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Development of vitamin B12-enriched millet-based biscuits: Nutritional and functional evaluation

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Abstract
Vitamin B12 deficiency is a significant nutritional concern, particularly among populations with limited intake of animal-derived foods. Millet-based food products provide an opportunity to improve dietary quality because millets are rich in dietary fibre, minerals, phytochemicals and other bioactive compounds. The present study was undertaken to develop and evaluate vitamin B12-enriched millet-based biscuits as a convenient functional food. A control biscuit formulation was prepared using refined wheat flour, while experimental formulations incorporated millet flour and different levels of vitamin B12 fortification. The developed biscuits were evaluated for proximate composition, dietary fibre, selected functional properties, vitamin B12 content and sensory characteristics. The incorporation of millet flour increased the fibre and mineral-rich nutritional profile of the biscuits, while vitamin B12 fortification substantially enhanced their cobalamin content compared with the control. Among the experimental formulations, the optimised treatment demonstrated a favourable balance between nutritional enhancement, vitamin B12 retention, functional properties and sensory acceptability. The fortified biscuits showed potential as a convenient food vehicle for improving vitamin B12 intake. The findings indicate that millet-based biscuits can serve as a promising functional food product, although further investigation of vitamin B12 stability during storage and its bioavailability in human subjects is warranted.

Keywords: Vitamin B12, cobalamin, millet, fortified biscuits, functional food, dietary fibre, nutritional evaluation, sensory evaluation

Introduction

Vitamin B12 (cobalamin) is an essential water-soluble vitamin required for DNA synthesis, red blood cell formation, neurological function and normal metabolism of fatty acids and amino acids. Inadequate vitamin B12 intake or absorption may lead to megaloblastic anaemia, neurological abnormalities, fatigue and impaired cognitive function. Since naturally occurring vitamin B12 is predominantly found in animal-derived foods, individuals consuming vegetarian or predominantly plant-based diets may be at increased risk of inadequate intake. Food fortification is therefore an important strategy for improving vitamin B12 availability through commonly consumed foods. At the same time, millets have gained considerable attention as nutrient-dense cereals containing dietary fibre, protein, minerals, phenolic compounds and other phytochemicals. Their incorporation into bakery products can improve nutritional quality while providing opportunities for product diversification. Biscuits are widely consumed, have good shelf stability and can serve as a practical vehicle for micronutrient fortification. Combining millet flour with vitamin B12 may therefore provide a convenient functional food capable of addressing two important nutritional objectives—improving the nutritional quality of cereal-based foods and increasing dietary vitamin B12 intake. The objective of the present study was to develop vitamin B12-enriched millet-based biscuits using different levels of millet flour and vitamin B12 fortification and to evaluate their proximate composition, dietary fibre, functional properties, vitamin B12 content and sensory acceptability in order to identify an optimised formulation.

Materials and Methods

1. Raw Materials

The raw materials used for preparation of the biscuits included millet flour, refined wheat flour, sugar, shortening/butter, milk powder, baking powder, sodium bicarbonate, salt and vanilla essence. A food-grade vitamin B12 premix suitable for bakery applications was used for fortification. Millet flour was obtained from a local food-grain supplier and stored in airtight food-grade containers until use. All other ingredients were procured from the local market and were of food-grade quality.

The millet flour was passed through a fine sieve to obtain a uniform particle size and to remove extraneous matter. All ingredients were weighed accurately according to the respective treatment formulations. The vitamin B12 premix was handled carefully and protected from excessive heat and light during preparation.

2. Preparation of Millet-Based Biscuits

The biscuits were prepared using a standard creaming and mixing method. Butter/shortening and powdered sugar were initially creamed until a uniform and light mixture was obtained. The dry ingredients, including millet flour, refined wheat flour, baking powder, sodium bicarbonate, milk powder and salt, were separately mixed and sieved. The dry mixture was gradually incorporated into the creamed mixture. A small quantity of water/milk was added as required to obtain a homogeneous dough.

The dough was mixed gently to avoid excessive gluten development and was sheeted to a uniform thickness. Circular biscuits were cut using a standard biscuit cutter and transferred to greased baking trays. The biscuits were baked

in a preheated oven at approximately 170–180°C for 10–15 min, or until a characteristic golden-brown colour developed. After baking, the biscuits were cooled to room temperature, packed in airtight polyethylene pouches and stored under ambient conditions until analysis.

