

## Iron Accessibility and Antioxidant Potential of Fortified Cereal-fruit Bar

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### ABSTRACT

Considering the increasing rates of anemia globally, and in view of increasing popularity of snack bars, study was planned with the objective to develop iron fortified cereal-fruit bars and evaluate different methods of iron fortification for *in vitro* iron accessibility and availability. Four types of bars with cereal-fruit nut combination were formulated using different fortification methods i.e. no fortification, bio-fortification, direct fortification and encapsulated fortification. Iron accessibility and availability were analyzed in terms of soluble and dialyzable iron. The developed bars were also analyzed for antioxidant potential and *in vitro* starch and protein digestibility. Iron fortification with encapsulation provided significantly higher ( $P < 0.05$ ) iron accessibility (78.23%) as well as availability (70.81%). The non fortified, bio fortified and direct fortified cereal-fruit bars, respectively, had 57.35, 57.39 and 76.69% accessibility. The iron availability, respectively, was 49.84, 49.85 and 66.62%. The developed bars presented a good nutritional and antioxidant profile also. The total phenolic content, total flavonoids content and DPPH radical scavenging activity in developed products ranged from 248.41 to 249.73 mg GAE/100 g, 124.63 to 126.30 mg RE/100g and 24.24 to 25.64 mg TE/100 g, respectively. The cereal fruit bar developed using biofortified wheat provided highest *in vitro* digestibility for protein (72.30%) and starch (52.56 mg maltose released/g). Anemia is fast becoming an unsolved global issue and a very pertinent national challenge for India. A nutrition filled iron fortified cereal fruit bar can be a readily acceptable approach to meet out their iron requirements. The present study explores different methods of fortification for better iron accessibility.

**Key words:** Health food, iron fortification, iron encapsulation, iron *in vitro* accessibility, *in vitro* digestibility

### INTRODUCTION

The last few decades have seen a major shift in food consumption habits of young adults with a shift towards ready-to-use products. However, there is also an increase in consumer awareness. Consumers now demand the product to be "healthy or functional" (Campisi *et al.*, 2024). Cereal snack bars are fast becoming a common food item for all age groups and have the potential to contribute towards a person's overall nutrition.

At the same time, anemia is fast becoming an unsolved global issue. In 2021, the global prevalence of anemia across all ages was 24.3%, corresponding to 1.92 billion prevalent cases and caused 52.0 million YLDs (years lived in disability) in 2021. The most common causes of anemia YLDs in 2021 were dietary

iron deficiency (Gardner *et al.*, 2023). Data from the National Family Health Survey (NFHS) 4 and 5 in India also indicate an increase from 54.1 to 59.1% anemic women in age group of 15-19 years. Similarly, the per cent non-pregnant anemic women in the reproductive age group have also increased from 53.2 to 57.2% (Maji *et al.*, 2023).

Fortification and biofortification are emerging as relatively cost-effective and enduring micronutrient supply approaches, which are profitable tactics in addition to intravenous and oral supplementation along with other dietary and nutritional education policies such as management of parasitic infections and dietary diversification (Mushtaq *et al.*, 2024; Hamad and Singh, 2025). Biofortification enhances the nutrient content of crops during their growth and is considered as a sustainable, long-term

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solution. Direct fortification adds nutrients to food during processing and is a quicker way to address current iron deficiency in any population. Direct fortification is, therefore, a more commonly applied approach. The encapsulation provides further protection and improved absorption to fortificants while reducing related negative gastric effects (Seck *et al.*, 2024; Swain *et al.*, 2024).

Considering the increasing rates of anemia globally, and in view of changing food trends, study was planned with the objective to develop cereal-fruit bars (CFB) using different methods of fortification and evaluate their iron accessibility. The sensory, nutritional and antioxidant properties of the iron-fortified cereal fruit bar (CFB) were also examined.

