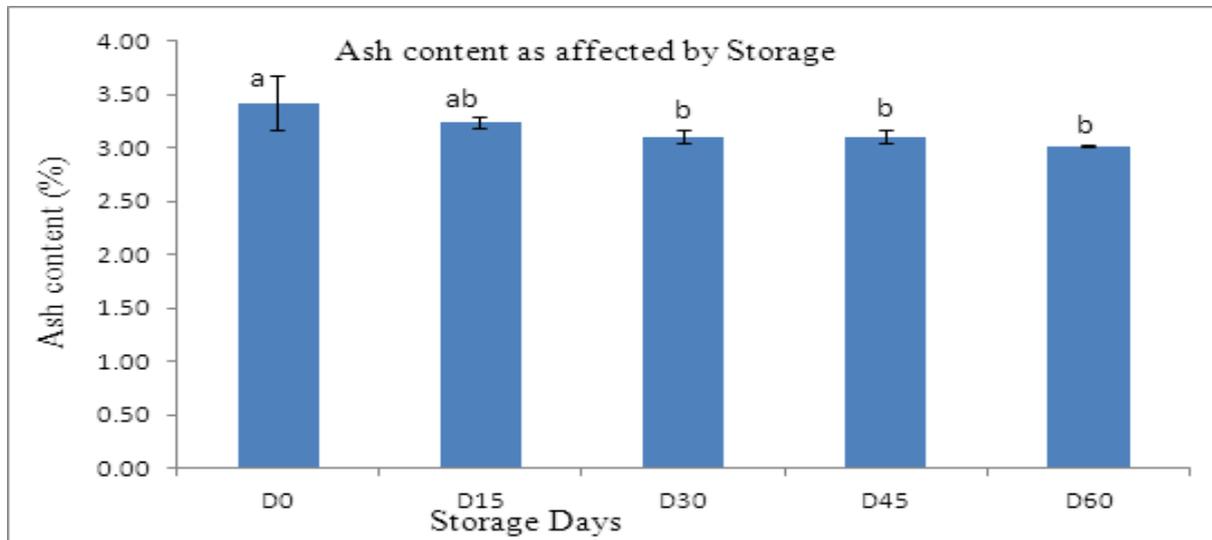


Fig. 6. Graphical representation for the reduction of Ash content (%) during storage.

Fiber content of protein enriched composite cereal bar

Analysis of variance for the fiber of cereal bar is presented in Appendix 5. The statistical results showed that the storage treatment significantly

($p < 0.05$) affected the fiber content of protein enriched composite cereal bar. According to the statistical results it has been shown that crude fiber affected significantly due to difference in the type of recipe composition.

