The Pharma Innovation Journal 2022; 11(4): 30-41

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(4): 30-41

www.thepharmajournal.com
Received: 07-02-2022
Accepted: 31-03-2022

Monika Mathur
Center of Food Science and Technology, College of Agricultural Engineering and Technology, CCS Haryana Agricultural University, Hisar, Haryana, India

Anju Kumari
Center of Food Science and Technology, College of Agricultural Engineering and Technology, CCS Haryana Agricultural University, Hisar, Haryana, India

Corresponding Author:
Monika Mathur
Center of Food Science and Technology, College of Agricultural Engineering and Technology, CCS Haryana Agricultural University, Hisar, Haryana, India

Development and evaluation of multigrain fibre and protein enriched composite bars

Monika Mathur and Anju Kumari

Abstract
The present investigation entitled "Development and evaluation of protein and fibre enriched composite bars" was carried out to evaluate the physico chemical and functional properties of grains and processed fruits & vegetables, for standardization of protein and fibre enriched composite bars. Composite bars were standardized by trial and error method using various proportions of puffed rice and barley, flaked maize and oat, popped amaranth and sorghum, roasted chickpea, groundnut and sesame seeds were used along with osmotic dehydrated candies of carrot, pumpkin, lemon peel and kinnow peel, chunked mango, and carrot and bottle gourd powders. Carrot powder (6%) and whey protein isolate (6%) were supplemented in rice, maize and amaranth based composite bars, for fibre enrichment, CB1, CB2, and CB3 and protein enriched, CB4, CB5 and CB6 composite bars respectively. Crude fat, ash and crude fibre were highest in CB3 and energy in CB6, whereas moisture and crude protein were highest in CB4 (6.99%) and CB5 (17.50%). Maximum hardness was observed in CB6 after control. Highest bulk density for CB2 and calcium, iron, zinc and total soluble fibre were highest in CB6, however, insoluble fibres found maximum in CB3. Lowest phytic acid and tannins were in CB1, however, maximum total phenolics and antioxidant activity was in CB3 and CB6, respectively. Amaranth based protein enriched bar (CB6) and fibre enriched (CB3) were most acceptable bars during storage. Maize based protein enriched composite bar (CB5) had the highest protein whereas, highest fibre was exhibited in fibre enriched composite bar CB3.

Keywords: Composite bar, protein enriched, fibre enriched, cereal

Introduction
Consumers have become increasingly concerned about their own and the planet's health in recent decades. Consumers are becoming more aware of how healthier food choices can help prevent future health problems, and as a result, they are requesting meals that provide specific health benefits (Topolska *et al.*, 2021) [57]. The highly demanding consumer of today has set the trend not only for convenient, but also for natural, nutritious, and sustainable food items, as evidenced by the introduction of COVID-19 (Lockyer, 2020; Rodrguez-P'erez *et al*., 2020) [33, 46]. The transition in individuals' lifestyles has resulted in a steady increase in the consumption of convenient foods (Nielsen, 2018) [37]. Snacks are becoming increasingly popular among convenience foods (Nielsen, 2018) [37]. In fact, many consumers regard them as a means of "maintaining" energy throughout the day, rather than as decadent treats (Damen *et al*., 2020; Forbes *et al*., 2016; de Saint Pol & H'ebel, 2021) [13, 19, 15]. As a result, the snack market has turned away from traditional snack options (such as chocolate bars) and toward the development of functional and more inventive items, such as cereal bars (Glanbia Nutritionals, 2021) [21]. Between 2021 and 2026, the global cereal bar market is predicted to increase at a CAGR of 8.5 percent, which is 4% more than the global chocolate market (CAGR of 4.5 percent) (Mordor Intelligence, 2020a, 2020b) [35, 36]. Cereal bars are frequently regarded or labelled as a healthy alternative to chocolate bars (Bucher *et al*., 2016; Huitink *et al*., 2020; Poquet *et al*., 2020; Vasiljevic *et al*., 2015) [9, 25, 44, 59].
People should consume food that really are high in vitamins and minerals, as well as balanced in terms of major nutrients like carbs, proteins, and fats. The number of healthful and nutritional food products available to children is extremely limited. This need must be filled by designing products that adhere to emerging nutraceutical and functional food trends (De Irala-Estevez *et al*., 2000) [14]. Food products made with dried fruits, processed cereals, legumes, millet and pseudocereals, and nuts would be a nutritious snack for schoolchildren, working professionals, and athletes who require a high protein, low calorie diet on a daily basis (Chávez-Jáuregui *et al*., 2003) [11].

From a nutritional standpoint, the bars can be divided into four categories: high protein, high energy, high fibre, and low-calorie diet bars. Diet bars have only 65 calories, are sugar-free or very low in sugar, and are a good choice for diabetic customers. High fibre bars are rich in fibre and glucose content, with an energy value of nearly 100kcal per unit, whereas diet bars have only 65 calories, are sugar-free or very low in sugar, and are a good choice for diabetic customers. Because they include less fibre and have a high caloric content, high-energy bars with 280kcal provide easily consumable energy. These bars are recommended for energy replacement after strenuous physical activity. High protein bars with 200 calories per unit include roughly 17 grammes of protein and have a lower fat content (Degaspari *et al*., 2008) [16].

Currently, Indian consumers are becoming more interested in locally produced bars such as horlicks multi cereal nutri bar, rite bite choco delite bars, low-fat bar, sugarless bar, woman snack bar, fruit choco bars, and so on. However, these bars are only available in superstores in major cities, and the selling price for these bars is also quite high. Wheat-soy snack bars have been designed in such a way that they contain appropriate nutrients for individuals on the go's health (Aramouni & Abu, 2011) [8], as well as high protein snack bars for athletes and dieters (Hogan *et al*., 2012) [24]. To boost the value addition and functional value of bars, fruits and vegetable by products, powders, and candies can be included. Humans benefit from eating fruits and vegetables because they improve their health. They're chock-full of vitamins and antioxidants (Njike *et al*., 2016) [38]. Multigrain bars have been designed to address the aforementioned issues. Multigrain cereal snack bars are ready-to-eat, handy food products that are readily available to consumers, giving nourishment and fulfilling their hunger. They are also available on the market with a variety of options to meet a variety of needs (Catherine & Johnston, 2012) [10]. These are made with whole-grain cereals, flaked grains, fruits, legumes, dehydrated or crystallised fruits, nuts, fruit and vegetable candies, chocolates, sugar, and other ingredients such as whole-grain cereals, flaked grains, fruits, legumes, dehydrated or crystallised fruits, nuts, fruit and vegetable candies, chocolates, sugar, and so on (Lobato *et al*., 2012) [32].