## MATERIALS AND METHODS

The collection of the food ingredients used in study complied with university guidelines. The required cereal for the development of cereal-fruit bar (CFB) i.e. non-biofortified wheat (HD 2967) and biofortified wheat (WH 1124) were obtained from Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar, India in a single lot. For direct fortification, the vitamin-mineral premix was obtained from the Nutaste Food and Drink Labs Pvt. Ltd., Gurugram. Additional ingredients i.e. the date fruit, almonds, cashew, sesame seeds and honey for the development of iron-fortified cereal fruit bar were procured in single lots from the local market in Hisar, Haryana, India.

Four types of iron-fortified Cereal Fruit Bar (CFB) were standardized detailed as:

- (i). CFB I (Non bio fortified wheat; no fortification)

- (ii). CFB II (Bio fortified wheat)  
 (iii). CFB III (Non bio fortified wheat with direct fortification)  
 (iv). CFB IV (Non bio fortified wheat with encapsulated fortification)

A vitamin-mineral premix comprising vitamin C, vitamin B12, folic acid and ferric pyrophosphate was used for fortification of the bars. Ferric pyrophosphate is WHO approved salt for fortification and was preferred because of its colour neutrality (Vatandoust and Diosady, 2022; Dehnad *et al.*, 2024). The level of fortification was adjusted to provide 50% of the recommended dietary allowances of iron i.e. 14.5 mg/day (ICMR, 2020) for the young adults (18-25 years) per recipe (Table 1). All the ingredients were folded together on a slow flame. The paste was rolled out evenly to a thickness of half inch, refrigerated and shaped into bars. One recipe culminated in three bars of 85±2 g. Since the developed bars were expected to be taken as snacks and not replace any meals, provision of 50% RDA avoided the risk of toxicity.

A patent application no. 202511005780 for the invention titled "iron granules for fortification of food" is under consideration at the patent office (India).

The developed iron fortified cereal fruit bars (CFB) were subjected to nutritional evaluation. For each analysis, three independent samples were drawn and each sample was analyzed in duplicate. The proximate parameters including moisture (%), crude fiber (%), crude protein (%), crude fat (%) and ash (%) were estimated according to the AOAC guidelines (AOAC, 2023).

The total carbohydrate was calculated by the difference method.

Total carbohydrate (%) = 100 – [Moisture (%) +

**Table 1.** Ingredients used for the development of cereal-fruit bar (CFB)

Ingredients	CFB I amount (g)	CFB II amount (g)	CFB III amount (g)	CFB IV amount (g)
Non bio-fortified wheat (broken and roasted)	50	-	50	50
Bio-fortified wheat (broken and roasted)	-	50	-	-
Dates (deseeded and made into paste)	100	100	100	100
Almonds	10	10	10	10
Cashew nuts	20	20	20	20
Sesame seeds	20	20	20	20
Honey	80	80	80	80
Cardamom powder	A pinch	A pinch	A pinch	A pinch
Ghee	5	5	5	5
Vitamin-mineral premix	-	-	420 mg	-
Vitamin-mineral premix (encapsulated)	-	-	-	850 mg

Crude protein (%) + Crude fat (%) + Total ash (%)]

The quantification of the total iron content was carried out using Atomic Absorption Spectrophotometer 2380, manufactured by Perkin Elmer (USA). Iron accessibility and availability were determined through modified *in vitro* assays employing the solubility and dialysis methods (Rebellato *et al.*, 2017). The method involved mimicking the gastrointestinal digestion, measuring the soluble fraction and further dialysis across a semipermeable membrane.

Iron availability (%) = Dialyzable iron/Total iron x 100

DPPH radical scavenging activity (Dinev *et al.*, 2021), total phenolic content, total flavonoid content and Vitamin C (AOAC, 2023) were also analyzed using standard methods. The phenolic content, flavonoids content and DPPH radical scavenging activity results were expressed, respectively, as Gallic Acid Equivalent (GE), Rutin Equivalents (RE) and Trolox equivalent (TE).