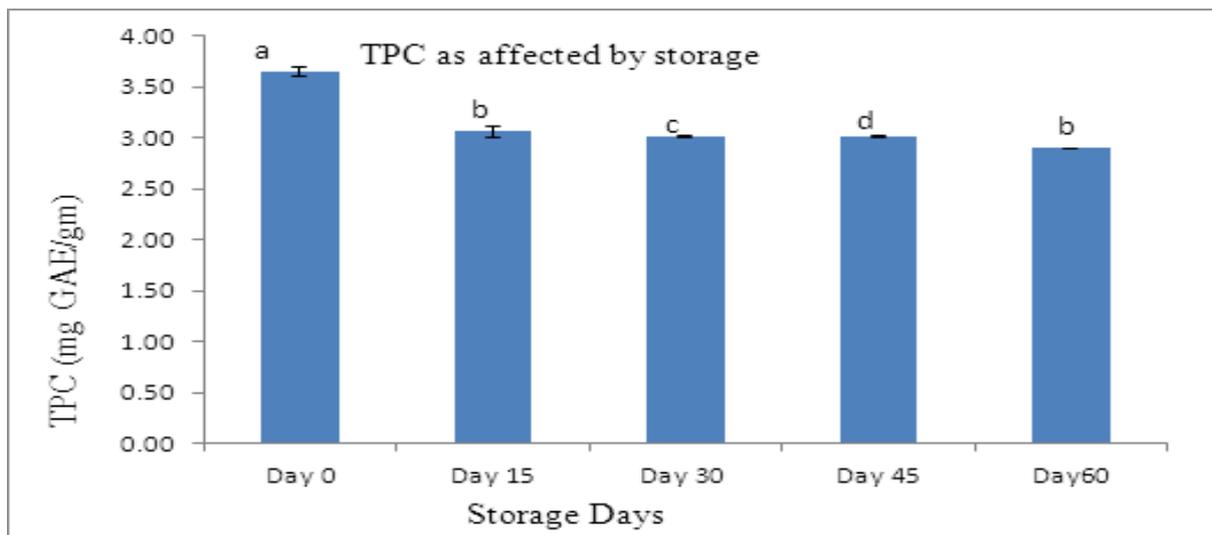


Fig. 7. Graphical representation for the reduction of TPC (mg GAE/gm) during storage.

The highest value of fiber was observed in 4.44 %. Fiber content of protein enriched bar at different storage time periods were between 4.4 to 4.2 %. Our findings were closely matched with the results of

Lobato *et al.*, (2012) who investigated 4.17% of crude fiber in high protein cereal bar. Other investigated results of (Freitas and Moretti, 2006) that found 4.17% fiber content in cereal bar. Further found

values 6.69 % fiber content in oat based cereal bar by Gutkoski *et al.*, (2007). Utilization of food that had high dietary fiber may also create hinder in the availability of zinc and iron .Product become more viscous and impart laxative effect on gastrointestinal tract of humans and help to maintain bowel moment and increased fecal bulk which help to reduce the cholesterol in blood. Recommended daily intake is 25 gm and 38 gm per day for women and men respectively. Oat contains b-glucan that controls

blood glucose and cardiovascular diseases. Oat exerts a potentially useful effect on plasma lipoprotein risk factors for cardiovascular disease. The incorporation of oat in daily diet is not only important from the nutrition standpoint but also for its therapeutic potential. Millet is gluten-free, an excellent option for people suffering from celiac diseases often irritated by the gluten content of wheat and other cereal grains.

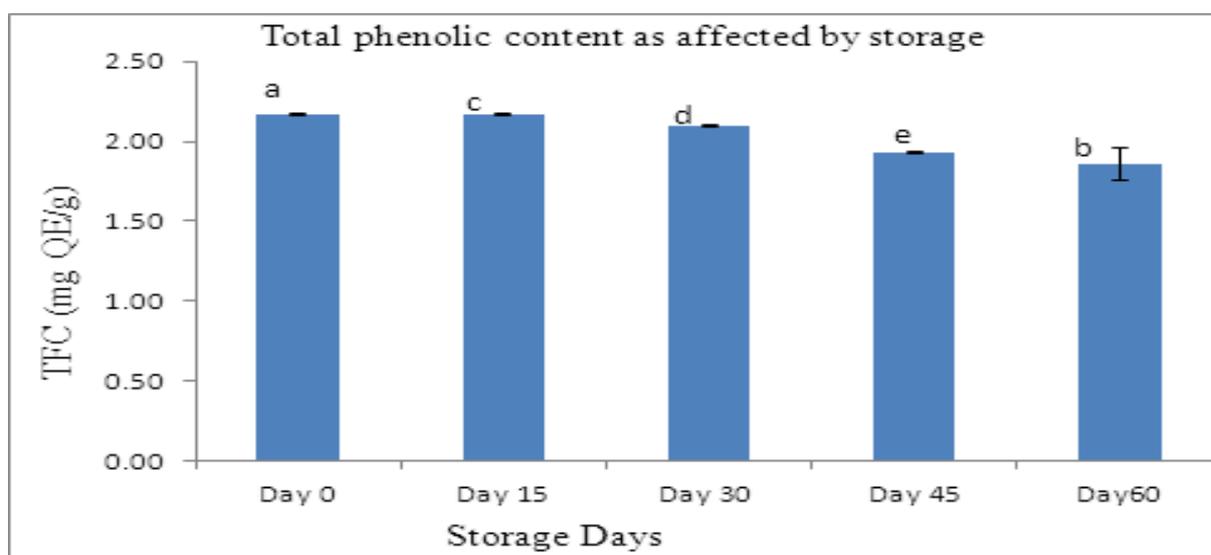


Fig. 8. Graphical representation for the reduction of TFC (mg QE/g) during storage.

