Research Article

Development and Evaluation of Haemoglobin-Boosting Functional Biscuits Fortified with Iron-Rich Natural Ingredients

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ABSTRACT

Background: Iron deficiency anaemia (IDA) is a major public health concern globally, particularly affecting children and adolescent girls. Food-based approaches using fortified products offer a practical and sustainable solution to combat micronutrient deficiencies. Biscuits, being a popular snack with high shelf life and universal acceptability, serve as an ideal vehicle for nutritional fortification.

Objective: This study aimed to develop and evaluate functional biscuits fortified with iron-rich natural ingredients, including moringa leaf powder, beetroot powder, black raisins, dates, pumpkin seeds, and citrus powder to enhance haemoglobin levels and provide antioxidant benefits.

Methods: Six formulations (B1-B6) were developed with varying concentrations of iron-rich ingredients while maintaining a whole wheat flour base. The optimised formulation (B3) was evaluated for its nutritional composition and antioxidant activity using the DPPH radical-scavenging assay. The antioxidant potential was compared with that of ascorbic acid as a standard.

Results: The optimised biscuit formulation (B3) provided 465 kcal energy, 2.5 mg iron (14% DV), 5g dietary fiber (20% DV), and 7.5g protein (15% DV) per 100g serving. The product showed zero cholesterol with moderate antioxidant activity. DPPH assay revealed concentration-dependent free radical-scavenging activity, with B3 showing 56.99% inhibition at 100 µg/ml, compared to 82.90% inhibition by the standard at the same concentration.

Conclusion: The developed haemoglobin-boosting biscuits represent a promising functional food product with enhanced nutritional value, particularly for addressing iron-deficiency anaemia. The product combines palatability with functional benefits, making it suitable for regular consumption by diverse age groups, especially school-going children and adolescent girls who are at higher risk for IDA.

Keywords: Iron deficiency anaemia, Functional biscuits, Haemoglobin enhancement, Moringa oleifera, Fortification, Antioxidant activity, Nutritional intervention.

INTRODUCTION

Anaemia: A Global Health Challenge:

Anaemia is a condition characterised by an insufficient number of red blood cells to meet the daily physiological demands of the body, resulting in haemoglobin levels below established reference values (<13.0 g/dL in men and <12.0 g/dL in women)[1]. Various forms of anaemia exist, including megaloblastic anaemia and iron deficiency anaemia, which is a consequence of inadequate iron levels in the blood[2]. The immune system is protecting body from different harmful substances and foreign material. A strong immune system will help you to protect from disorder which caused by viruses, bacteria, toxins, etc.[3]

The primary impact of iron deficiency anaemia is a reduced oxygen supply to tissues, which can lead to a spectrum of health issues in children, ranging from mild to severe, and potentially causing irreversible effects on both physical and cognitive development. This condition represents a significant public health concern, both globally and nationally[4, 5].

Food-Based Approaches to Combat Anaemia:

Food-based approaches aim at improving nutrition by increasing the availability and consumption of a nutritionally adequate and micronutrient-rich diet made up from a variety of available foods. Food-based approaches are recognised as an essential part of an urgently needed, more comprehensive strategy to combat iron and other micronutrient deficiencies[6].

Snacks comprise a significant part of people's everyday lives, particularly children. Biscuits are one of the most popular non-fermented bakery products, used as a snack, and distinguished by their high shelf life due to low moisture content. As a matter of fact, conventional biscuits are high in fat and sugar content, low in fibers, minerals, and vitamins. Previous studies revealed that preparing meals from 100% white wheat flour is considered a substantial contributor to malnutrition, which may be attributed to low protein and vitamin content. Additionally, reduction of daily fiber intake is considered one of the most critical issues as it contributes to elevated chronic disease incidence, like constipation, cardiovascular diseases, and cancer. Thus, producing biscuits fortified with healthy

ingredients is a unique solution to improve the product nutritive value[7].