The developed samples were also studied for *in vitro* protein and starch digestibility, phytic acid and trypsin inhibitor activity.

The results were based on the interpretation of the appropriate statistical analysis. One-way analysis of variance (ANOVA) and Duncan's multiple range tests were used to determine the significant difference ( $P < 0.05$ ) for the experiment data. Statistical software package SPSS (Version 24) was used. Values were expressed as mean  $\pm$  SD. Critical difference (CD) was calculated using online statistical package OPSTAT (CCSHAU, India).

## RESULTS AND DISCUSSION

The comprehensive assessment of sensory attributes, including colour, appearance, flavour, texture, taste and overall acceptability, was conducted by a panel of 10 semi-trained

judges using a 9-point Hedonic Rating Scale for the evaluation. All the developed variants scored between 7.10 and 8.50 for aroma, colour, appearance, taste and texture of all the parameters analyzed. The scores lied between "like moderately" and "like very much".

The developed bars had moisture per cent ranging from  $28.51 \pm 0.12$  (CFB III) to  $30.73 \pm 0.08$  g/100 g (CFB I) with significant ( $P < 0.05$ ) differences among them (Table 1). The amount of moisture in cereal- fruit bar is a crucial factor since it influences a product's taste, texture and appearance as well as its shelf life. The crude protein content for CFB II (11.61%) was observed to be significantly higher to other cereal-fruit bar variants, while no significant difference was observed among CFB I, II and III. The significantly higher protein content of CFB II might be due to difference in wheat variety in CFB II considering there was no quantitative or qualitative difference in other ingredients. The fat content of different variants of cereal-fruit bars ranged from  $20.31 \pm 0.32$  to  $20.93 \pm 0.33$  g/100 g with no statistically significant difference among them.

The crude fiber content for CFB II bar was 4.85% and was significantly higher to other types of developed cereal-fruit bars. Prominent crude fiber content may be ascribed to the bars being derived from whole grains and dates. The ash content was 3.55, 3.89, 3.67 and 3.95% for CFB I, II, III and IV, respectively. CFB III cereal fruit bar had significantly higher total carbohydrate content i.e. 36.71%, respectively, as compared to CFB I, II and IV cereal fruit bar which contained 34.47, 34.91 and 34.88%, respectively. The proximate composition of any food reveals its basic chemical makeup and is a crucial tool for assessing and comparing the nutritional profiles of various food items.

The total iron content of CFB I, II, III and IV fortified cereal-fruit bar was observed as 9.59, 10.07, 35.44 and 35.60 mg/100 g, respectively.

**Table 2.** Proximate composition of developed iron fortified cereal fruit bar (% , db)

Treatments	Moisture	Crude protein	Crude fat	Crude fiber	Ash	Total CHOs
CFB I	$30.73 \pm 0.08^a$	$10.37 \pm 0.01^b$	$20.88 \pm 0.08^a$	$4.40 \pm 0.02^b$	$3.55 \pm 0.20^b$	$34.47 \pm 0.06^b$
CFB II	$29.28 \pm 0.06^b$	$11.61 \pm 0.18^a$	$20.31 \pm 0.32^a$	$4.85 \pm 0.03^a$	$3.89 \pm 0.06^a$	$34.91 \pm 0.14^b$
CFB III	$28.51 \pm 0.12^c$	$10.56 \pm 0.19^b$	$20.55 \pm 0.02^a$	$4.13 \pm 0.03^b$	$3.67 \pm 0.13^{ab}$	$36.71 \pm 0.03^a$
CFB IV	$29.48 \pm 0.17^b$	$10.76 \pm 0.12^b$	$20.93 \pm 0.33^a$	$4.25 \pm 0.06^b$	$3.95 \pm 0.12^a$	$34.88 \pm 0.37^b$
C. D. ( $P < 0.05$ )	0.37	0.47	0.75	0.35	0.30	0.66

Values are mean  $\pm$  SE of six independent replications. Values with different superscript in the same columns are significantly different ( $P \leq 0.05$ ).

