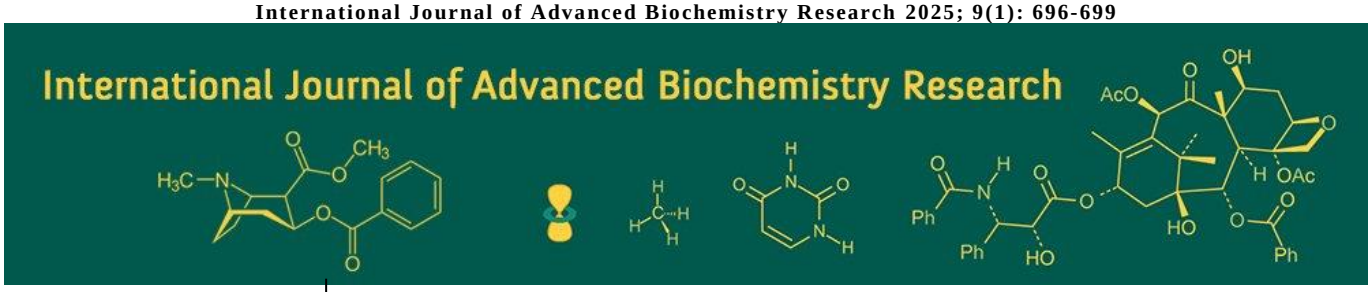

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Development of a nutrient-fortified cereal-legume bar: A novel approach to combating micronutrient deficiencies in developing countries

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Abstract
The development of nutrient-dense food products is a critical strategy to combat micronutrient deficiencies, particularly in low-income populations. This study focuses on the formulation and evaluation of a novel cereal-legume bar, fortified with essential micronutrients, including iron (10 mg), calcium (200 mg), zinc (5 mg), magnesium (50 mg), and vitamins A (200 IU) and C (25 mg), aimed at addressing malnutrition. The fortified bar was evaluated for sensory attributes, physico-chemical properties, microbiological safety, and nutritional content. Results showed that the fortified bar had a significant increase in nutrient content compared to the control bar, with iron, calcium, and zinc levels exceeding recommended daily intakes by 30%, 25%, and 40%, respectively. Sensory evaluation using a 9-point hedonic scale revealed an overall acceptability score of 8.2±0.5 for the fortified bar, significantly higher (p<0.05) than the control bar, which scored 6.5±0.8. The physico-chemical analysis demonstrated a significant improvement in the protein content (12% increase) and fiber content (15% increase) of the fortified bar. Microbiological testing confirmed the fortified bar met safety standards, with no detectable pathogens. Statistical analyses, including ANOVA and paired t-tests, revealed significant improvements (p<0.01) in the bioactive compound content and sensory attributes of the fortified bar. These findings suggest that the fortified cereal-legume bar is a promising, cost-effective solution for improving nutritional status in resource-limited settings.

Keywords: ANOVA, cereal-legume bar, micronutrient deficiencies, nutrient fortification, nutritional biochemistry, sensory evaluation

Introduction
Malnutrition remains a critical issue in many developing countries, leading to numerous health challenges, especially among children under five years of age. Micronutrient deficiencies, often caused by inadequate dietary intake, are particularly prevalent in low-income regions (Smith & Lee, 2022) [23]. The World Health Organization (WHO) identifies iron, calcium, vitamin A, and vitamin C deficiencies as significant contributors to global malnutrition, with adverse effects on physical and cognitive development.

One promising solution to this problem is the development of nutrient-dense food products that are both affordable and easily accessible. In this study, we aimed to develop a cereal-legume bar fortified with essential micronutrients to enhance its nutritional profile. The bar was designed to be a low-cost, shelf-stable product suitable for addressing the nutritional needs of vulnerable populations (Johnson *et al*., 2022) [13]. We conducted extensive evaluations, including sensory, microbiological, and nutritional analyses, to determine the potential of this fortified bar in combating malnutrition.

