International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 5 Sep - Oct 2024, pp: 107-115 www.ijprajournal.com ISSN: 2456-4494

Nutraceutical Characterization of formulated Contemporary Cereal Nutri bar Endowed with Pumpkin Seed Powder and Whey Protein concentrate

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Date of Submission: 05-09-2024 Date of Acceptance: 15-09-2024

ABSTRACT
Introduction: Nowadays, health-conscious consumers attend to nutritional, health, and easy-to-use products. Demand for healthy snacks is significantly increasing. Present study aimed to develop contemporary cereal nutri bars by incorporating pumpkin seed powder and whey protein concentrate powder and assess their nutraceutical characterization**.**

Study methods: We analyzed seventeen cereal nutri bars samples for sensory analysis and the best cereal nutri bar was carried out for further mineral, amino acids, sugar content and bioactive components. Mineral and amino acids content were performed following the AAS and HPLC method. Bioactive compounds of the cereal nutri bars were determined by the 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging and Ferric reducing antioxidant power (FRAP) by modified method, respectively.

Results and discussion: Results of sensory evaluation showed the T9 cereal nutri bar scored the highest mean score among all seventeen treatments of cereal nutri bars. Mineral content analysis revealed that cereal nutri bar have a significant amount of copper, iron, zinc and calcium. Essential amino acids such as cysteine, threonine, phenylalanine, lysine, methionine, tyrosine and BCAA have significant amounts in cereal nutri bar. Cereal nutri bar had significantly higher total phenolic content, total flavonoid content and antioxidant activity respectively.

Conclusion: This study showed that developed contemporary cereal nutri bar increase market's revenue and enable the snack market to develop new type snack bars.

Keywords: Cereal nutri bar, pumpkin seed powder, whey protein concentrate, sensory evaluation, amino acids, bioactive components

I. INTRODUCTION

There is a current preference for health-related foods among consumers, including a low-calorie diet containing higher amounts of protein, fiber, and antioxidants, whilst also being easy to handle, store, and consume **(Solan, 2020).** The high value of ready-to-eat products, such as snack bars, can be attributed to their ease of use. New opportunities for protein product development are offered by the growing food protein market, but they are also adding new challenges to food protein research and innovation **(Szydłowska et al., 2022).** Human growth and maintenance require energy and essential amino acids, both of which are found in food proteins. Products from natural sources have been used for centuries as nutraceutical foods **(Ranjha et al., 2022)**. In recent years, seeds and nuts have received growing attention due to the high nutraceutical and therapeutic value of their bioactive components **(Dotto and Chacha, 2020).** Pumpkin seeds, generally considered agro-industrial waste, are an extraordinarily rich source of bioactive compounds with interesting nutraceutical properties **(Bardaa, 2016).** Pumpkin (Cucurbita pepo L.) belongs to the family Cucurbitaceae generally grown in the regions of the globe as a vegetable. Pumpkin seeds are harvested worldwide due to their economical and environmentally friendly properties **(Ayyildiz et al., 2019).** In many countries, pumpkin is used as a medicine for its anti-inflammatory, antioxidant, antiviral, and antidiabetic properties. Pumpkin seeds are a good source of carotene, minerals, vitamins and bioactive components that are beneficial to health. It is believed that bioactive compounds of pumpkin have a protective role against many diseases, including hypertension,

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diabetes, and cancer and coronary heart diseases **(Batool et al., 2022).**

Nowadays,food industries and consumers are looking for ingredients, which can provide nutritional properties for formulation of various value added food products. The food industry has come to realize that whey proteins in particular have potential to improve the quality of food products. Whey is obtained from milk processing, produced as a by-product in the manufacture of cheese casein, cottage cheese (paneer) and channa **(Minj and Anand, 2020).** Whey represents a heterogeneous pool of nutrients with a wide range of physico- chemical properties. Nutritionally the most valuable component of whey is protein. Its protein has long been established as an exceptionally rich and balanced source of amino acids containing higher concentration of essential amino acids along with sulphur containing amino acids than other conventional protein sources like egg, soy and meat protein **(Madureira et al., 2007).** Minerals like calcium, phosphorus, potassium, sodium and magnesium are available as constituents of whey, which is required for normal health and supplementation in sports supplements for the athletes. According to US Food and Drug Administration, WPC is the substance obtained by the removal of sufficient non-protein constituents from whey so that finished dry product contains not less than 25% protein. Whey protein concentrates are available with protein levels ranging from 35 to 75% proteins **(Gonzalez et al., 2023).**