Significant difference was observed in total iron content of CFB I and CFB II. This might be explained by use of different wheat varieties. While HD 2967 (CFB I) is a conventional wheat variety; WH 1124 (CFB II) has been developed at CCS Haryana Agricultural University, Hisar, India through selection. Higher total iron content of CFB III and IV can be explained as result of fortification. CFB IV with encapsulated fortification had significantly higher soluble iron content (27.85 mg/100 g) (Table 3).

CFB I, II, III and IV fortified cereal-fruit bars contained 4.78, 5.02, 23.61 and 25.21 mg/100 g of dialyzable iron content, respectively. No significant difference was observed in soluble or dialyzable iron content of CFB I and CFB II. The dialyzable iron content of CFB IV (25.21 mg/100 g) fortified cereal fruit bar was found significantly higher to CFB III (23.61 mg/100 g), CFB II (5.02 mg/100 g) and CFB I (4.78 mg/100 g) cereal-fruit bars. The per cent iron availability after dialysis through semi-permeable membrane was found to be 49.84, 49.85, 66.62 and 70.81% in the CFB I, II, III and IV cereal-fruit bars, respectively (Table 3). The total iron content of foods is not an indication of its accessibility. The absorption of iron from any food depends on certain factors including the chemical form of iron, presence of inhibitory factors such as phytates and enhancing factors such as presence of Vitamin C (Pannu *et al.*, 2025).

Despite non-significant difference in total iron content of CFB III and CFB IV, accessibility in terms of both soluble and dialyzable iron was higher for CFB IV which contained encapsulated iron premix along with Vitamin C. This may be ascribed to reduced interactions of fortificant with external environment of food matrix due to encapsulation. Furthermore, the presence of ascorbic acid is known to enhance the absorption of iron from plant foods. It forms

a chelate with ferric iron  $Fe^{3+}$  at acidic pH of stomach. This chelate remains soluble in the alkaline environment of small intestine also and is easily absorbed. It also increases the iron absorption by reducing ferric iron to its ferrous form which is the preferred form absorbed by enterocytes (Piskin *et al.*, 2022; Pepi *et al.*, 2024).

These results are in agreement to previous studies in terms of iron availability who have reported the relative iron bioavailability of ferric pyrophosphate (micronized) between 82 to 92% when used in powder form (Man *et al.*, 2022). Another study by Baldelli *et al.*, (2023) reported development of spray-dried iron gluconate microparticles for food fortification. Encapsulation reduced the toxicity to Caco-2 and Hep-G2 cell lines and reached iron bioavailability of 60%.

The total phenolic content of CFB IV (249.73 mg GAE/100 g) cereal fruit bar was highest followed by CFB I (248.66 mg GAE/100 g), CFB III (248.47 mg GAE/100 g) and CFB II (248.41 mg GAE/100 g) cereal fruit bar (Table 4). The total flavonoids content of CFB IV (126.30 mg RE/100 g) bar was higher as compared to CFB I, II and III. The differences were statistically significant.

DPPH radical scavenging activity of CFB IV (25.64 mg TE/100g) bar was higher as compared to other developed variants. The vitamin C content was also higher in CFB IV (178.48 mg/100g) followed by CFB III (1.87 mg/100 g), CFB I (1.80 mg/100 g) and CFB II (1.63 mg/100 g) cereal-fruit bars (Table 5). The differences were statistically significant.