Whole grains provide health-promoting characteristics in food, such as anti-carcinogenic, antibacterial, and antioxidant capabilities (Adebo & Gabriela, 2020) [4]. Vitamins, fatty acids, phytosterols, proteins, dietary fibre, carotenoids, lignin, and sphingolipids are all physiologically relevant substances found in whole grains that promote greater health either alone or in combination (Schaffer, 2017) [48]. These whole cereal grains, legumes, millets, and other oilseeds are combined to create specific characteristics such as colour and appearance, aroma, taste, and other physical properties such as texture. They are also fortified with functional ingredients to improve their nutritional properties in terms of vitamins, minerals, herbs, and energy-rich ingredients in order to create multigrain food products (Shaheen *et al*., 2013) [49].

Customers wanted a larger piece size and a lower price, therefore researchers tried to construct the bar using locally accessible cereal grain crops that are not only very nutritious but also cheaper (Ho *et al*., 2016) [23]. (Pinto *et al*., 2018) [43]. Nutritional bars made from processed cereal grains and enriched with whole cereals, dehydrated or crystallised fruits (such as carrot candy, mango candy, pumpkin & bottle gourd candy, orange and lemon peel candy), nuts, and functional ingredients, which provide varying amounts of calories, fat, proteins, bioactive components, and other essential nutrients (Peuckert *et al*., 2010) [44]. The combination of popped sorghum, amaranth seed, groundnut, roasted split chickpea, dark chocolate, corn flakes, gulkand, jaggery, and liquid glucose was liked by people (Ravindra & Sunil, 2018) [44].

Material and Method

Standardization of ingredients for bars

The main ingredient for the preparation of bars was cereal flakes and completely roasted or popped grains. The cereal grains were used at a level of 30 to 40% in different treatments. Dried fruits like dehydrated mango chunks, raisins and nuts like peanuts and cashew nuts were used up to 10%. For enriching the bars, carrot powder, bottle gourd powder, psyllium husk, whey protein concentrate, and soya protein concentrate are used.

The standardized quantity of different ingredients used for the preparation of 100g of composite bars using Jaggery, honey and cane sugar are furnished in table 1 and 2, respectively.

Table 1: List of ingredients used for optimization of composite bars

Cereal grain and pulses	**Oat, barley, sorghum, chickpea, maize, finger millet, rice (puffed), amaranth**
Nuts and oil seeds:	groundnut, flaxseed and sesame seed
Functional & fibre source	Psyllium husk, carrot, bottle gourd, pumpkin, banana, mango, kinnow peel, lemon peel
Sugar source	Cane sugar, honey and jaggery
Protein source	Whey protein concentrate / soy protein isolate / soy protein concentrate.

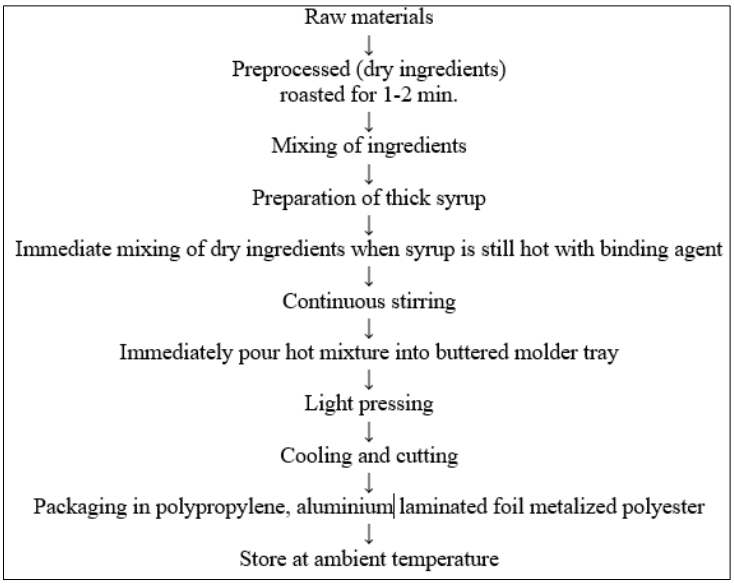

Fig 1: Procedure for preparation of composite bars

Table 2: Composition of ingredients in high fibre and high protein enriched composite bars (g/100g)

Ingredients	High fibre composite bar 1 (CB1)	High fibre composite bar 2 (CB2)	High fibre composite bar 3 (CB3)	High Protein composite bar 4 (CB4)	High Protein composite bar 5 (CB5)	High Protein composite bar 6 (CB6)
Puffed rice	10	-	-	10	-	-
Maize flakes	-	10	-	-	10	-
Puffed amaranth	-	-	10	-	-	10
Oat	10	10	10	10	10	10
Chickpea	14	14	14	14	14	14
Groundnut	14	14	-	14	14	-
Sesame seed	-	-	14	-	-	14
Fruit and veg. candy	4	4	4	4	4	4
Carrot powder	6	6	6	-	-	-
Whey protein isolate	-	-	-	6	6	6
Jaggery	40	40	40	40	40	40
Gum acacia	2	2	2	2	2	2
Total	100	100	100	100	100	100

Quality evaluation of prepared bar

-Sensory evaluation of multigrain bars

The multigrain bar samples were subjected to sensory evaluations by 10 semi trained panelists using 9-point hedonic scale (from like extremely to dislike extremely) to determine the acceptability of product with respect to colour, flavour, taste, texture and overall acceptability (Obatolu *et al.*, 2006) [39].

Physical Properties: Texture analysis of the multigrain bars

A texture analysis is primarily concerned with measurement of the mechanical properties of a product. Texture analyzer performs this test by applying controlled force to the product and recording its response in the form of force, deformation and time. Hardness is the force necessary to attain a given deformation of the material or it is the force required to bite through the sample with molars (Itagi *et al.*, 2013) [26]. Texture of multigrain bar was analyzed using stable micro system texture TA-XT plus texture analyzer and method adopted by Nadeem *et al.* (2012) [27]. TPA is a "one-bite" test, which includes the compression cycles. The cycle indicates the force *vs* time data during the compression of the product by the instrument probe.

Nutritional evaluation of prepared bar

Proximate composition

Moisture

Moisture content was calculated by employing the standard methods of analysis (AOAC, 2000) [3].