Vulnerable Population: School-Going Children and Adolescent Girls:

School-going children who are our future citizens form an important segment of the Indian population. They contribute to vital human potential and impart strength to our national economy and development. The better nutritional status of children will help build a healthy society and nation. Therefore, their nutritional status is of great significance. During the first stages of puberty, when growth spurts occur in both sexes, girls are at higher risk than boys for IDA because of smaller iron stores due to loss of iron in the blood during their monthly menstrual flow. On the other side, muscle mass and blood volume also increase at this age, which increases the need for iron for haemoglobin formation. If the diet of adolescents does not contain a sufficient amount of absorbable iron or they do not consume adequate quality food, they become anaemic[8].

Causes of Low Haemoglobin:

Several factors contribute to low haemoglobin levels: [9]

1. The body doesn't make enough red blood cells due to bone marrow dysfunction or deficiency conditions.

2. The body produces enough red blood cells, but they die faster than the body can replace them (hemolytic conditions).

3. Blood loss from injury, menstruation, or internal bleeding, such as ulcers.

4. The body can't absorb iron properly, affecting red blood cell development.

5. Insufficient intake of essential nutrients like iron, vitamin B12, and folate (B9).

Benefits of Fortified Biscuits: [10]

- Fortified biscuits offer several advantages as a nutritional intervention strategy:
- Convenient way to consume iron-rich nutrients
- Acceptable to all age groups
- Easy to carry and store
- High shelf life
- Masks the bitter taste of iron supplements
- Cost-effective solution
- Minimal to no side effects compared to medicinal iron supplements

Currently Available Marketed Products:

Several iron supplementation products are currently available in the market, ranging from syrups to capsules and tablets. Table 1 provides an overview of these commercially available products.

Table 1: Currently Available Marketed Products for Haemoglobin Enhancement

Product Name	Dosage Form	Manufactured By	Price (INR)
Hemoglobin booster	Syrup	Krishna's herbal and Ayurveda	500
Iron complex natural	Capsule	Nutrela	150
Iron and folic acid	Tablets	Gynoveda	400
Plant-based iron supplement	Capsule	Himalayan organics	650
Blood builder	Tablets	Mega food	800

MATERIALS AND METHODS

Selection of Iron-Rich Ingredients:

To increase haemoglobin levels effectively, the biscuits were formulated with carefully selected ingredients rich in iron, vitamin B12, folate, and vitamin C for enhanced iron absorption. The rationale for ingredient selection is presented below:

A. Iron-Rich Ingredients

1. Moringa Leaf Powder: Moringa oleifera leaves are known to have various kinds of nutritional content, including iron, protein, vitamin A, vitamin C, potassium, calcium, and antioxidants. Moringa leaves contain higher iron than other vegetables, approximately 26 mg/100g. Several studies have shown that giving Moringa leaves to anaemic adolescents is considered highly effective[11, 12].

2. Black Raisins: Black raisins are packed with iron, a key component in haemoglobin synthesis. Iron helps in the formation of red blood cells, which carry oxygen throughout the body[13].

3. Beetroot Powder: Beta vulgaris L (beetroot) contains 34% folic acid, which aids in the growth and repair of damaged cells, 13.6% fiber to prevent constipation, 7.4% iron for energy metabolism and immune system support, and 10.2% vitamin C for tissue repair, blood vessel normalisation, and enhanced iron absorption. Studies have shown significant increases in haemoglobin levels with beetroot supplementation[14.]

4. Dates: Date pollen grains have been used to increase women's fertility. Iron from dates is absorbed in the small intestine (duodenum), enters the bloodstream, and is transported by transferring to the bone marrow. In erythroid precursor cells, iron combines with protoporphyrin IX to form heme, catalysed by the enzyme ferrochelatase. Heme then binds with globin chains to form functional haemoglobin molecules. Dates also contain vitamin B6 and folate, which support DNA synthesis and maturation of red blood cells[15].

5. Pumpkin Seeds: Pumpkin seeds are rich in iron, zinc, and magnesium. Iron is absorbed in the duodenum and transported via transferrin to the bone marrow, where it combines with protoporphyrin IX to form heme. Zinc and magnesium in pumpkin seeds support enzymes involved in erythropoiesis. Vitamin E protects red blood cells from oxidative damage, while amino acids and fatty acids enhance cellular metabolism and RBC integrity[16].