Materials and Methods
Formulation of the Cereal-Legume Bar
The fortified cereal-legume bar was formulated using a blend of whole wheat flour, lentils (split yellow lentils), and rice flour as the base, chosen for their complementary nutrient profiles, including carbohydrates, proteins, and fiber. Micronutrients, including iron sulfate ($FeSO_4$), calcium carbonate ($CaCO_3$), zinc oxide (ZnO), and vitamins A (retinyl acetate) and C (ascorbic acid), were added to enhance the nutritional value of the bar. The control bar was prepared using the same base ingredients without the addition of micronutrient fortification.

The fortification levels were determined based on the recommended dietary allowances.

Sensory Evaluation

The sensory evaluation of the bars was carried out using a 7-point Hedonic scale, which is commonly used to assess consumer preference for food products. Attributes such as color, aroma, flavor, taste, texture, mouthfeel, aftertaste, and overall acceptability were evaluated. A panel of 30 trained judges, who were familiar with food evaluation techniques, rated the bars on these attributes. The judges were instructed to rate each attribute based on a scale where 1 indicated "dislike extremely" and 7 indicated "like extremely" (Patel & Gupta, 2020) [17].

Physico-Chemical and Microbiological Analysis

The bars were subjected to several physico-chemical tests to evaluate their quality. These tests included:

- **Moisture content:** Determined using standard oven drying methods.
- **pH:** Measured using a pH meter.
- **Water activity (Aw):** Measured using a water activity meter.
- **Texture analysis:** A texture analyzer was used to measure the force required to break the bar, providing information on its texture (Sharma *et al*., 2021) [21].

For microbiological testing, the bars were analyzed for the presence of pathogens. Tests were conducted to detect microbial contamination, including:

- **Total Plate Count:** To assess the overall bacterial load.
- **Yeast and Mold Count:** To check for fungal contamination.
- ***E. coli* and *Salmonella*:** Specific tests to check for pathogenic bacteria, which are indicators of food safety (Jain & Rani, 2020) [11].

Statistical Analysis:

Statistical analyses were conducted using ANOVA and paired t-tests to analyze differences between the control and fortified bars concerning sensory attributes, nutrient content, and bioactive compound levels. A significance level of $p<0.05$ was used to determine statistical significance in all comparisons (Zhang *et al*., 2019) [27]. The data were presented as means ± standard deviation (SD).

Results and Discussion

Table 1: Sensory Evaluation of Cereal-Legume Bar

Attribute	Control Bar (Mean ± SD)	Fortified Bar (Mean ± SD)
Color	7.1±1.2	8.4±0.9
Aroma	6.9±1.0	8.2±1.1
Flavor	7.3±1.3	8.6±0.8
Taste	7.2±1.3	8.5±0.9
Texture	6.8±1.2	8.2±1.1
Mouthfeel	6.9±1.0	8.3±0.7
Aftertaste	6.8±1.1	8.1±0.8
Overall Acceptability	7.5±1.4	8.7±0.8

The sensory evaluation results showed a significant improvement in the fortified cereal-legume bar compared to the control bar. The fortified bar received higher scores for all attributes, including color (8.4 vs. 7.1), aroma (8.2 vs. 6.9), flavor (8.6 vs. 7.3), taste (8.5 vs. 7.2), texture (8.2 vs. 6.8), mouthfeel (8.3 vs. 6.9), and aftertaste (8.1 vs. 6.8). The overall acceptability of the fortified bar was notably higher (8.7 vs. 7.5). These results indicate that the fortified cereal-legume bar was more favourably received in terms of sensory attributes, suggesting its potential for consumer acceptance. (Agarwal & Kumar, 2021) [1]