Procedure: Ten gram sample was weighed in a petri dish and dried in an oven at 60°C temperature for six hours or till a constant weight was obtained. The sample was reweighed after cooling in desiccators.

$$\text{Moisture (\%)} = \frac{\text{Loss in weight (g)}}{\text{Weight (g) of sample}} \times 100$$

Crude Protein

Crude protein was estimated by standard Micro-kjeldahl method of analysis (AOAC, 2000) [3] using automatic KEL-Plus CLASSIC-DX apparatus.

Reagents

1. Hydrochloric acid (N/10)
2. Boric acid (4%)
3. Sulphuric acid (concentrated)
4. 40% NaOH (Sodium Hydroxide) solution

5. Digestion mixture: Potassium sulphate (10g) and copper sulphate (2g) were mixed together.
6. Mixed indicator solution: Dissolved 0.3g of bromocresol green and 0.2g of methyl red in 400ml 90% ethanol and the solution was adjusted with drops of dilute NaOH to bluish purple colour.

Procedure

A 200mg sample was taken and digested with 10ml concentrated H_2SO_4 and 3g of digestion mixture was added to the sample. Digestion tubes were loaded into the digester and heated the block at 420 °C. Digestion was carried out for about one hour and forty minutes till the contents became colorless or light green. The digested samples were cooled at room temperature and distilled in Classic-DX. During distillation, the digested samples were heated by passing steam and 40% of sodium hydroxide was discharged to liberate ammonia. Liberated ammonia was titrated against 0.1 N HCl until the endpoint was indicated by change of color to light ink. Titrated volume of a blank solution of boric acid and mixed indicator was also determined.

$$\text{Nitrogen (\%)} = \frac{\text{Titre volume (S-B)} \times \text{Normality} \times 14}{1000 \times \text{W}} \times 100$$

Crude protein (%) = Nitrogen (%) × conversion factor

Where

W = Weight of sample taken (g)
S = Volume (ml) of HCl (N/10) used in titration for sample
B = Volume (ml) of HCl (N/10) used in titration for blank

Conversion factor of 6.25, 5.7, 5.83 and 5.3 was used for maize, wheat, oat & barley to calculate.

Crude Fibre

Crude fibre was estimated by the standard method of analysis (AOAC, 2000) [3] using automatic Fibra-Plus apparatus.

Reagents

1. Sulphuric acid stock solution (10%, w/v): Diluted 55ml concentrated sulphuric acid to one litre.
2. Sulphuric acid working solution (1.25%): Diluted 125ml of stock solution to 1 litre.
3. Sodium hydroxide stock solution (10%) w/v: Dissolved 100g of NaOH in distilled water and diluted to one litre.
4. Sodium hydroxide working solution (1.25%): Diluted 125ml stock solution to one litre with distilled water.

Procedure

One gram of fat free dried sample was weighed and transferred into crucible. The crucibles were fixed in apparatus and proper sealing was ensured. Individual valves were kept in close position. Dilute acid was poured from the top of the extractor through a funnel. The power supply was switched on in the control panel. The temperature was set to 500 °C and when boiling was ensured, the temperature was reduced to 400 °C and boiling was carried out for 45 min. After that, oven supply was switched off. All the knobs were put in open position and the suction pump apparatus was switched on. Filtration of acid reagent was ensured through the crucibles. The sample was washed with distilled water. Similar process was repeated with dilute alkali. The crucibles were removed and dried in a hot air oven at 70 °C for overnight. The crucibles were cooled in desiccators and weighed. The residue was ignited in a muffle furnace at 500 °C for 2 h. The crucibles were cooled in desiccators and weighed again. Crude fibre then was determined by loss in weight due to ignition:

$$\text{Crude Fibre (\%)} = \frac{W_1 - W_2}{W} \times 100$$

Where

W = Weight (g) of sample
W_1 = Weight of crucible + weight of treated sample after oven drying
W_2 = Weight (g) of crucible + weight of sample after ashing

Crude Fat

Crude fat was estimated by standard method (AOAC, 2000) [3] using the automatic SOCS plus Solvent extraction system.

Procedure

The beakers were washed thoroughly and dried in a hot air oven at 60 °C. Weight of the empty beaker was taken. Dried sample (2gm) transferred to an extraction thimble. The thimble holder along with the sample was kept into the beaker along with 100ml of petroleum ether (boiling point 60-80 °C). Loaded the beakers into the system and set 90 °C temperature in the control panel. The extraction was carried out for one hour at 90 °C. When the extraction period got completed. The temperature was raised at 180 °C; the stopper was closed in order to collect the solvent in the solvent compartment. The beaker containing fat was removed and kept in a hot air oven 60 °C temperature, until a constant weight was obtained. The beaker was weighed after cooling it in a desiccator.

$$\text{Fat (\%)} = \frac{W_2 - W_1\,(g)}{W\,(g)} \times 100$$

Where

W_1 = Weight of empty beaker
W_2 = Weight of beaker after extraction
W = Weight of sample

Ash

Ash content in the sample was estimated by employing the standard method of analysis (AOAC, 2000) [3].

Procedure

Five gram of dried sample was taken in a weighed crucible and ignited until no charred particles left back in the crucible and then the crucible was put in a muffle furnace (550 °C) for 6 h or until a white ash was obtained. Thereafter, the crucible was cooled in a desiccator and reweighed. The loss in weight represents the organic matter residue, the ash content, which was calculated using following formula

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

W = Weight (g) of sample
W_1 = Weight (g) of crucible
W_2 = Weight (g) of the crucible + ash

Nitrogen free extract
Carbohydrates content was calculated by difference method AOAC (1995) [2] on dry basis using following formula:

Total carbohydrates % = 100 – (crude fat % + crude protein % + ash % + crude fibre %)

Energy
Energy was calculated by factorial method on a dry basis using the following formula.

Energy (kcal) = 4.0 x protein (g) + 4.0 x carbohydrate (g) + 9.0 x fat (g).

Mineral content
Mineral content of the sample was determined by a wet digestion method.
One g flour was weighed and dispersed in a 150ml conical flask. 25-30ml diacid mixture (HNO_3**:**$HCLO_4$) in ratio 5**:**1 was added in flask and kept for overnight. The contents were digested by heating until clear white precipitates snuggled down at the bottom. The crystals left were dissolved by adding double distilled water. Then the contents were filtered through whatman # 42 filter paper and the filtrate was made to 50ml volume by using double distilled water and used for the determination of trace minerals: iron and zinc, using atomic absorption spectrophotometer (Lindsey & Norwell, 1969), in the Department of Soil Science, CCS Haryana Agricultural University, Hisar.

$$\text{Minerals (mg/100g)} = \frac{\text{Reading (conc. µg/ml)} \times \text{volume made}}{\text{Weight of sample (g)} \times 1000} \times 100$$

Calcium
Total calcium in acid digested samples was determined by the method of Chopra & Kanwar (1979) [12].