Bars are magnificent as far as supplement availability because of the favorable position of compactness, palatability and ease. Because of low moisture content they have a longer time span of usability and less microbial decay. Cereal nutri bars are commonly reinforced by utilizing different energy and protein or vitality rich ingredients **(Siddique et al., 2018)**. The developing acknowledgement of health, changing socio-economic needs and inadequate time to cook food with balanced nutrition leads to rapid increase demand for the processed and convenience foods. Cereal nutri bars are one of the nutritionally balanced convenient foods which has gained abundant popularity since the 1980s **(Yadav and Bhatnagar, 2016)**. TechSci Research (2019) published a report on nutrition bars market in India, stated that the Compound Annual Growth Rate (CAGR) is expected to grow at a rate of more than 30% in 2024, taking into consideration the growth of the working population, alarming increase of lifestyle diseases, better awareness of health and nutrition, rising per capita expenditure, and soaring youth population (**Radhakrishnan, 2005**). Keeping this in view the present research aimed to formulate and analyze the mineral content, amino acids, sugar content and bioactive compounds of cereal nutri bar from underutilized protein source- pumpkin seeds and whey protein concentrate which is economical agri waste as well as nutritious.

II. MATERIALS AND METHODS

Procurement of raw material

All raw ingredients used in this study such as rolled oats, dates, jaggery, rice crispies and 80% whey protein concentrate was purchased from the supermarket of Indirapuram, Ghaziabad, New Delhi. All chemicals used were analytical grade and the results were depicted as the mean value of the three replicates on a dry weight basis.

Processing of pumpkin seeds

Fresh indigenous varieties of Pumpkin (Cucurbita pepo L.) were collected from the Indian Agriculture Research Institute (IARI) New Delhi (India). Pumpkins were taken to separate the seed. After separating the seeds from the pumpkin, seeds were shade dried for 7 consecutive days and crushed into a fine powder. The powdered material was dried at 60°C for 3hr in the oven.

Formulation of cereal nutri bar

Seventeen variants of the cereal nutri bar were prepared by combining different proportions of the pumpkin seeds, whey protein concentrate and rolled oats. Peanuts (7g) rice crispies (5g), jaggery (10g) and dates (5g) were constant in all formulations. The experimental design of the variations for the preparation of cereal nutri bars depicted in **Table 1**.

Table 1 Experimental design of cereal nutri bar

Treatments	Pumpkin Seeds	Whey Protein Concentrates	Rolled Oats
T1	10	12.5	7
T2	10	10	6

T3	10	15	6
T4	10	12.5	5
T5	10	10	6
T6	12.5	12.5	6
T7	10	10	6
T8	12.5	10	5
T9	12.5	15	5
T10	10	10	6
T11	12.5	12.5	6
T12	12.5	15	7
T13	10	10	6
T14	15	12.5	7
T15	15	12.5	5
T16	15	15	6
T17	15	10	6

Preparation of cereal nutri bar

The cereal nutri bar was prepared using pumpkin seed powder, whey protein concentrate, rolled oats, rice crispies, peanuts, dates and jaggery as depicted in **Figure 1** and packed in oriented polypropylene pouches and stored at refrigeration temperature for further analysis.