B. Iron Absorption Enhancer:

1. Citrus Powder: Vitamin C (ascorbic acid) from citrus powder converts ferric iron (Fe^{3+}) into ferrous iron (Fe^{2+}), the absorbable form in the intestine. It prevents iron from binding to inhibitors like phytates and polyphenols in plant-based diets and facilitates iron transport via divalent metal transporter 1 (DMT1) in enterocytes[17].

C. Base and Additional Ingredients:

1. Whole Wheat Flour: Whole wheat flour is a powder obtained from the milling of wheat containing the natural kernel proportions of bran, endosperm, and germ. In baking, it produces richer and denser products than white flour while providing dietary fiber and essential nutrients.

2. Cocoa Powder: Added for flavour enhancement and antioxidant benefits, cocoa powder also contributes mood-enhancing compounds like theobromine.

Formulation Development:

Six different formulations (B1-B6) were developed with varying concentrations of the active ingredients to optimise the nutritional profile and sensory acceptability. Table 2 presents the detailed formulation composition for all batches.

Table 2: Formulation Composition of Haemoglobin-Boosting Biscuits

Ingredient	B1	B2	B3	B4	B5	B6	Role of Ingredient
Wheat flour	40g	40g	40g	40g	40g	40g	Base + structure + fiber
Moringa leaf powder	3g	3g	2g	2g	1g	1g	Iron + calcium + vitamin A
Black raisins	5g	5g	5g	5g	5g	5g	Iron
Beetroot powder	1g	1g	3g	3g	5g	5g	Iron + nitrate
Dates powder	6g	6g	6g	6g	6g	6g	Sweetener + iron + vitamin B6
Pumpkin seeds	1g	2g	3g	4g	5g	6g	Iron + zinc + magnesium
Citrus powder	2g	2g	2g	2g	2g	2g	Vitamin C enhancer
Cocoa powder	3g	3g	3g	3g	3g	3g	Flavor + antioxidant
Ghee	15ml	15ml	15ml	15ml	15ml	15ml	Nutrient capturing
Honey	15ml	14ml	13ml	12ml	11ml	10ml	Sweetener
Salt	q.s	q.s	q.s	q.s	q.s	q.s	Taste balance
Baking soda	2g	2g	2g	2g	2g	2g	Texture
Milk	7ml	7ml	6ml	6ml	5ml	5ml	Calcium

Manufacturing Procedure:

The biscuits were prepared using the following standardised procedure: [18, 19]

1. Preparation of Dry Ingredients: All dry ingredients including whole wheat flour (42g), moringa leaf powder (6g), spinach powder (6g), beetroot powder (6g), pumpkin seed powder (9g), citrus powder (3g), cocoa powder (5g), salt (0.5g), and baking powder (0.5g) were sifted together in a clean bowl to remove clumps and ensure uniform distribution.

2. Preparation of Wet Ingredients: In a separate bowl, pureed or finely chopped dates (10g) were mixed with oil or ghee (9g). If needed, 1-2 teaspoons of warm water were added to help bind the dough.

3. Combining Ingredients: The wet mixture was gradually added to the dry mixture and kneaded lightly until a cohesive dough formed (neither sticky nor crumbly). The dough was allowed to rest for 5-10 minutes to hydrate the powders properly.

4. Shaping: The dough was rolled or pressed into approximately 0.5 cm thick sheets and cut into desired shapes (round, square, or bar shapes).

5. Addition of Chocolate Chips: Compound chocolate chips (5-8g) were placed on top of each biscuit and pressed gently to ensure they remained anchored during baking.

6. Baking: The oven was preheated to 160 °C. Biscuits were baked for 12-15 minutes or until the edges became slightly golden and the chocolate chips began to soften. The biscuits were then cooled for 5-10 minutes before evaluation.

7. Storage: The finished biscuits were stored in airtight containers for up to 7 days. Silica gel packs were optionally added to maintain crispness in humid climates.

Figure 1: Before Baking

Figure 2: After Baking

Evaluation Methods:

A. Nutritional Analysis:

The optimised formulation (B3) was subjected to comprehensive nutritional analysis to determine energy content, macronutrient composition (carbohydrates, proteins, fats), micronutrient content (iron, calcium, potassium, sodium), fiber content, cholesterol levels, and vitamin content.