The presence of dates, nuts and honey well explains the antioxidant profile of developed cereal fruit bars. Dates are preferred choice for developing snack bars because of their exceptional nutritional and functional properties. While providing a binding medium, dates also add to the antioxidant profile of any

**Table 3.** Total, soluble and dialyzable iron content of iron fortified cereal fruit bars (db)

Treatments	Total iron (mg/100 g)	Soluble iron (mg/100 g)	Dialyzable iron (mg/100 g)
CFB I	9.59±0.06 <sup>c</sup>	5.50±0.09 <sup>c</sup> (57.35)	4.78±0.06 <sup>c</sup> (49.84)
CFB II	10.07±0.03 <sup>b</sup>	5.78±0.14 <sup>c</sup> (57.39)	5.02±0.08 <sup>c</sup> (49.85)
CFB III	35.44±0.04 <sup>a</sup>	27.18±0.06 <sup>b</sup> (76.69)	23.61±0.09 <sup>b</sup> (66.62)
CFB IV	35.60±0.06 <sup>a</sup>	27.85±0.09 <sup>a</sup> (78.23)	25.21±0.07 <sup>a</sup> (70.81)
C. D. (P≤0.05)	0.33	0.34	0.25

Values are mean ± SE of six independent replications. Values with different superscript in the same columns are significantly different (P≤0.05). Figures in parentheses under the column soluble iron indicate the per cent accessibility. Figures in parentheses under the column dialyzable iron indicate the per cent availability.

**Table 4.** Antioxidant profile of developed iron fortified cereal fruit bar (db)

Variables	Total phenolic content (mgGAE/100 g)	Total flavonoids (mgRE/100 g)	DPPH radical scavenging activity (mgTE/100 g)	Vitamin C (mg/100 g)
CFB I	248.66±0.04 <sup>b</sup>	124.64±0.08 <sup>b</sup>	24.65±0.07 <sup>b</sup>	1.80±0.10 <sup>b</sup>
CFB II	248.41±0.09 <sup>c</sup>	124.64±0.13 <sup>b</sup>	24.24±0.06 <sup>c</sup>	1.63±0.21 <sup>b</sup>
CFB III	248.47±0.04 <sup>b<sup>c</sup></sup>	124.63±0.13 <sup>b</sup>	24.48±0.07 <sup>b</sup>	1.87±0.05 <sup>b</sup>
CFB IV	249.73±0.06 <sup>a</sup>	126.30±0.10 <sup>a</sup>	25.64±0.06 <sup>a</sup>	178.48±0.04 <sup>a</sup>
C. D. (P≤0.05)	0.20	0.35	0.20	0.39

Values are mean ± SE of six independent replications. Values with different superscript in the same columns are significantly different (P≤0.05).

developed product (Maqsood *et al.*, 2020). Antioxidants are known to preserve health against the harmful effects of free radicals by reducing or repairing the damage. Oxidative stress by free radicals has often been implicated in pathology of many diseases such as cancer, metabolic syndrome, atherosclerosis, as well as neurodegenerative diseases (Kiran *et al.*, 2023). A high antioxidant content of cereal fruit bars is, therefore, a major consumer appeal for consumers (Kosicka *et al.*, 2022; Manisha *et al.*, 2023) bringing them under the umbrella of functional foods.

The CFB IV sample in the study, had high Vit C content due to its addition in encapsulated form. Despite a much higher Vit C content, the corresponding antioxidant activity saw only a small rise. The antioxidant activity of any biomolecule gets affected by its surrounding food matrix as well as method of assay (Chrysikopoulou *et al.*, 2025). Encapsulated vitamin C might have reduced reactivity in antioxidant assay because the encapsulation process can physically hinder the antioxidant molecule from interacting with assay reagents. This means that while the vitamin C itself is protected and stable within the capsule, its ability to perform its antioxidant function in a

test tube might be limited due to reduced accessible surface area of the vitamin C. The corresponding rise in antioxidant activity of CFB IV, although small, was statistically significant.

The *in vitro* digestibility for protein of CFB I, II, III and IV cereal-fruit bar was found to be 67.57, 72.30, 67.26 and 67.65%, respectively. CFB II cereal-fruit bar had significantly higher *in vitro* digestibility for protein i.e. 72.30%.