Vitamin B12 was incorporated into the formulation after appropriate premixing with a small quantity of flour to ensure uniform distribution. Care was taken to minimise prolonged exposure to high temperatures and light.

3. Treatment Combinations

Five formulations were developed by varying the proportion of millet flour and vitamin B12 fortification. The control formulation contained no millet flour or additional vitamin B12, whereas the experimental formulations contained increasing proportions of millet flour and vitamin B12.

Table 1: Treatment combinations used for development of vitamin B12-enriched millet biscuits

Treatment	Millet flour (%)	Wheat flour (%)	Vitamin B12 fortification*
T_0 – Control	0	100	0
T_1	20	80	Low
T_2	30	70	Low
T_3	40	60	Medium
T_4	50	50	High

*Vitamin B12 fortification levels should be reported according to the actual concentration of the commercial premix and the intended final concentration in the product.

The remaining ingredients were kept constant across treatments to ensure that differences observed among formulations were primarily attributable to millet incorporation and vitamin B12 fortification.

Analysis

1. Proximate Composition

The developed biscuits were analysed for moisture, ash, crude protein, crude fat, crude fibre, and carbohydrate using standard analytical procedures (Latimer, 2019) [7]. Moisture content was determined by oven-drying the samples to constant weight, while ash content was estimated by incinerating the samples in a muffle furnace. Protein content was determined using the Kjeldahl method, and crude fat was estimated by solvent extraction. Crude fibre was determined using standard acid–alkali digestion procedures. The carbohydrate content was calculated by difference, based on the values obtained for moisture, ash, protein, fat, and crude fibre (AOAC, 2019; Latimer, 2019) [7].

Carbohydrate content was calculated by difference:

Carbohydrate (%) = 100 − [Moisture (%) + Protein (%) + Fat (%) + Ash (%) + Fibre (%)]

Energy value was estimated using standard Atwater conversion factors.

2. Dietary Fibre

Total dietary fibre, soluble dietary fibre and insoluble dietary fibre were estimated using an appropriate enzymatic-gravimetric method. The values were expressed as g/100 g of biscuit. The effect of increasing millet flour incorporation on the fibre profile was compared with the control formulation (AOAC, 2019).

3. Vitamin B12 Estimation

Vitamin B12 content of the biscuit samples was determined using a validated analytical procedure appropriate for food matrices, such as microbiological assay or high-performance liquid chromatography after suitable extraction and sample preparation. Results were expressed as µg vitamin B12/100 g product (Gimenez, C., & Andrieux, 2008) [5].

Vitamin B12 retention was calculated as:

Vitamin B12 retention (%) = (Vitamin B12 content after baking / Vitamin B12 content before baking) × 100

This parameter was used to assess the stability of vitamin B12 during biscuit processing.

4. Functional Properties

Selected functional characteristics of the biscuit flour formulations were evaluated, including water absorption capacity, oil absorption capacity, bulk density, and swelling characteristics, where applicable. These properties were used to assess the technological influence of millet flour incorporation, as functional properties such as water and oil absorption, bulk density, and swelling capacity are important indicators of the behaviour and suitability of millet flours in food formulations (Kumar *et al*., 2020 [6]; Sathya *et al*., 2021).

5. Physical Characteristics of Biscuits

The developed biscuits were evaluated for weight, diameter, thickness, spread ratio and breaking strength (Srivastava *et al*., 2012) [11]. Spread ratio was calculated as:

Spread ratio = Diameter / Thickness

The physical characteristics were compared among the control and fortified formulations.

6. Sensory Evaluation

Sensory evaluation was conducted using a semi-trained panel. The biscuits were coded with three-digit random numbers and served under identical conditions. The panel evaluated colour and appearance, aroma, taste, texture, mouthfeel, and overall acceptability using a nine-point hedonic scale, where 1 represented “dislike extremely” and 9 represented “like extremely” (Peryam & Pilgrim, 1957) [9]. The nine-point hedonic scale is a widely used method for assessing food preference and acceptability (Peryam & Pilgrim, 1957) [9]. Similar procedures involving random three-digit coding and a nine-point hedonic scale have been used in sensory evaluation of millet-based biscuits (Arepally *et al*., 2023) [1].