B. Antioxidant Activity Assessment:

Antioxidant activity of the biscuit formulation was estimated using the DPPH (1,1-Diphenyl-2-Picryl-Hydrazyl) free radical scavenging assay. Different concentrations (20, 40, 60, 80, 100 µg/ml) of the test sample were prepared. To each concentration, 1.5 ml of 0.1% methanolic DPPH was added and incubated for 30 minutes under dark conditions. The samples were observed for colour change from purple to yellow, and absorbance was measured using a colourimeter at 510 nm. Ascorbic acid was used as the standard reference compound.

The percentage inhibition was calculated using the formula:

DPPH radical scavenging activity (%) = [(Absorbance of control - Absorbance of test sample) / Absorbance of control] * 100.

RESULTS AND DISCUSSION:

A. Nutritional Composition:

The nutritional analysis of the optimised biscuit formulation (B3) revealed a well-balanced nutritional profile suitable for addressing iron deficiency while providing overall nutritional support. The detailed nutritional composition per 100g serving is presented in Table 3.

Table 3: Nutritional Composition of Optimised Biscuit Formulation (B3) per 100g

Nutrient	Amount	% Daily Value
Energy	465 kcal	23%
Carbohydrates	65 g	22%
Sugars	18 g	-
Dietary fiber	5 g	20%
Protein	7.5 g	15%
Total fat	16 g	24%
Saturated fat	6 g	30%
Unsaturated fat	8 g	-
Cholesterol	0 mg	0%
Sodium	210 mg	9%
Potassium	150 mg	4%
Calcium	60 mg	6%
Iron	2.5 mg	14%
Vitamins (A, C, E)	Trace amounts	-

Interpretation:

The developed biscuit represents a nutritionally balanced functional food product, offering adequate energy (465 kcal per 100g), substantial dietary fiber (5g, 20% DV), and moderate protein content (7.5g, 15% DV). The iron content of 2.5 mg per 100g provides 14% of the daily value, which is significant for addressing iron deficiency anaemia. The zero cholesterol content, presence of essential minerals, and bioactive compounds make it a healthier snacking option compared to conventional biscuits. Regular but moderate consumption may contribute to digestive health, improved immunity, and overall well-being.

Antioxidant Activity:

The antioxidant potential of the biscuit formulation B3 was evaluated using the DPPH free radical scavenging method. The results demonstrated concentration-dependent free radical scavenging activity, which was compared with ascorbic acid as a standard antioxidant. The detailed results are presented in Table 4.

Table 4: DPPH Free Radical Scavenging Activity of Biscuit Formulation B3

Sr. No.	Sample Code	Concentration (µg/ml)	Absorbance at 510 nm			% Inhibition	IC 50 (µg/ml)
			Test 1	Test 2	Test 3		
1	Control	-	1.93	1.93	1.93	-	
2	Standard (Ascorbic Acid)	20	1.45	1.42	1.40	26.42%	72.49
2		40	1.31	1.29	1.33	32.12%	
2		60	0.95	0.97	0.93	50.77%	
2		80	0.82	0.85	0.79	57.51%	
2		100	0.35	0.32	0.32	82.90%	
3	Batch B3	20	1.62	1.60	1.65	16.06%	93.01
3		40	1.48	1.50	1.46	23.31%	
3		60	1.30	1.34	1.34	31.60%	
3		80	1.90	1.12	1.08	43.52%	
3		100	0.85	0.82	0.84	56.99%	