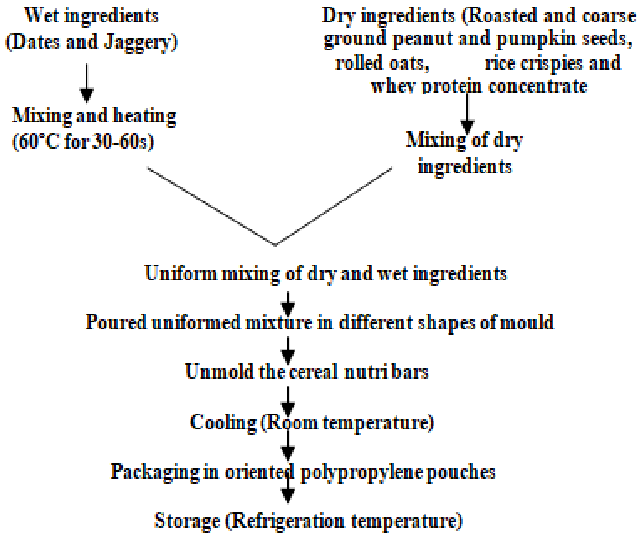

Figure 1 Flow chart of cereal nutri bar development

Sensory analysis

Sensory analysis of developed cereal nutri bar conducted according to the procedure explained by **Momanyi et al., (2020)** The mean sensory score of 30 semi-trained panelists for each sensory attribute color, appearance, taste, odor, texture, crunchiness was assessed on a 5 point composite score and overall acceptability was recorded on a 9-point hedonic scale. Best formulation of cereal nutri bar was selected on the basis of sensory evaluation and carried for further analysis respectively.

Minerals composition

Analyses of minerals were done by dry ashing and atomic absorption spectrophotometry (AAS) **(Garcia et al., 2014).** 5 g of sample weighed put into a clean and dry crucible. The crucibles were placed on a hot plate under a fume hood and the temperature increased slowly until smoking cease and the samples were thoroughly charred. They were then put in a muffle furnace and temperature increased gradually to 250° and heated for 1 h. The temperature was increased to 550° and incinerated for about 5 h. The temperature was then decreased to 300°, the crucibles removed and cooled to room temperature. The ash was transferred quantitatively to a 100 ml beaker using 20 mL of 1 N HCl and filled to the mark using 1 N HCl. The absorbance of the solutions was read by Atomic Absorption Spectrophotometer (AAS). The various mineral standards were also prepared to make the calibration curve.

Amino acids composition

The amino acids composition in the cereal nutri bar was analyzed. The analysis of amino acids was based on the method of sample hydrolysis and the analysis of the individual amino acids using HPLC (High-Performance Liquid Chromatography) (Shimadzu Nexera X2) with automated on-line Pre Column Derivatization (with the use a liquid sampler and Poroshell 120 column HPH-C18 **(Rutherfurd and Gilani, 2009).**

Analysis of sugar content

Total sugar, reducing sugar, and non-reducing sugar contents were also determined by a Lane & Eynon titration using Fehling's solution as described in **AOAC (2019)**

Analysis of bioactive compounds

Total phenolic content determination

Total phenol content (TPC) was estimated using the Folin–Ciocalteu reaction, as described by **Flores et al., 2014** with minor modifications. In brief, 0.30 mL of extract and 1.20 mL of Folin–Ciocalteu reagent were combined (diluted 1:10). After 5 min, 1.50 mL of a 7.50% Na_2CO_3 solution was added to the mixture, which was then incubated in the dark for 2 h at room temperature. A UV/Visible spectrophotometer was used to measure the absorbance at 765 nm. The TPC was calculated by extrapolating the calibration curve, which was created by preparing a gallic acid solution (0–200 µg/mL). The experiment was repeated three times, and the results were reported in milligrams of gallic acid equivalents per gram of dry weight (GAE mg/g).

Total flavonoid content determination

The total flavonoid content (TFC) of powdered cereal nutri bar extract was determined by **Kiranmai et al., (2011)**. Briefly, one milliliter of a 2% $AlCl_3$ solution was mixed with one milliliter of extract. The absorbance of the mixture was measured at 430 nm after 10 min. The total flavonoid content was expressed in milligrams of quercetin equivalents (mg QE/g). The experiment was carried out three times.

DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay

For the estimation of DPPH radical scavenging activity, 1 mL methanolic extract was added in a test tube and added 1 mL tris buffer, followed by the addition of 2 mL DPPH (2,2-diphenyl-1- picryl hydrazyl). Test tubes were incubated for 30 min in dark. Absorbance was measured at 517 nm using a spectrophotometer **(Szydłowska et al., 2020).**

FRAP (Ferric Ion Reducing Antioxidant Power) assay

The FRAP method was adopted to estimate the antioxidants according to the protocol mentioned by **Sahni and Sharma, (2020).** Ferrous sulfate (Fe) was used as standard, and results were expressed as µmol Fe (II)/100 g of dry matter.

Statistical analysis

All statistical analyses were performed using SPSS Statistics 26 Software package. The analyses were done in triplicates and the data are subjected to descriptive statistics such as mean and standard deviation. Independent't' test at ($p<0.05$) is adopted to determine the significant difference between the parameters.

III. RESULTS AND DISCUSSION

Sensory analysis

Consumer sensory testing of the cereal nutri bar was necessary in evaluating the level of liking of the snack bars. The 9 point hedonic scale and 5 point composite score approach was used to determine the sensory characteristic of the developed cereal nutri bar as depicted in **Figure 2 and 3.** Among the 17 treatments of cereal nutri bars T9 (4.56±0.49) and T14 (4.46±0.91) scored the highest mean value in appearance respectively. Mean score of taste in cereal nutri bars T9 (4.46±0.78) and T14 (4.56±0.50) scored the highest score. In odor attributes of sensory evaluation T14 (4.36±0.85) and T9 (4.23±0.67) scored the highest mean score. In texture attribute T16 (4.66±0.54) and T9 (4.56±0.62) scored higher mean scores, similarly in crunchiness of cereal nutri bars T16 (4.66±0.54) and T9 (4.46±0.59) scored higher mean scores. In overall acceptability T1 (8.53±0.62) and T14 (8.23±1.19) scored the highest mean score. Similar results were reported by **Damasceno et al., 2016** studied the replacement of 0, 50 and 100% raisin by grape bagasse in cereal bars. Lower sensory acceptance scores were observed with increasing the concentrations of grape bagasse, and the formulation containing 100% bagasse had the lowest score, despite no differences were observed among the different treatments for the attribute appearance.