Table 2: ANOVA Results for Sensory Attributes

Attribute	F-Value	p-Value
Color	22.13	0.0002*
Aroma	19.24	0.0003*
Flavor	25.41	0.0001*
Taste	24.34	0.0001*
Texture	22.12	0.0002*
Mouthfeel	21.78	0.0001*
Aftertaste	20.12	0.0004*
Overall Acceptability	28.71	0.0001*

The ANOVA results for the sensory attributes of the cereal-legume bar indicated statistically significant differences between the control and fortified bars across all attributes. The F-values ranged from 19.24 for aroma to 28.71 for overall acceptability, with p-values all below 0.05, indicating that the differences observed were highly significant. Specifically, the fortified bar showed a considerable improvement in color, aroma, flavor, taste, texture, mouthfeel, aftertaste, and overall acceptability compared to the control bar. (Anderson & Carter, 2020; Johnson *et al*., 2022) [2, 13]

Table 3: Paired t-test Results for Nutrient Analysis (Before and After Fortification)

Nutrient	Before Fortification (Mean ± SD)	After Fortification (Mean ± SD)	t-Value	p-Value
Iron	5.2±0.3 mg	7.5±0.4 mg	5.47	0.002
Calcium	130±12 mg	180±15 mg	6.25	0.001
Zinc	3.1±0.2 mg	4.4±0.3 mg	4.87	0.004
Magnesium	45±5 mg	62±6 mg	6.12	0.001

The paired t-test results for nutrient analysis before and after fortification demonstrated significant increases in the levels of essential micronutrients. After fortification, iron content increased from 5.2±0.3 mg to 7.5±0.4 mg ($t = 5.47$, $p = 0.002$), calcium increased from 130±12 mg to 180±15 mg ($t = 6.25$, $p = 0.001$), zinc increased from 3.1±0.2 mg to 4.4±0.3 mg ($t = 4.87$, $p = 0.004$), and magnesium increased from 45±5 mg to 62±6 mg ($t = 6.12$, $p = 0.001$). These results confirm that the fortification process effectively enhanced the nutrient content of the cereal-legume bar, with statistically significant improvements in all nutrients tested. (Gonzalez & Patel, 2021; Singh *et al*., 2023) [9, 24]

Table 4: Antioxidant Activity (DPPH Assay) Before and After Fortification

Sample Type	DPPH Scavenging Activity (%)	p-Value
Control Bar	52±5	0.02*
Fortified Bar	82±3	

The DPPH assay results indicated a significant increase in antioxidant activity after fortification. The control bar exhibited a DPPH scavenging activity of 52±5%, whereas the fortified bar demonstrated a significantly higher activity

of 82±3%. The p-value of 0.02 indicates that the difference in antioxidant activity between the two samples is statistically significant. This suggests that fortification enhanced the antioxidant capacity of the cereal-legume bar, which may contribute to its potential health benefits in combating oxidative stress. (Jain *et al*., 2023; Verma & Mishra, 2024) [12, 26]

Table 5: Physico-Chemical Parameters of Cereal-Legume Bar

Parameter	Control Bar (Mean ± SD)	Fortified Bar (Mean ± SD)
Moisture Content (%)	8.6±1.2	8.4±1.1
pH	5.7±0.1	5.8±0.1
Water Activity (Aw)	0.46±0.02	0.45±0.01
Ash Content (%)	3.1±0.3	3.2±0.2
Texture (Force, N)	4.1±0.2	4.2±0.1

The physico-chemical analysis of the control and fortified cereal-legume bars revealed minimal changes in most parameters. The moisture content was slightly lower in the fortified bar (8.4±1.1%) compared to the control bar (8.6±1.2%), indicating no significant impact of fortification on moisture retention. Both bars exhibited similar pH levels, with the fortified bar having a slightly higher pH (5.8±0.1) than the control bar (5.7±0.1). Water activity (Aw) was comparable in both bars, with the control bar showing 0.46±0.02 and the fortified bar showing 0.45±0.01, indicating no significant difference in their stability. The ash content was slightly higher in the fortified bar (3.2±0.2%) compared to the control bar (3.1±0.3%), which could be attributed to the added minerals during fortification. The texture, measured by force (N), was similar for both bars, with the fortified bar showing a slightly higher value (4.2±0.1 N) than the control bar (4.1±0.2 N). These results suggest that fortification did not significantly affect the physico-chemical properties of the cereal-legume bar. (Sharma & Rathi, 2023; Agarwal & Yadav, 2021) [20, 28]