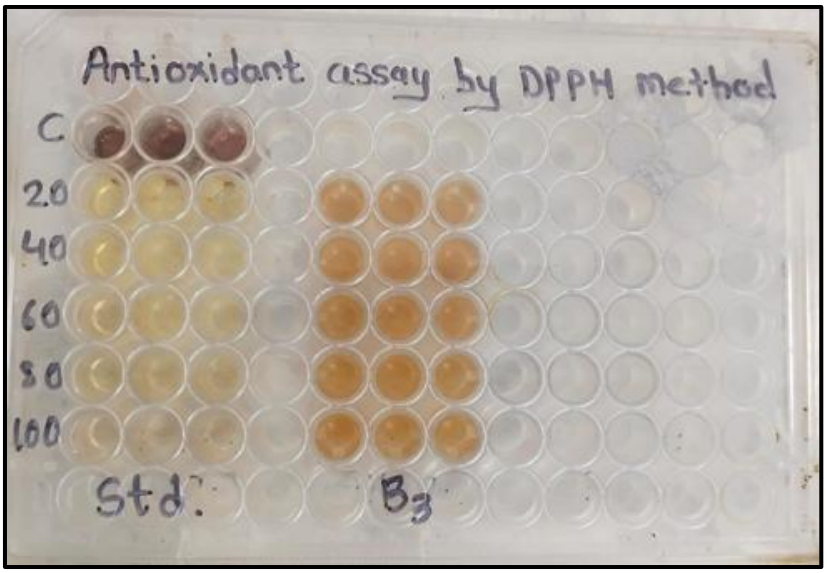

Figure 3: Anti-oxidant activity

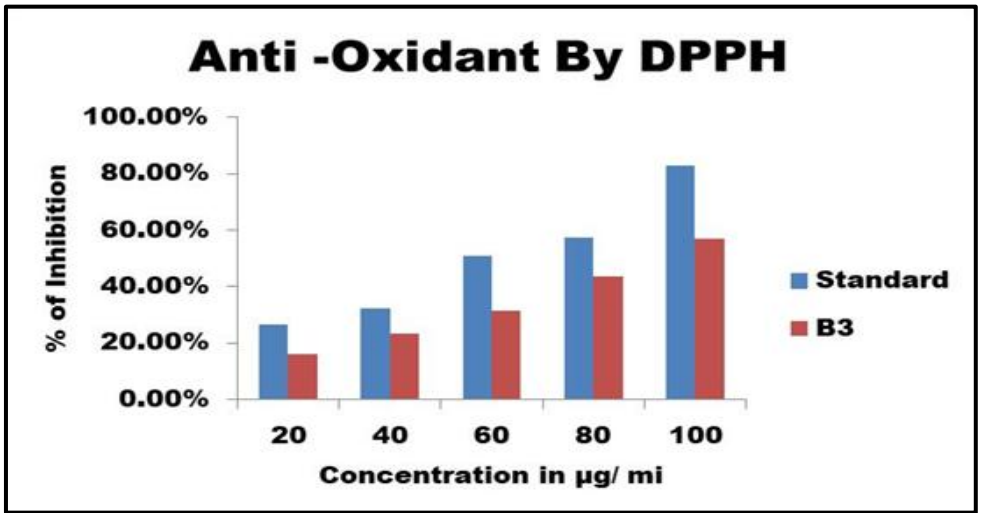

Figure 4: Antioxidant by DPPH Method

Interpretation:

The antioxidant activity of the test sample B3 was evaluated using the DPPH free radical scavenging method and compared with ascorbic acid as a standard antioxidant. The results demonstrated a concentration-dependent increase in percentage inhibition for both B3 and the standard, indicating enhanced free radical scavenging at higher concentrations. However, B3 consistently showed lower inhibition values than the standard at every tested concentration.

At 100 µg/ml, the standard exhibited approximately 82.90% inhibition, whereas B3 showed 56.99% inhibition. At the lowest concentration (20 µg/ml), the inhibition was approximately 26.42% for the standard and 16.06% for B3. These findings suggest that B3 has moderate antioxidant potential, attributed to the presence of moringa, beetroot, and cocoa powder, though it is less effective compared to pure ascorbic acid under identical conditions. The antioxidant activity contributes to the overall health-promoting properties of the biscuit by helping to combat oxidative stress.

Benefits of the Developed Product:

1. Haemoglobin and Iron Support: Moringa, spinach, beetroot, and pumpkin seeds are rich in iron, folate, and vitamin C. These nutrients help boost haemoglobin levels, making the biscuits ideal for people with anaemia or low energy levels.

2. Immune System and Antioxidant Boost: Citrus powder adds vitamin C, which enhances iron absorption and supports immunity. Cocoa and moringa are loaded with antioxidants that help fight oxidative stress and reduce cellular damage.

3. Brain and Heart Health: Pumpkin seeds contain magnesium, zinc, and omega-3 fatty acids, which support cognitive function and cardiovascular health. Cocoa contributes to improved mood and blood flow through its bioactive compounds.