Reagents
1. **Standard solution of Ca (0.01 N):** 0.5g of well dried pure $CaCO_3$ was transferred to 400ml beaker and added 10ml of 1N HCl in it and boiled to expel CO_2. Cooled and 200ml distilled water was added to dissolve the contents. It was transferred to a one liter volumetric flask and made up the volume. Stored in a glass reagent bottle
2. **Standard solution of EDTA (0.01N):** 2g of EDTA-di-sodium salt was dissolved in sufficient volume of distilled water in a 400ml beaker and was transferred to one liter volumetric flask. To this, 0.05g of $MgCl_2.6H_2O$ was added and made up the volume to one liter. The normality of this solution was standardized as under.
3. **Standardization of EDTA solution:** 5ml of standard calcium solution (0.01N) was pipetted in 100ml china dish. It was diluted to 10ml with distilled water. Ten drops each of NaCN and hydroxylamine hydrochloride solutions were added. Then 2.5ml of NaOH solution and 10-15 drops of calcon indicator were added. Pink colour appeared. In another china dish, a blank solution was prepared in the same manner, taking 5ml of distilled water instead of the standard calcium solution. The blank solution was blue in colour or it turned blue with the addition of 2-3 drops of EDTA solution. This blue blank was kept alongside for comparing the end point. Then a china dish containing the pink colored standard calcium solution was placed on a magnetic stirrer plate. A glass coated iron needle was put in it and stirred. Solution was titrated with EDTA until the colour of the solution changed from pink to blue matching the blank. The volume of EDTA used was noted. Based on calculation, N1V1 = N2V2, necessary dilutions were made so that the normality of the EDTA solution was exactly equal to 0.01 N.
4. **Calcon indicator solution (0.5%):** 0.5g of calcon reagent (Solochrome dark blue and Eriochrome blue-black R) was dissolved in 100ml 95% ethanol, stored in a plastic bottle.
5. Sodium hydroxide solution (16% or 4 N)
6. Sodium cyanide solution (2%)
7. Hydroxylamine hydrochloride solution (5%)

Procedure: 5ml of digested sample was added in a 100ml china dish and diluted to 10ml with distilled water. To these 10 drops each of 2% NaCN and 5% hydroxylamine hydrochloride solution, 2.5ml of 4N NaOH solution and 10-15 drops of calcon indicator were added. Pink colour appeared. In another china dish, a blank solution was prepared exactly the same manner by taking 5ml of distilled water instead of the test solution. In blank blue colour appeared, if not, 2-3 drops of EDTA solution were added and this blue blank was used for comparing the endpoint of the test solution. Test solution was placed on a magnetic stirrer top and a glass coated iron needle was placed in it and stirred. The solution was titrated with 0.01 N EDTA till the pink colour changed to blue matching the blank.

Calculations

$$\text{Ca (mg per 100g)} = \frac{\text{Vol. (ml) of EDTA used} \times \text{Normality of EDTA} \times \text{Dilution factor} \times 100}{\text{Vol. (ml) of aliquot taken for titration}}$$

Dietary Fibre
Total, soluble and insoluble dietary fibre constituents were determined by the enzymatic method given by Furda (1981). The sum of insoluble dietary fibre and soluble dietary fibre contents were calculated for determining total dietary fibre content.

Total dietary fibre = Insoluble dietary fibre + Soluble dietary fibre

Insoluble dietary fibre
Reagents
1. 0.005 N HCl
2. Phosphate buffer (pH 10)
3. EDTA
4. Enzymes: Alpha amylase and protease enzymes
5. Ethanol (75% and absolute)
6. Acetone

Procedure

1. Sample preparation: Five gram samples of less than 1mm particle size food material was defatted on a Soxhlet apparatus.
2. Extraction of water-soluble material: The prepared sample weighing about 2.0g was dispensed in 200ml of 0.005N HCl and boiled for 20 minutes. The suspension was then cooled down to 60 °C; 0.3g of disodium EDTA was added and then adjusted to pH 5.0-6.5 with 12ml of phosphate buffer pH 10. The extraction was continued for an additional 40 min at 60ºC to ensure the extraction of pectin with minimal degradation.
3. Starch and protein hydrolysis: pH was adjusted 6.0-6.5 to bring the solution closer to the pH optimum of amylase and protease. Suspension was cooled to 20-30ºC before incubation overnight with 10mg of bacterial alpha-amylase and 10mg of bacterial protease. The incubation was continued by slow stirring with a magnetic bar.
4. Isolation of insoluble dietary fibre: The suspension was filtered through a coarse – tarred gooch filtering crucible containing glass wool and the insoluble residue was washed with a small amount of water. The filtrate was saved for the next step.
5. The insoluble residue was then washed with water, alcohol and acetone before being dried at 70 °C in a vacuum oven overnight. The dry residue constitutes insoluble dietary fibre.

Soluble dietary fibre

Precipitation and isolation of soluble dietary fibre

The saved filtrate was acidified with a few drops of concentrated hydrochloric acid to pH 2-3; this pH tended to enhance the rapid precipitation of polysaccharides. Four volumes of ethanol were slowly added and left suspended to stand for about 1 h. Filtered the precipitate on a tarred, coarse gooch crucible containing glass wool, then washed with 75% ethanol, absolute ethanol, and acetone before drying at 70 °C in a vacuum oven overnight. The residue was weighed in the crucible to give the soluble dietary fibre content of the original material. The soluble dietary fibre fraction was corrected for ash and for co-precipitated protein.

Total dietary fibre (TDF): the sum of insoluble dietary fibre was calculated.

TDF = Insoluble dietary fibre + soluble dietary fibre

Anti-nutritional factors

Samples of different cereal flour like barley, oat, sorghum & chickpea were analyzed for Anti nutrition factor: Phytic acid, Polyphenols.

Phytic acid

Phytic acid was determined by the method of Haugh & Lantzsch (1983) [22].