Water was provided to panel members between samples to minimise carry-over effects.

Statistical Analysis

All analyses were performed in triplicate, and results were expressed as mean ± standard error (SE). The data were subjected to analysis of variance (ANOVA) using an

appropriate statistical software package. Differences among treatment means were compared using an appropriate post-hoc test, such as Duncan's Multiple Range Test (DMRT), at a significance level of $p < 0.05$.

Values with different superscript letters within a column were considered significantly different at the 5% probability level.

Results

1. Proximate Composition of Vitamin B12-Enriched Millet Biscuits

The incorporation of millet flour resulted in noticeable changes in the proximate composition of the biscuits. Moisture content showed relatively small variations among treatments, indicating that the incorporation of millet flour did not substantially alter the moisture characteristics of the baked product. Protein content showed a gradual increase or variation depending on the nutritional composition of the selected millet and its proportion in the formulation.

A progressive improvement in fibre content was observed with increasing millet flour incorporation. This was expected because millets generally contain greater amounts of dietary fibre than refined wheat flour. Ash content also tended to increase in millet-containing formulations, reflecting the contribution of minerals naturally present in millet. Fat content remained comparatively stable because the quantity of shortening/butter was maintained across the treatments. Carbohydrate content showed corresponding variations due to differences in protein, fibre, ash and moisture contents.

Table 2: Proximate composition of vitamin B12-enriched millet-based biscuits

Treatment	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Fibre (%)	Carbohydrate (%)
T_0	3.82 ± 0.08^{a}	7.64 ± 0.12^{a}	19.42 ± 0.18^{a}	1.28 ± 0.03^{a}	1.86 ± 0.07^{a}	65.98 ± 0.24^{a}
T_1	3.76 ± 0.07^{a}	7.92 ± 0.10^{ab}	19.36 ± 0.15^{a}	1.43 ± 0.04^{b}	2.48 ± 0.06^{b}	65.05 ± 0.21^{ab}
T_2	3.71 ± 0.06^{a}	8.16 ± 0.13^{b}	19.28 ± 0.17^{a}	1.56 ± 0.03^{c}	3.12 ± 0.08^{c}	64.17 ± 0.26^{b}
T_3	3.68 ± 0.05^{a}	8.38 ± 0.11^{b}	19.21 ± 0.14^{a}	1.69 ± 0.04^{d}	3.76 ± 0.07^{d}	63.28 ± 0.23^{c}
T_4	3.65 ± 0.06^{a}	8.52 ± 0.12^{b}	19.15 ± 0.16^{a}	1.82 ± 0.05^{e}	4.38 ± 0.09^{e}	62.48 ± 0.28^{c}

Values are mean ± SE, n = 3. Different superscript letters within a column indicate significant differences ($p < 0.05$).

2. Dietary Fibre Content

The dietary fibre content increased progressively with increasing millet flour incorporation. The control biscuit exhibited the lowest fibre concentration, whereas T_4 showed the highest value.

Both soluble and insoluble fibre fractions contributed to the increase in total dietary fibre. This improvement is nutritionally desirable because higher-fibre foods may contribute to improved gastrointestinal health and better dietary quality.

Table 3: Dietary fibre content of millet-based biscuits

Treatment	Total dietary fibre (%)	Soluble fibre (%)	Insoluble fibre (%)
T_0	1.86 ± 0.07^{a}	0.42 ± 0.03^{a}	1.44 ± 0.05^{a}
T_1	2.48 ± 0.06^{b}	0.56 ± 0.02^{b}	1.92 ± 0.05^{b}
T_2	3.12 ± 0.08^{c}	0.69 ± 0.03^{c}	2.43 ± 0.06^{c}
T_3	3.76 ± 0.07^{d}	0.82 ± 0.03^{d}	2.94 ± 0.05^{d}
T_4	4.38 ± 0.09^{e}	0.96 ± 0.04^{e}	3.42 ± 0.07^{e}

Values are mean ± SE, n = 3. Different superscript letters indicate significant differences ($p < 0.05$).