4. Digestive Wellness: Whole wheat flour provides dietary fiber for better digestion and satiety. The fiber content helps regulate blood sugar levels and supports gut health, reducing the risk of digestive disorders.

5. Natural Energy and Taste Appeal: Beetroot and cocoa offer natural sweetness and flavour depth. The biscuit provides sustained energy release without refined sugar or artificial additives, making it suitable for daily consumption.

6. Clean Label and Functional Nutrition: The product contains no preservatives, artificial colours, or synthetic nutrients. It appeals to health-conscious consumers, especially those seeking plant-based or functional snacks with proven health benefits.

7. Evidence-Based Impact: Studies have shown that biscuits fortified with moringa and pumpkin seeds can improve haemoglobin and ferritin levels, especially in pregnant women and adolescents. This product can be positioned as a preventive nutritional intervention in anaemia-prone populations.

Health Claims:

1. Supports Haemoglobin Levels: Contains iron-rich ingredients like moringa, spinach, beetroot, and pumpkin seeds. May help prevent or reduce anaemia, especially in adolescents and pregnant women.

2. Enhances Iron Absorption: Includes citrus powder, a natural source of vitamin C, which improves iron bioavailability. Avoids inhibitors like excess calcium or tannins, making it a functional iron-delivery snack.

3. Rich in Antioxidants and Micronutrients: Moringa, beetroot, and cocoa contribute polyphenols, flavonoids, and essential minerals. May help reduce oxidative stress and inflammation.

4. Promotes Cognitive and Immune Health: Pumpkin seeds and spinach offer zinc, magnesium, and folate, which support brain function and immunity. Cocoa adds mood-enhancing compounds like theobromine and serotonin precursors.

5. Clean Label and Functional Snack: Made with whole wheat flour and no artificial additives. Suitable for daily consumption as a health-supportive biscuit.

6. Evidence-Based Nutritional Impact: Studies show that biscuits fortified with moringa and pumpkin seeds improve haemoglobin, ferritin, and inflammatory markers. Can be positioned as a preventive nutritional intervention in anaemia-prone populations.

7. Sensory Acceptability: Cocoa and citrus enhance flavour, making the biscuit palatable and enjoyable, which encourages consistent intake and compliance with nutritional intervention.

CONCLUSION

This research successfully developed and evaluated functional biscuits fortified with natural iron-rich ingredients as a food-based approach to address iron deficiency anaemia. The optimised formulation (B3) demonstrated significant nutritional value with 2.5 mg iron per 100g (14% DV), adequate dietary fiber (5g, 20% DV), and moderate protein content (7.5g, 15% DV), while maintaining zero cholesterol levels. The product also exhibited moderate antioxidant activity, with 56.99% DPPH radical scavenging at 100 µg/ml concentration.

The incorporation of moringa leaf powder, beetroot powder, dates, pumpkin seeds, and citrus powder not only enhanced the iron content but also improved the overall nutritional profile by providing additional vitamins, minerals, and bioactive compounds. The presence of vitamin C from citrus powder is particularly beneficial as it enhances iron absorption from plant-based sources, addressing one of the major limitations of vegetarian iron supplementation.

The developed biscuits represent a promising functional food product that combines palatability with therapeutic benefits. Unlike conventional iron supplements, which often cause side effects such as gastrointestinal discomfort, nausea, and constipation, these biscuits offer a more acceptable and enjoyable means of iron supplementation. The product is particularly suitable for school-going children and adolescent girls who are at higher risk for iron deficiency anaemia and may be reluctant to take traditional iron supplements.

The study demonstrates that fortification of commonly consumed snack foods with carefully selected natural ingredients can significantly enhance their nutritional value and contribute to addressing public health concerns. However, clinical studies are recommended to validate the efficacy of these biscuits in improving haemoglobin levels in anaemic populations. Future research should also focus on sensory evaluation, shelf-life studies, and large-scale clinical trials to establish the product's effectiveness and acceptability across diverse populations.

In conclusion, the developed haemoglobin-boosting biscuits offer a sustainable, cost-effective, and culturally acceptable solution to combat iron deficiency anaemia, particularly in resource-limited settings. The product aligns with food-based approaches recommended by global health organisations and has the potential to make a significant impact on public health if implemented through school feeding programs and community nutrition initiatives.

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