Reagents

1. Phytate reference solution: Exactly 30.54mg sodium phytate (5.5% water, purity and containing 12 Na/mole) was dissolved in 100ml of 0.2 N HCl, which gave a solution containing 200 µg phytic acids per/ml.
2. Ferric ammonium sulphate solution: Ferric ammonium sulphate (0.2g) was dissolved in 100ml of 2N HCl and made the volume of 1000ml with distilled water.
3. Bipyridine solution: Ten gram 2-2 bipyridine and 10ml thioglycolic acid were dissolved in distilled water and volume was made to 1000ml.

These solutions are stable for several months at room temperature.

Extraction

A fine ground sample (0.5g) was extracted with 25ml of 0.2 N HCl for 3 hours continuous shaking in a shaker. Thereafter, it was filtered through Whatman # 1 filter paper.

Estimation: An aliquot (0.5ml) of the above extract was pipetted into a test tube fitted with a round glass stopper. One ml of ferric ammonium sulphate was added. The tube was heated in a boiling water bath for 30 minutes. The contents of the tube were mixed and centrifuged at 3,000 rpm for 30 minutes. One ml of supernatant was transferred to another test tube and 1.5ml bipyridine solution was added. The absorbance was measured at 519 nm against distilled water. For plotting a standard curve, different concentrations (0.2 to 1.0ml) of standard sodium phytate solution containing 40-200 µg phytic acid were taken and made to 1.4ml with water O.D. of 0.342 corresponded to 80µg phytic acid.

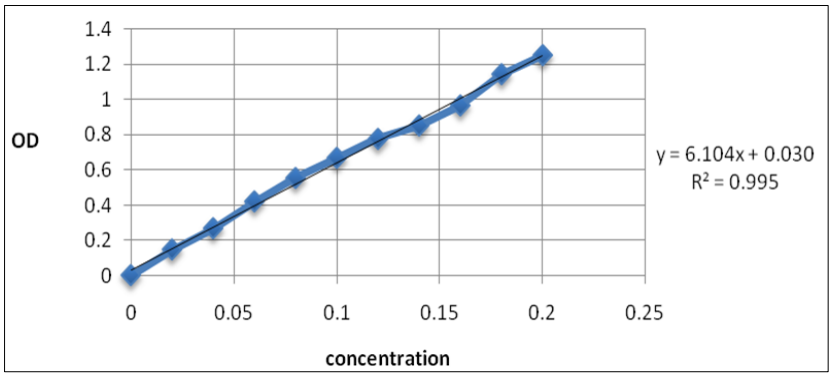

Fig 2: Standard curve of phytic acid

Polyphenols

Total polyphenols were extracted by the method of Singh & Jambunathan (1981) [52]. Defatted sample (500mg) was recirculated with 50ml methanol containing 1% HCl for four hours. The extract was concentrated by evaporating on a hot water bath and brought the final volume to 25ml with methanolic–HCl. The amount of polyphenolic compounds was estimated as tannic acid equivalent according to Folin-Davis procedure (Swain & Hillis, 1959) [54].

Reagents

1. Folin-Denis reagent: To 750ml water, 100g sodium tungstate, 20g phosphomolybdic acid and 50ml phosphoric acid were added, and heated and then refluxed for 2 hours. It was cooled and diluted to one liter.
2. Tannic acid (stock solution): 100mg of tannic acid was dissolved in water and made up to one liter. In order to have a working standard solution, 20ml stock solution was further diluted to 100ml with water.
3. Saturated aqueous sodium carbonate solution: Dissolved 350g sodium carbonate in one liter hot distilled water at 70ºC to 80 ºC, cooled and filtered through glass wool.

Procedure

Test solution (1.5ml) was diluted with distilled water upto 8.5ml in a graduated test tube. After thoroughly mixing, Folin-Denis reagent was added 0.5ml and the tubes were well shaken. Exactly after 3 minutes, one ml of saturated sodium carbonate, solution was added and the tubes were thoroughly shaken again. After an hour, the absorbance was read at 725nm on UV-VIS Spectrophotometer, using a suitable blank. If the solution was cloudy or precipitates appeared, it was centrifuged before readings were taken. A standard curve was plotted by taking 0.5ml to 4.0ml working tannic standard solution containing 10 to 80µg tannic acid.

$$\text{Polyphenols (mg/100g)} = \frac{M \times V \times 100}{W \times V_1 \times 1000}$$

Where

M = Concentration of extract elute obtained from graph
V = Volume made of extract (ml)
W = Weight (g) of the sample
V_1 = Volume of extract aliquot taken (ml)

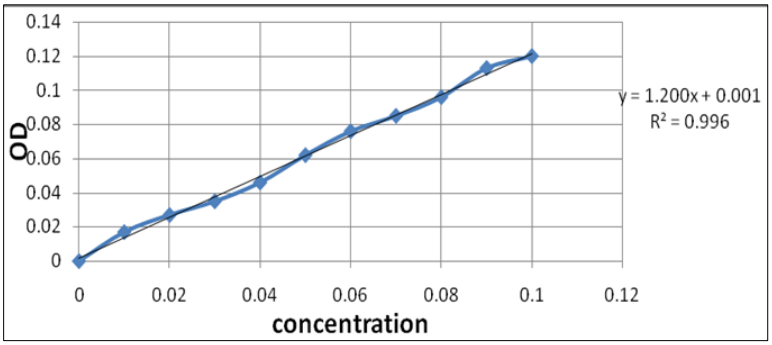

Fig 3: Standard curve for polyphenol

Total phenolic content (TPC)

The TPC in raw and cooked foods was determined according to the method of Singleton and Rossi, (1965) and modified by Saucier & Waterhouse (1999) [47]. The results were expressed in gallic acid equivalent (GAE) mg/100gm of fresh weight.

Sample Preparation

The food samples were first homogenized using mortar and pestle. The 5gm of sample was taken in a test tube for extraction overnight with 20ml of 80% methanol for raw foods and 100% methanol for cooked foods. The test tube was vortexed for two minutes and kept for overnight extraction. After the overnight extraction, the test tube was centrifuged for 10-15 min at 2000 rpm. The supernatant obtained, after centrifugation, was used for the analysis of TPC.