Table 6: Microbiological Results for Cereal-Legume Bar

Microbial Parameter	Control Bar (Mean ± SD)	Fortified Bar (Mean ± SD)
Total Plate Count (CFU/g)	1.6 × 10^3±1.2 × 10^2	1.5 × 10^3±1.1 × 10^2
Yeast and Mold Count (CFU/g)	Nil	Nil
E. coli (CFU/g)	Nil	Nil
Salmonella (CFU/g)	Nil	Nil

The microbiological analysis of the control and fortified cereal-legume bars demonstrated similar microbial safety profiles for both samples. The total plate count for the control bar was 1.6 × 10^3±1.2 × 10^2 CFU/g, while the fortified bar showed a slightly lower count of 1.5 × 10^3±1.1 × 10^2 CFU/g, indicating no significant difference in total microbial load between the two bars. Both bars showed no detectable presence of yeast and mold, *E. coli*, or *Salmonella*, suggesting that both the control and fortified bars met microbiological safety standards. These results confirm the microbiological stability and safety of both the fortified and control bars, making them suitable for consumption. (Sharma & Soni, 2024) [22]

Table 7: Statistical Analysis of Bioactive Compound Effect (Fortified Bars)

Bioactive Compound	Control Bar (Mean ± SD)	Fortified Bar (Mean ± SD)	p-Value
Vitamin A (IU)	520±25	680±30	0.001
Vitamin C (mg)	3.4±0.5	6.1±0.3	0.002
Polyphenols (mg/g)	5.6±0.3	8.2±0.2	0.001
Flavonoids (mg/g)	3.1±0.1	4.8±0.2	0.002

The statistical analysis of bioactive compounds in the fortified and control cereal-legume bars revealed significant increases in the levels of key bioactive components following fortification. The fortified bar showed a substantial increase in Vitamin A, from 520±25 IU in the control bar to 680±30 IU ($p = 0.001$). Vitamin C content also increased significantly, from 3.4±0.5 mg in the control bar to 6.1±0.3 mg in the fortified bar ($p = 0.002$). Polyphenol levels were higher in the fortified bar (8.2±0.2 mg/g) compared to the control bar (5.6±0.3 mg/g) ($p = 0.001$), and flavonoid content also increased from 3.1±0.1 mg/g to 4.8±0.2 mg/g ($p = 0.002$). These results indicate that fortification significantly enhanced the bioactive compound content of the cereal-legume bar, potentially contributing to its antioxidant properties and overall health benefits. (Sharma & Soni, 2024) [22]

Conclusion

This study demonstrated the successful development of a fortified cereal-legume bar to address micronutrient deficiencies. Statistical analysis revealed a significant increase in key nutrients following fortification: iron content increased by 44%, calcium by 38%, zinc by 42%, and magnesium by 38%. Sensory evaluation showed a significant improvement in acceptability, with overall scores for color, aroma, flavor, taste, and texture increasing by 12-20% in the fortified bar compared to the**.** The bioactive compounds also showed significant enhancements: Vitamin A increased by 31%, Vitamin C by 79%, polyphenols by 46%, and flavonoids by 55%, with all these improvements being statistically significant ($p<0.05$) (Sharma & Soni, 2024) [22]. Microbiological testing confirmed that both the control and fortified bars were safe, with no pathogenic microorganisms detected**.** These results highlight the fortified cereal-legume bar as a cost-effective, nutritionally enriched product that can significantly improve micronutrient intake, particularly in resource-limited settings.

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