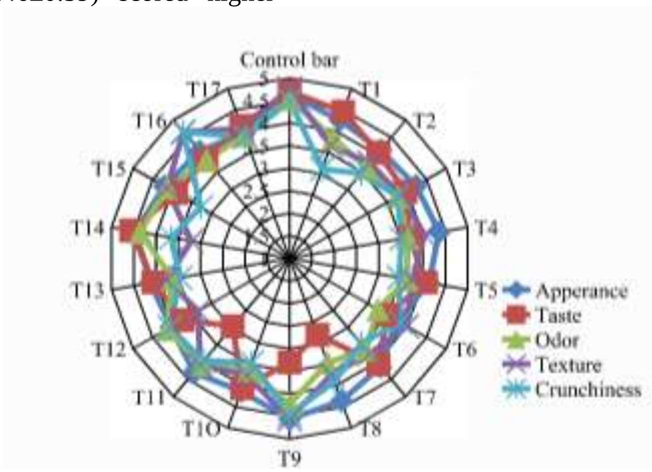

Figure 2 Sensory analysis of cereal nutri bar

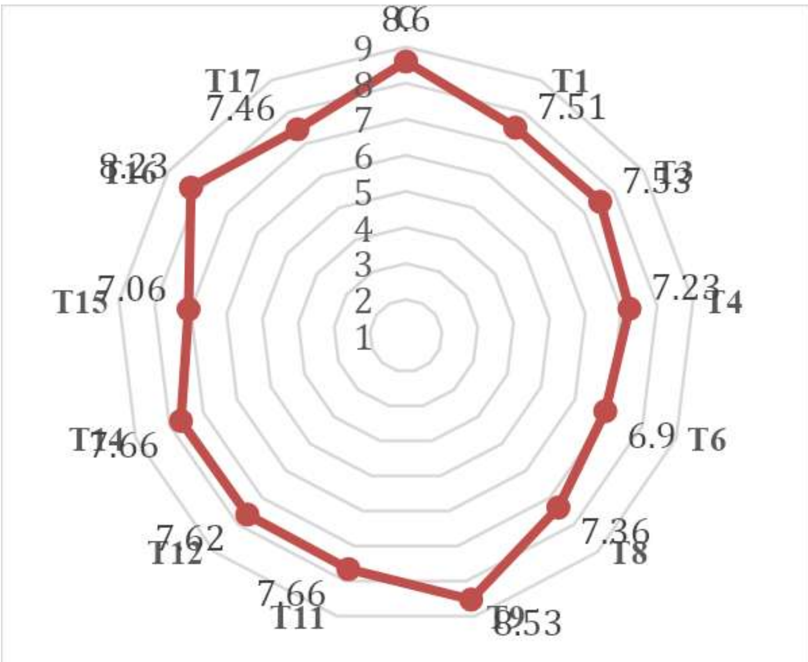

Figure 3 Overall acceptability of cereal nutri bar

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 5 Sep - Oct 2024, pp: 107-115 www.ijprajournal.com ISSN: 2456-4494

Minerals content

The mineral composition of the optimized cereal nutri bar was found significant at ($p<0.05$) when compared to the control bar **Table 2** shows the minerals content of control and optimized cereal nutri bar. The copper content of the optimized cereal nutri bar (1.48±0.05) was significantly high when compared to the control bar (0.18 ±0.03). The iron content of the optimized cereal nutri bar (10.08±0.12) was significantly high whereas the control bar consisted of a low amount (6.14±0.49) of iron content. Zinc content of optimized cereal nutri bar (4.24±0.27) was significantly higher as compared to the control (3.40±0.18) bar respectively. Calcium content in optimized cereal nutri bar (38.73±0.70) was significantly higher as compared to (33.04±1.04) control bar. Magnesium (49.30±0.56), sodium (38.92±0.13) and potassium (163.90±0.74) content in optimized cereal nutri bar were lower followed by the control bar (50.63±1.77), (41.06 ±1.38) and (177.60±2.58) respectively. **Paiva et al., 2012** studying cereal bars elaborated with residues of rice, soy, pineapple, and brown pequi, found copper 1.69, zinc 3.33 and iron 5.58 respectively stated that these differences are probably due to the type of residue used in the preparation of the cereal bars.

Table 2 Minerals content of cereal nutri bar

Minerals Content (mg/100g)	Variants		p value
	Control bar	Optimized bar	
Copper	0.18 ±0.03	1.48±0.05	0.04*
Iron	6.14±0.49	10.08±0.12	0.04*
Zinc	3.40±0.18	4.24±0.27	0.00*
Magnesium	50.63±1.77	55.30±0.56	0.03*
Sodium	41.06 ±1.38	35.92±0.13	0.13NS
Potassium	167.60±2.58	163.90±0.74	0.00*
Calcium	33.04±1.04	40.73±0.70	0.01*

Values are expressed as mean ± standard deviation. The means within column followed by different superscripts are significantly different ($p <0.05$)

Amino acids composition

The amino acid profile of optimized cereal nutri bar was found to be advantageous because it has a significant amount of essential amino acids depicted in **Table 3.** The essential amino acids as cystine (24.43±0.45), threonine (62.92±1.19) and phenylalanine (29.63±0.45) were found significantly higher in optimized cereal nutri bar than the control bar (19.5±0.36), (55.0±0.98), (25.4±0.04) respectively. BCAA as isoleucine (50.89±0.78), valine (45.32±0.73) and leucine (62.43±0.64) were significantly high in optimized cereal nutri bar when compared to the control bar (47.02±0.79), (39.16±0.45) and (55.24±1.38) respectively. Similarly lysine, methionine and tyrosine essential amino acids in optimized cereal nutri bar were consisted significant amount (72.26±1.16), (19.55±0.35) and (24.40±0.21) when compared to control bar (69.38± 1.20), (16.6±0.35) and (20.5±0.39) respectively.**Szydłowska et al., (2022)** conduct a study on novel organic high-protein bars developed and produced from organic ingredients such as prebiotic and pro-healthy additives or whey protein concentrate. A total of 17 amino acids, including 10 essential amino acids, were identified in high-protein bars. The concentrations of all essential amino acids (89.4%) whereas the non-essential amino acids score (100.7%) were higher than the FAO/WHO/UNU (2007) pattern.