Reagents

1. Gallic acid (GA) standard solution (100mg)
   Stock solution: 100mg gallic acid dissolved in 100ml distilled water (D/W)
   Working solution: Took 1ml stock and volume made up 100ml with D/W
2. Folin-Ciocalteu (FC) reagent (50%): 1:1 dilution with D/W
3. Sodium carbonate (7.5%): Dissolved 7.5g Na_2CO_2 in 100ml D/W

Procedure

For each analysis, six replicates were taken and more than 1.5ml of water was pipette into each test tube. A 20µl sample solution and 20 µl of water was pipette into standard, sample and blank test tubes and mixed thoroughly. For standard solution, 1mg of gallic acid was dissolved in 1ml of distilled water. Different aliquots (2, 4, 6, 8, 10, 12, 14, 16, 18 and 20µl) were taken for obtaining standard graphs. The standard solution was freshly prepared each time. The 100 µl of FC-reagent was added to each test tube and the solutions were mixed again. A 300 µl of a 20% sodium carbonate (Na_2CO_3) solution was added to each test tube after 30 see and before 8 min. The solution was left at room temperature for 2 hr. Then the absorbency of the developed blue color was determined at 700 nm with the help of spectrophotometer. The amount of light absorbed is proportional to the amount of oxidizable material present, *viz*, phenolic compounds. For control, methanol is used in the place of extract.

Antioxidant activity (%)

Antioxidant activity was measured using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) dye as per the procedure described by Shimada *et al*. (1992) [50].

Preparation of dye

25mg DPPH dye was weighed and 10ml of methanol was added to it with vigorous shaking until the dye got dissolved. One ml of this solution was then made up to a volume of 100ml in a volumetric flask (working solution).

Extraction

500mg of sample were macerated in 10ml methanol and centrifuged at 4000 rpm. The supernatant was used to measure antioxidant activity.

Estimation

Three ml of dye working solution was mixed with 0.5ml of supernatant and incubated for 30 minutes at 25 to 30 °C in water bath and absorbance was measured with spectrophotometer at 517 nm. Dye mixed with methanol was used as a blank. Lower absorbance of reaction mixture indicated higher free radical scavenging activity.

$$\text{Antioxidant activity (\% scavenging of DPPH)} = \frac{A_0 - A_1}{A_0} \times 100$$

Where

A_0 = absorbance of blank

A_1 = absorbance of sample

Results and Discussion

Sensory evaluation for fibre and protein enriched composite bars

Sensory evaluation for fibre and protein enriched composite bars is presented in figure 4. Six different samples of composite bars along with control were analyzed for colour and appearance, aroma, taste and texture. Overall acceptability observed maximum for CB6, followed by CB3, CB4, CB2, CB1, CB5, and control, respectively.

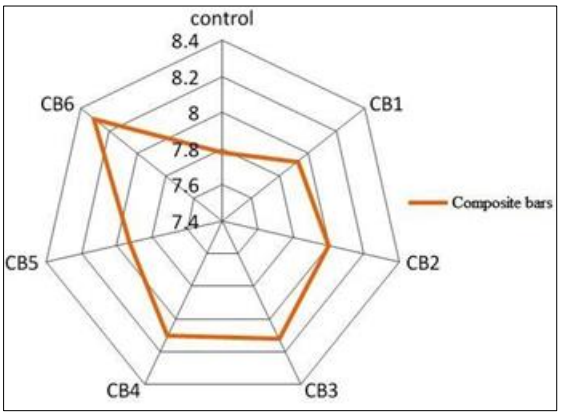

Fig 4: Sensory evaluation for fibre and protein enriched composite bars

Sensory evaluations for fibre and protein enriched composite bars are presented in figure 4. Six different samples of composite bars along with control were analyzed for colour and appearance, aroma, taste and texture. Overall acceptability was observed maximum for CB6, followed by CB3, CB4, CB2, CB1, CB5 and control respectively. Nadeem *et al.* (2012) [27] developed high protein cereal bars whose sensory evaluation revealed that protein level, texture, and taste were very much enhanced by adding 6.05% whey protein concentrate and 4.35% vetch protein isolates in date bar without disturbing any sensory parameters. Verma *et al.* (2018a) [59] and Ahmad *et al.* (2017) [5] both prepared cereal bars by using different cereal, pseudocereals, millet and legumes which are preprocessed *viz.* puffing, roasting and flaking similar to present study. Tanska *et al.* (2007) [55] reported that the best treatment from a rheological and organoleptical point of view was 5% addition of carrot pomace in wheat bread. Chilkawar *et al.* (2017) reported sensory evaluation of protein rich multigrain bar, puffed amaranth based at the rate 0, 10, 20 and 30% instead of rice crisp, reported results indicated that bar with 30% puffed amaranth was most acceptable in all sensory characteristics. Appelt *et al.* (2015) [7] reported that cereal bars were developed using oat flakes and rice flakes with fruits and vegetables by product, showed higher moisture content, fibre, fat, lower protein, and ash content.

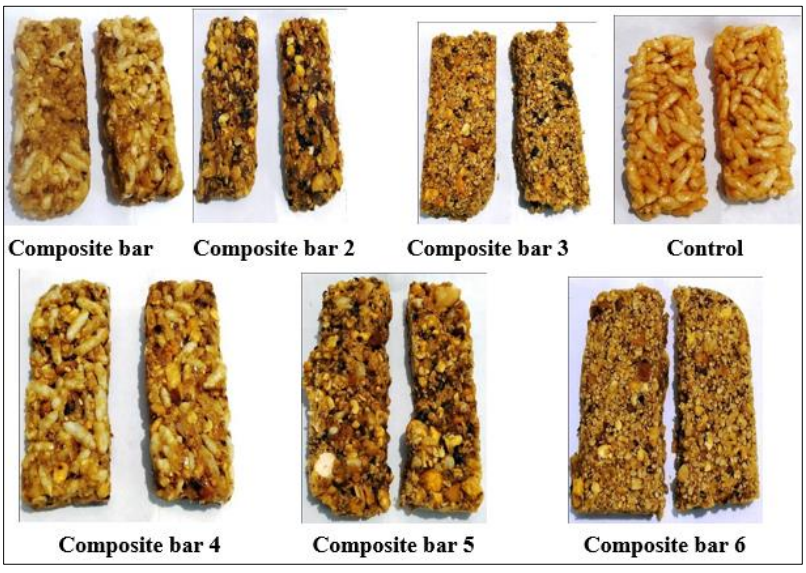

Plate 1: Fibre and protein enriched composite bars prepared from various cereals, pulses, nut and oilseeds

Physical properties of protein and fibre enriched composite bars

Table 3 exhibits the physical properties as texture and bulk density of protein and fibre enriched composite bars. All samples of the composite bars along with control were analyzed for texture and bulk density. For texture, maximum value was observed in control samples and minimum for CB2. The maximum and minimum values of bulk density were observed for CB2 (0.61g/ml) and control (0.30g/ml), respectively. Joy *et al.* (2016) [28] reported that water absorption and swelling power capacity was higher for oat bar whereas yellow maize granola had the highest value for dispersibility and bulk density. Solubility was highest for popped corn granola bars. No significant difference was observed in bulk density and water absorption capacity. Pallavi *et al.* (2013) [40] reported hardness of freshly prepared cereal bars ranged from 183 to 301 N, with gradual increase in hardness during storage. According to Nadeem *et al.* (2012) [27] protein bars having a_w (water activity in the range of 6.0–6.5%) indicated that fracturability strength amplified and continued storage period, and during storage period, rate of chemical reaction might be decreased and protein particles packed together resulting in precipitation of soluble protein due to moisture migration. Similar results were reported as texture of snack bars affected by water activity (Katz & Labuza, 1981) [29].