Ash content of protein enriched composite cereal bar

The analysis of variance (ANOVA) for ash content of cereal bar has been given in Appendix 2. The statistical results showed that there is significant variation ($p < 0.05$) in ash content among the storage days interval.

The ash content ranged from 3.01 to 3.41 % in protein bar during storage. The findings are in line with the results of Padmashree *et al.*, (2012) that shows 1.7% total ash in cereal bar. A 100 g of peanut provide RDA levels of 127% copper, 84% manganese, 57% iron, 54% phosphorus and 42% magnesium. Ash refers to the inorganic residue remaining after either ignition or complete oxidation of organic matter in a food sample. It helps determine the amount and type of minerals in food. The amount of minerals can determine physiochemical properties of foods, as well as retard the growth of microorganisms. The mineral

intake reduced inflammation and a decreased risk of metabolic syndrome and diabetes (Song *et al.*, 2005) .High fiber content present in product also has ability to bind minerals. Inorganic material present in product is quantitatively analyzed through ash content.it was found that ash content decreased significantly with the increases in the number of days during storage interval.

The differences in ash content of all samples can be attributed to the differences in the level of individual mineral contents of all samples which in turn related to varietal differences, growing conditions and production year of ingredients Gutkoski *et al.*, (2007).

Phytochemical analysis of protein enriched composite cereal bar

The analysis of variance (ANOVA) for phytochemical analysis of cereal bar has been given in Appendix 6, 7

and 8. The statistical results showed that there is significant difference ($p < 0.05$) in phytochemical analysis during the storage day's interval.

Table 6, 7 and 8 illustrates the mean values of TPC, TFC and antioxidant activity of protein bar of tested sample during storage interval. The amount of total phenolic content was determined by comparing with the calibration curve of Gallic acid and results were

expressed as $\mu\text{g GAE/g}$. The mean values showed the significant variation in protein bar value during storage, the maximum values of TPC in protein bar was noticed at the preparation day of protein bar that was $3.6433 \mu\text{g GAE/g}$ while the minimum value was noted in protein bar $2.0100 \mu\text{g GAE/g}$. A standard curve of quercetin was prepared for the calculation of the amount of total flavonoids in protein bar during different storage interval.

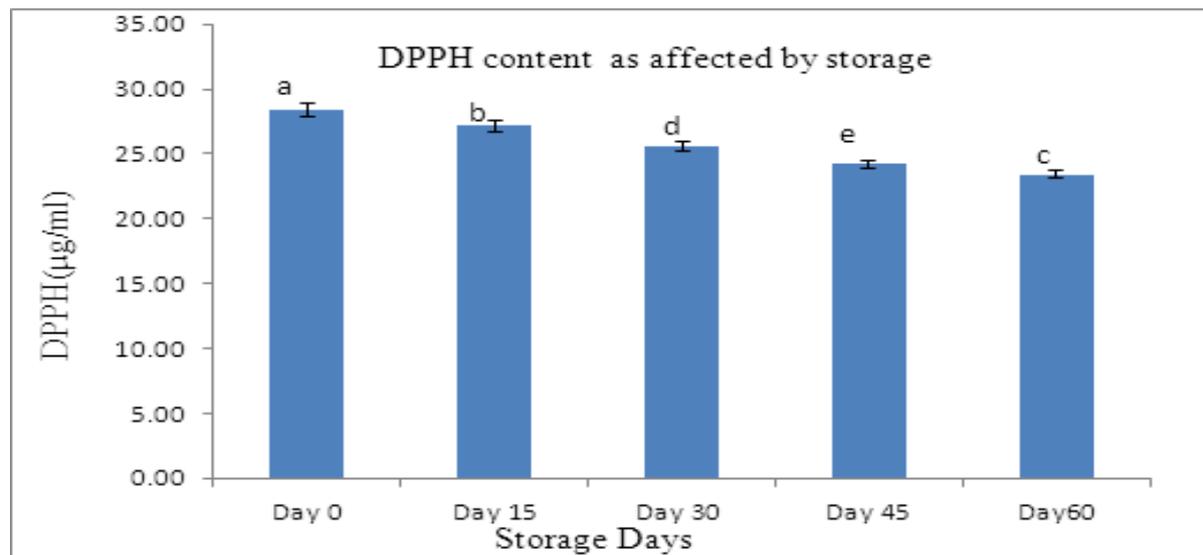


Fig. 9. Graphical representation for the reduction of DPPH ($\mu\text{g/ml}$) during storage.