Table 3 Amino acids composition of cereal nutri bar

Essential amino acids	Variants		
	Control bar	Optimized bar	p value
Lysine	69.38± 1.20	72.26±1.16	0.02*
Methionine	16.6±0.35	19.55±0.35	0.03*
Cystine	19.5±0.36	24.43±0.45	0.04*
Threonine	55.0±0.98	62.92±1.19	0.00*

Isoleucine (BCAA)	47.02±0.79	50.89±0.78	0.03*
Valine (BCAA)	39.16±0.45	45.32±0.73	0.03*
Leucine (BCAA)	55.24±1.38	62.43±0.64	0.00*
Histidine	10.5±0.23	14.72±0.19	0.01*
Phenylalanine	25.4±0.04	29.63±0.45	0.02*
Tyrosine	20.5±0.39	24.40±0.21	0.01*

Values are expressed as mean ± standard deviation. The means within column followed by different superscripts are significantly different (p <0.05)

Analysis of sugar content

Table 4 depicted the total, reducing, and non-reducing sugar in the control cereal bar was in the range of (11.85±0.09, 16.53±0.11) and (28.38±0.18) found significantly low followed by the optimized cereal nutri bar (15.69±0.09, 20.12±0.15) and (35.81±0.2) respectively.

Table 4 Sugar content of cereal nutri bar

Parameters	Control bar (%)	Optimized bar (%)	p value
Reducing sugar	11.85±0.09	15.69±0.09	0.00*
Non-reducing sugar	16.53±0.11	20.12±0.15	0.00*
Total Sugar	28.38±0.18	35.81±0.21	0.01*

Values are expressed as mean ± standard deviation. The means within column followed by different superscripts are significantly different (p <0.05)

Analysis of bioactive compounds

Total phenol and flavonoid content observed for optimized cereal nutri bar and control bar has been presented in **Table 5.** The results for total phenol revealed the significant increase in the optimized cereal nutri bar 63.50±0.43mg GAE/g as compared to the control bar (52.04±0.17mg GAE/g). However, the total flavonoid content of the optimized cereal nutri bar has significantly increased (56.48±0.39mg QE/g) than (39.12±0.12mg QE/g) of the control bar. **Kaur, 2014** reported the phenolic content of 79.00mgGAE/100g in dry soup mix containing 46.296 % whole barley flour and 23.148 % roasted flax seed powder.

Table 5 Bioactive compounds of cereal nutri bar

Antioxidant content	Variants		
	Control bar	**Optimized bar**	**p value**
TPC (mgGAE/g)	52.04±0.17	63.50±0.43	0.01*
TFC (mgQE/g)	39.12±0.12	56.48±0.39	0.02*
DPPH(%inhibition)	65.71±0.31	74.38±0.30	0.01*
FRAP (µmolfe/g)	66.45±0.50	71.55±0.56	0. 01*

Values are expressed as mean ± standard deviation. The means within column followed by different superscripts are significantly different (p <0.05)

Optimized cereal nutri bar (74.38±0.30µg/ml) was highly effective in scavenging DPPH free radicals when compared to the control bar (65.71±0.31µg/ml). The reducing ability of the optimized cereal bars was determined through FRAP (**Table 5**). Results revealed that FRAP activity of optimized cereal nutri bar (80.55±0.56µmolfe/g) significantly increased at (p<0.05) as compared to the (66.45±0.50µmolfe/g) control bar respectively.

IV. CONCLUSION

The results of the research have shown that it is possible to develop the contemporary cereal nutri bars using whey protein and pumpkin seeds. The novelty of the developed products in comparison with others available on the market is that they can be considered as concentrated, contemporary food products with a high sensory acceptance and good nutritional value. The results revealed that the developed cereal nutri bar was rich in mineral and antioxidant content when compared to the control bar. It was also observed that the snack bar had good inclination towards essential amino acid composition. This type of contemporarycereal nutri bar increases the market's revenue with boosts immunity, and is advantageous to people of all ages.

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