Loveday *et al.* (2010) suggested that the role of chemical reaction was less as compared to variation in microstructure resulted in moisture migration in hardening of protein bars. Mechanical properties of snack bars are associated with change in water activity of bar, which were probably associated with the differences in a product's microstructure and chemical composition (Lewicki *et al*., 2004) [30].

Table 3: Physical properties of protein and fibre enriched composite bars

Samples	Texture (N)	Bulk Density (g/ml)
Control	3.78±0.35	0.30±0.01
CB1	3.69±0.04	0.59± 0.01
CB2	3.44±0.38	0.61±0.01
CB3	3.73±0.07	0.32±0.01
CB4	3.68±0.15	0.59±0.01
CB5	3.50±0.07	0.60±0.02
CB6	3.70±0.02	0.32±0.01
CD at 5%	N.S	0.015

Value are mean + SD of three observations

Proximate composition of protein and fibre enriched composite bars

Proximate composition of protein and fibre enriched composite bars is shown in table 4. Six different samples of composite bars along with control were analyzed for moisture content, crude protein, crude fat, ash, crude fibre, carbohydrates, and energy.

Proximate composition of protein and fibre enriched composite bars shown in table 4. Six different samples of composite bars along with control were taken into account. All these samples were analyzed for moisture content, crude protein, crude fat, ash, crude fibre, carbohydrates and energy. Results revealed that the moisture content for CB4 (6.99g/100g), crude protein for CB5 (17.50g/100g), crude fat for CB3 (8.21g/100g), ash for CB3 (2.85g/100g), and crude fibre for CB3 (5.48g/100g) were found highest. While, for control bar, minimum values were reported for moisture (6.51g/100g), protein (5.46g/100g), fat (1.34g/100g), ash (1.46g/100g), and fibre (2.30g/100g), respectively. Carbohydrate content was found maximum for control (90.43) and minimum values for CB5 (69.53g/100g). Energy content was found maximum for CB1 (327.5 kcal) while the values for CB6 (418.9 kcal). High protein content in cereal bars was due to incorporation of groundnut, chickpea and whey protein isolate; this improves the fibre and protein quality of composite bars. Higher fat content in CB3 and CB6 were due to the presence of sesame seed and groundnut.

Ferreira *et al.* (2015) [18] shows the cereal bar formulated with addition of fruits and vegetable flour exhibited the proximate composition of cereal bar of both sweet and salty bar moisture 136.2g/kg to 274.6g/kg on dry matter basis, ash 35.7g/kg. Similar moisture content of snack bars (5.6 -11.5%) was observed by Sun-waterhouse *et al.* (2010) [53] and Pereira *et al*. (2019) [41]. Ahmad *et al*. (2017) [5] reported that protein content of granola bars was in the range of 9.7-12.51%, and similar fat content was observed 5.83-10.34%, low moisture content 2.68 to 2.69%, ash 1.54 to 1.81% and fibre 2.05 to 3.45%. Low moisture content in cereal bars leads to long shelf life without use of preservatives (Pallavi *et al.*, 2013) [40].

Table 4: Proximate composition of protein and fibre enriched composite bars

Sample	Moisture (g/100g	Crude Protein (g/100g)	Crude Fat (g/100g)	Ash (g/100g)	Crude Fibre (g/100g)	Carbohydrate (g/100g)	Energy (kcal)
Control	6.51±0.28	5.46±0.07	1.34±0.01	1.46±0.02	2.30±0.02	90.43	395.69
CB1	6.86±0.37	11.60±0.08	7.86±0.03	2.05±0.13	5.07±0.01	73.41	327.5
CB2	6.64±0.04	12.20±0.12	7.68±0.15	1.94±0.02	5.39±0.01	72.84	408.5
CB3	6.60±0.22	10.45±0.04	8.21±0.01	2.85±0.24	5.48±0.13	73.00	409.7
CB4	6.99±0.32	17.04±0.05	7.78±0.02	1.63±0.04	3.29±0.08	70.54	397.8
CB5	6.89±0.42	17.50±0.04	7.64±0.04	1.78±0.05	3.48±0.02	69.53	413.6
CB6	6.74±0.50	16.11±0.16	8.00±0.17	2.33±0.03	3.59±0.03	69.96	418.9
CD at 5%	0.566	0.163	0.167	0.195	0.134		

Value are mean + SD of three observations

The composition of oat and other cereal and legume results in high fibre and dietary fibre in products (Ahmad *et al*, 2017) [5]. Toan and Vinh (2018) [56] revealed the proximate values of nutritional bars, high moisture content, fat, protein, fibre, carbohydrate and energy whereas similar ash content were reported. Ravindra & Sunil (2018) [47] revealed that best acceptable sample three C of cereal bar (popped sorghum, amaranth seed, groundnut, roasted split chickpea, dark chocolate, corn flakes, gulkand, jaggery and liquid glucose) contains higher value for crude fibre 9%, lower value for ash 1.12%, moisture content 3.6%, fat content 5.1%, protein content 3.32%, similar carbohydrates 77.86% and energy of puffed cereal bar was 369.6 kcal. Yadav & Bhatnagar (2016) [60] reported similar results for defatted soya flour cereal bars. The results are in agreement with the findings of Aleem *et al*. (2012) [6]. Verma *et al*. (2018a) [59] reported higher moisture, protein, fat, fibre but similar ash content in cereal bars. Ho *et al*. (2016) [23] developed snack bar that contains higher value for moisture 13.23%, lower value for ash content 13%, crude protein 6.36%, 1.16% of crude fibre, 56.89% of total carbohydrate, and 454.51 kcal of energy whereas higher value for crude fat 22.39%, were observed.