The present research revealed that the TFC in protein bar were noted in the range of 1.1233 mg QE/g to 2.1833 mg QE/g . The antioxidant potential of protein bar was evaluated as percent inhibition of DPPH free radicals. DPPH radical scavenging activity of protein bar was found in range of 19.790 to $28.380 \mu\text{g/ml}$ during storage interval.

The results were in line with the finding of Asharani *et al.* (2010) have shown that the millet based products contain $199 \mu\text{g}/100 \text{ g}$ of carotenoids that shows oat and millet consists of phytochemicals and bioactive compounds including phenolic, carotenoids, vitamin E, γ -oryzanol, dietary fiber, and β -glucan may be responsible for the health benefits of whole grain consumption in the prevention of chronic diseases. Rao and Prabhavathi (1982) found 0.36% tannin (catechin equivalents) and McDonough *et al.* (1986) observed 0.55 - 0.59% total polyphenols and 0.17 - 0.32% tannins (catechin equivalent) in millet

based cereals. According to Sriprya *et al.* (1996) the total polyphenol contents of products having brown variety of the millet (0.1%) was higher than the white variety (0.003%). Very recently Chethan and Malleshi (2007) also found 1.3 to 2.3% polyphenols as Gallic acid equivalents in brown varieties and 0.3 - 0.5% in white varieties.

Sensory evaluation

To evaluate the palatability of protein bar sensory evaluation test was performed. For the purpose of evaluation the products was prepared and allows to cool at room temperature then packed in air tight plastic bags. Sensory evaluation tested by 9 hedonic scales with the following number of qualitative characteristics is equal to extremely dislike and 9 is equal to extremely good. Tastes texture color and flavor and overall acceptability was the main component of sensory evaluation. Analysis of variance for the sensory content of protein enriched

cereal bar indicated that treatments significantly ($p < 0.05$) affected the sensory content of protein cereal bar. Storage has no significant effect on the sensory content of protein bar. The average of the sensory evaluation assigned by the judge's range between 6.43 to 9.73 % that showed there is significant difference during storage interval. As the syrup of brown sugar as binder intensify the color of product and great mouth feel. In the product the color was developed due to mallard reaction .Though the mallard reaction the color which was produced

depends upon type of sugar added .Our results closely resemble with the results of (Padmashree *et al.*, 2012) and (Vijayakumar and Simon 2009) that showed 7.5% with respect to flavor.

As far as texture was concerned the maximum score of 8.2% achieved. For taste criteria, comparatively texture of the bar was deteriorated much faster and it ranged from 7.7 to 5.2% and 5.0 % respectively on a 9 point Hedonic scale.