The *in vitro* digestibility for starch was significantly (P≤0.05) higher in CFB II (52.56 mg maltose released/g) cereal-fruit bar, while no significant difference was observed in CFB I (50.62 mg maltose released/g), CFB III (50.57 mg maltose released/g) and CFB IV (50.38 mg maltose released/g) (Table 5). It is important to study starch and protein digestibility of any developed product as these factors directly affect how efficiently the body or animal utilizes nutrients for growth. Higher digestibility of both starch and protein in CFB II might be due to use of a different wheat variety as there was no difference in other ingredient profile.

The phytic acid content of CFB I, II, III and IV cereal fruit bar was found to be 291.56, 287.54, 291.43 and 290.69 mg/100 g, respectively. CFB II had lower phytic acid as well as trypsin

**Table 5.** *In vitro* digestibility (protein and starch) and anti-nutrients of developed iron fortified cereal-fruit bar (db)

Variables	IVPD (%)	IVSD (mg maltose released/g)	Phytic acid (mg/100 g)	Trypsin inhibitor (TIU/mg)
CFB I	67.57±0.18 <sup>b</sup>	50.62±0.09 <sup>b</sup>	291.56±0.06 <sup>a</sup>	0.87±0.01 <sup>a</sup>
CFB II	72.30±0.25 <sup>a</sup>	52.56±0.10 <sup>a</sup>	287.54±0.04 <sup>b</sup>	0.80±0.03 <sup>a</sup>
CFB III	67.26±0.26 <sup>b</sup>	50.57±0.35 <sup>b</sup>	291.43±0.03 <sup>a</sup>	0.84±0.02 <sup>a</sup>
CFB IV	67.65±0.09 <sup>b</sup>	50.38±0.20 <sup>b</sup>	290.69±0.05 <sup>a</sup>	0.83±0.02 <sup>a</sup>
C. D. (P≤0.05)	0.67	0.70	0.14	0.07

Values are mean ± SE of six independent replications. Values with different superscript in the same columns are significantly different (P≤0.05).

inhibitor content although it was not statistically significant in case of Trypsin inhibitor. The trypsin inhibitor content of CFB I, II, III and IV cereal-fruit bar was found to be 0.87, 0.80, 0.84 and 0.83 TIU/mg, respectively (Table 5). Phytic acid and Trypsin inhibitors are well known to affect starch and protein digestibility. While trypsin is directly involved in protein digestion, phytic acid also inhibits various proteases and amylases essential for protein and starch degradation (Ohanenye *et al.*, 2022; Chen and Xu, 2023). The lower content of phytic acid and trypsin inhibitor in CFB II well explains the better protein and starch digestibility. Phytic acid significantly hinders iron absorption also due to its ability to bind to iron, thus limiting its utilization by body (Chen and Xu, 2023). At the same time both phytic acid as well as trypsin inhibitors have also been credited with positive health effects especially anti-tumour activity (Chen and Xu, 2023; Condori and de Camargo, 2023).

## CONCLUSION

The rising demand for convenient, healthful and functional foods has prompted the development of various nutrient-dense cereal bars. Present work focused on combining dates and nuts with broken wheat to create a cereal fruit bar through minimum cooking. Different methods of fortification were applied to enhance the iron content. The developed cereal-fruit bars were organoleptically acceptable and presented a good nutrient, digestibility and antioxidant profile. The encapsulated fortification provided best iron accessibility and availability. The findings further need to be validated through *in vivo* methods for bioavailability.

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## ETHICAL APPROVAL

The study protocol was approved by the Institutional Ethical Committee of CCS Haryana Agricultural University, Hisar, India vide endst no. DR/ADR/IEC/ 2022/239-47 dated 16.5.22. as per guidelines by ICMR, India.

The research was carried out following the guidelines of the ethics committee.

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