Mineral composition and dietary fibre of protein and fibre enriched composite bars

The Mineral content of various fruits and vegetables candies (lemon, kinnow, carrot and pumpkin), chunks of mango and powders lemon, kinnow, carrot and pumpkin, depicted in table 5. Six different samples of composite bars along with control were analyzed for calcium, iron and zinc content. Results revealed that the calcium, iron and zinc content were found highest in CB6 (245.70mg/100g), (8.76mg/100g) and (2.97mg/100g), respectively. The values for calcium (31.06mg/100g) and iron (6.41mg/100g) were found lowest for control. In addition, the zinc content was lowest for CB2 (1.79mg/100g). Ahmad *et al.* (2017) [5] reported lower value for calcium and iron content in all formulations of cereal bars, on the other hand similar values observed in case of zinc content. High calcium content in CB3 and CB6 composite bars would be due to amaranth, as amaranth is high in calcium content. Yadav & Bhatnagar (2016) [60] reported similar calcium content and iron content. Verma *et al*. (2018a) [59] exhibited the calcium content (78.27mg/100g) of cereal bar, similar iron content (6.29mg/100g) and similar results were reported in present study for iron content for CB1, CB2 and CB3.

Table 5: Mineral compositions and dietary fibre (mg/100g) of protein and fibre enriched composite bars

Sample	Calcium	Iron	Zinc	Total soluble	Total insoluble
Control	31.06±1.72	6.41±0.03	2.68±0.03	1.01±0.02	2.42±0.09
CB1	64.10±0.89	6.71±0.04	2.17±0.08	4.76±0.10	1.39±0.03
CB2	59.93±0.24	6.58±0.06	1.79±0.03	5.41±0.04	2.33±0.04
CB3	227.71±3.70	7.96±0.05	2.43±0.04	5.12±0.06	5.03±0.10
CB4	99.95±0.36	7.15±0.04	1.97±0.04	4.68±0.04	1.34±0.11
CB5	98.99±0.64	6.83±0.18	1.88±0.04	5.26±0.04	1.97±0.04
CB6	245.70±2.25	8.76±0.18	2.97±0.03	5.67±0.02	5.04±0.05
CD at 5%	3.216	0.185	0.086	0.099	0.135

Value are mean + SD of three observations

Dietary fibre present in protein and fibre enriched composite bars depicted in table 5. Six different samples of composite bars along with control were taken in account and the above said samples were analyzed for total soluble and insoluble fibre. Maximum values of total soluble and insoluble fibres were found in CB6 (5.67mg/100g) and CB3 (5.03mg/100g), respectively. While the minimum values were observed for control bars (1.01mg/100g) and CB4 (1.34mg/100g) respectively. Verma *et al.* (2018a) [59] revealed the dietary fibre of sorghum based protein rich cereal bars, similar to the line of present study for CB3 and CB6 composite bars. CB3 and CB6 contain higher value of insoluble fibre because of higher value of insoluble fibre present in amaranth seeds. Silva de Paula *et al.* (2013) [51] observed lower value for soluble and similar insoluble dietary fibre in cereal bar. On the other hand, Marques *et al.* (2015) [34] reported significantly higher value for insoluble dietary fibre and similar dietary fibre. Faber & Yuyama (2015) [17] reported higher value for both soluble and insoluble dietary fibre in cereal bars.

Bioactive Components present in protein and fibre enriched composite bars

Bioactive components present in protein and fibre enriched composite bars are depicted in table 6 All the above said samples were analyzed for phytic acid, tannins, total phenolic and antioxidant activity. Six different samples of composite bars along with control were taken into account. The highest phytic acid content, tannins, total phenolic and percentage antioxidant activity were found in CB2 (137.04mg/100g), CB6 (68.89mg/100g), CB3 (2.55mg/100g) and CB6 (40.70%). The highest phytic acid content, tannins, total phenolic and percentage antioxidant activity was found in CB2 (137.04mg/100g), CB6 (68.89mg/100g), CB3 (2.55mg/100g) and CB6 (40.70mg/100g), respectively. While, the lowest values of phytic acid content (82.44mg/100g), tannins (32.37mg/100g), total phenolic (0.98mg/100g) and antioxidant activity (17.43mg/100g) was observed in control bar. Among fibre enriched composite bars, CB1 had lowest phytic acid, tannins, total phenolic activity and antioxidant activity. CB2 contains the highest amount of phytic acid among fibre and protein enriched composite bars. CB6 contained a higher amount of phytic acid among protein-enriched bars. CB1 contained the lowest level of tannins 59.55mg/100g TAE among all composite bars. CB3 and CB6 exhibited higher amounts of total phenolic activity, *i.e.* (2.55mg/100g GAE and 2.42mg/100g GAE). The higher value of antioxidant activity for CB6 (40.70%) was observed than followed by CB5 (38.20%). Among fibre enriched (CB1, CB2, CB3) CB3 showed higher antioxidant activity, whereas among protein (CB4, CB5, CB6) enriched bars CB6 had highest antioxidant activity.

Table 6: Bioactive components present in protein and fibre enriched composite bars

Sample	Phytic acid (mg/100g)	Tannins (mg/100g) (TAE)	Total phenolic activity (mg/100g) (GAE)	Antioxidant activity (DPPH)%
Control	82.44±3.48	32.37±0.94	0.98±0.06	17.43±1.41
CB1	125.8±0.46	59.55±0.47	1.76±0.04	28.30±0.68
CB2	137.04±1.07	61.86±0.26	1.82±0.02	30.12±0.08
CB3	133.51±1.59	68.21±0.29	2.55±0.12	33.04±0.54
CB4	131.21±1.06	60.71±0.81	1.52±0.04	31.53±1.22
CB5	131.88±0.73	62.95±0.45	1.49±0.03	38.20±1.27
CB6	135.06±0.34	68.89±0.46	2.42±0.03	40.70±0.83
CD at 5%	2.811	1.027	0.034	0.520

Value are mean + SD of three observations

Verma *et al.* (2018a) [59] reported high total polyphenols in cereal bars which are known to possess antioxidant properties. Toan and Vinh (2018) [56] reported total phenolic content of cereal bars ranges from 2.08 to 2.41mgGAE/g, respectively. Verma *et al.* (2018a) [59] revealed the higher phytic acid (465.59mg/100g) and polyphenols (578.58mg/100g) for cereal-based protein enriched cereal bars. They also found higher phenolic activity of bar prepared from HC308 (and total antioxidant activity HC308 (126.75mg/100g), HJ513 (127.26mg/100g) of protein enriched cereal bars.

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