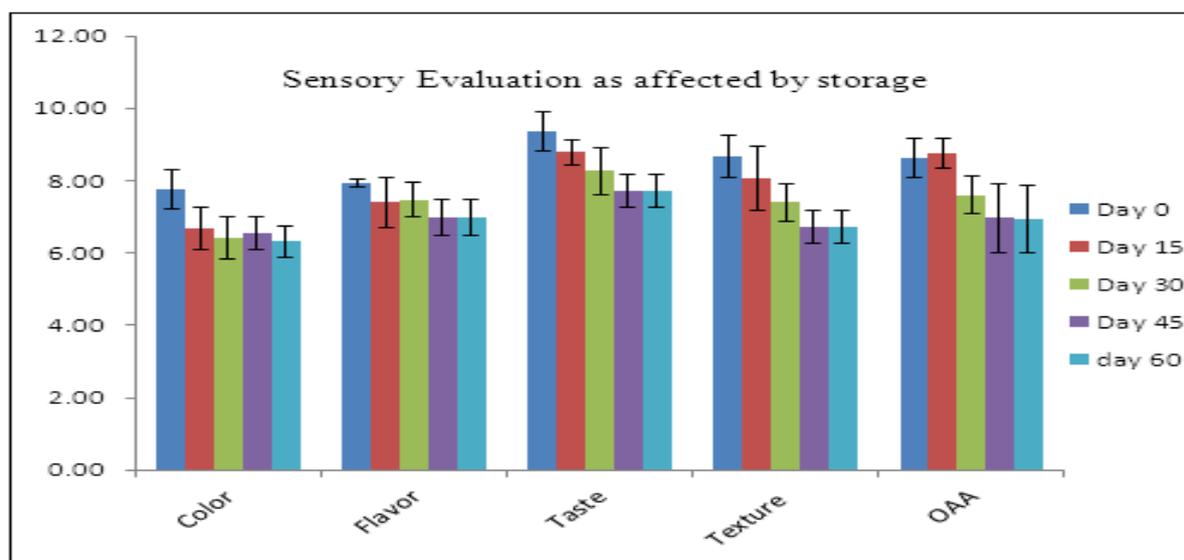


Fig. 10. Graphical representation of Sensory Evaluation of Protein bar during storage.

The range of flavor of supplemented food is 7 to 7.93 %. The highest flavors scored at day of preparation of protein cereal bar. The storage showed a significant variation during storage interval. Flavor is mixture of smell and taste also influence on user liking, textural features as well as appearance of product .The change in flavor due to fat content of peanut that cause bitter flavor during storage. The mean value of taste 9.37% to 7.73% percent range was measured in protein enriched cereal bar. However the lowest score showed among the 45th day of storage. Taste and aroma are closed properties and taste is a sense that's perceived through tongue, also influenced by texture, formulation and flavor of the products that impart important factor in food preparation. Last two intervals has changed significantly 8.27 to 7.73% it is due to taste of sesame seed which become soft with passage of time and due to high fat content of sesame

seed may developed after taste. As value of texture of product was presented revealed that significantly highest appreciated texture of protein bar at preparation day but other days of storage also showed significant variation with range between 8.67 to 6.73%. Commercially available protein bars normally got hard texture during storage because of moisture content but protein bar does not show this defect as composition consists no use of water throughout recipe.

Texture is such property that can only calculate and define only by man itself Sanchez *et al* (2002).

It is also an important characteristic of liking towards the product. During storage interval of protein bar, over all acceptability affected by texture, taste, flavor and color of the product.

Conclusion

Malnutrition is a situation that results from eating a diet in which nutrients are either not enough or too much that causes health problems. Pakistan has an alarmingly high level of malnutrition that means deficiency of macronutrients and micronutrients, 24 percent of the population is undernourished, 37.5 million people in Pakistan do not receive the adequate nutrition that causes double growth. Malnutrition is usually associated to the condition of poverty in Pakistan and the main contributory factors which includes the low consumption of produce that nourish the body. Foods with low nutritional value are one of the other causes of malnutrition.

In this study protein bar is prepared with honey, almonds, oats, sesame seeds, puffed millet, black cumin seeds. All these ingredients are readily available and affordable. Selecting the organic ingredients from non GMO and gluten free sources insures purity and quality. This bar is intended to be packaged for long term storage and its application demands least resources from the consumer. Then it tested for that how much it is storage stable for two months. Complete proximate analysis phytochemical analysis and sensory evaluation of protein bar is performed to evaluate its nutritional value. And results found that bar contains 13 to 14 % of protein in 1gm of sample and that's higher with the bars available in market. Protein enriched composite cereal bar is provide consumer about 160.6 kcal/32gm of energy and protein energy was found 16.64 kcal/32 gm.

The purpose was to provide consumer a ready to eat protein food that majority of people in Pakistan cannot afford because of its high cost. People on diet can also take advantage from this formulation. Phytochemical study investigates the bar about its total phenolic content and total flavonoid content and DPPH activity. One of the profound issues seen in protein bar is the hardening of outer texture with passage of time in storage which is caused by moisture content. Resulting into limited shelf life and makes the product unappetizing and unsatisfactory

for consumers. To insure this protein bar maintains its texture no water is used in preparation of its syrup thus maintaining moisture level at less than 10%. Protein Bar shows very negligible changes in its proximate analysis and phytochemical analysis.

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