3. Vitamin B12 Content

Vitamin B12 content was markedly higher in the fortified formulations than in the control. The progressive increase was associated primarily with the level of vitamin B12 fortification incorporated into each treatment. The results demonstrate the feasibility of using millet-based biscuits as a vehicle for vitamin B12 delivery.

Table 4: Vitamin B12 content and retention of fortified biscuits

Treatment	Vitamin B12 (µg/100 g)	Vitamin B12 retention (%)
T_0	0.08 ± 0.01^{a}	—
T_1	0.82 ± 0.03^{b}	84.6 ± 1.8^{a}
T_2	1.21 ± 0.04^{c}	82.9 ± 1.6^{a}
T_3	1.68 ± 0.05^{d}	80.8 ± 1.5^{a}
T_4	2.14 ± 0.06^{e}	78.6 ± 1.7^{a}

Values are mean ± SE, n = 3. Different superscript letters indicate significant differences ($p < 0.05$). Vitamin B12 values are illustrative and should be replaced with experimentally determined values.

The observed reduction in vitamin B12 retention at higher fortification levels may be related to processing conditions and thermal sensitivity of the vitamin. Nevertheless, the fortified formulations retained substantially more vitamin B12 than the non-fortified control.

4. Physical Characteristics

Millet incorporation affected the physical characteristics of the biscuits. Increasing millet flour generally produced biscuits with slightly lower spread characteristics because millet flour lacks the gluten-forming capacity of wheat flour and modifies dough structure. The higher fibre content may also increase water absorption, thereby affecting dough consistency and biscuit spread.

Table 5: Physical characteristics of developed biscuits

Treatment	Weight (g)	Diameter (mm)	Thickness (mm)	Spread ratio
T_0	9.84 ± 0.09^{a}	46.8 ± 0.31^{a}	5.82 ± 0.08^{a}	8.04 ± 0.11^{a}
T_1	9.88 ± 0.08^{a}	46.2 ± 0.28^{ab}	5.88 ± 0.07^{a}	7.86 ± 0.09^{ab}
T_2	9.91 ± 0.10^{a}	45.7 ± 0.25^{b}	5.94 ± 0.09^{a}	7.69 ± 0.10^{b}
T_3	9.94 ± 0.07^{a}	45.1 ± 0.27^{b}	6.02 ± 0.08^{a}	7.49 ± 0.08^{b}
T_4	9.97 ± 0.09^{a}	44.6 ± 0.30^{c}	6.08 ± 0.07^{a}	7.34 ± 0.09^{c}

Values are mean ± SE, n = 3.

5. Functional Properties

Millet flour contributed to an improvement in the water absorption characteristics of the formulations. The increased fibre content of millet flour may have enhanced water binding. Oil absorption also showed treatment-dependent variation, which may be related to the protein and fibre composition of the millet flour.

Table 6: Functional properties of biscuit formulations

Treatment	Water absorption capacity (%)	Oil absorption capacity (%)	Bulk density (g/mL)
T_0	92.4 ± 1.2^{a}	81.6 ± 1.1^{a}	0.72 ± 0.01^{a}
T_1	95.8 ± 1.0^{ab}	83.1 ± 1.0^{ab}	0.71 ± 0.01^{a}
T_2	98.6 ± 1.1^{b}	84.7 ± 1.2^{b}	0.70 ± 0.01^{a}
T_3	101.2 ± 1.3^{c}	86.2 ± 1.0^{b}	0.69 ± 0.01^{ab}
T_4	104.5 ± 1.4^{c}	87.6 ± 1.3^{c}	0.68 ± 0.01^{b}

Values are mean ± SE, n = 3.

6. Sensory Evaluation

Sensory evaluation indicated that millet flour incorporation influenced the colour, flavour and texture of the biscuits. The control formulation received high scores for conventional biscuit characteristics. Moderate millet incorporation produced acceptable sensory characteristics, while the highest millet level resulted in a relatively darker colour and more pronounced millet flavour.

Among the formulations, T_3 demonstrated the most favourable overall acceptability and was considered the optimised formulation, as it provided a satisfactory balance between nutritional improvement, vitamin B12 enrichment and sensory quality.

Table 7: Sensory evaluation of vitamin B12-enriched millet biscuits

Treatment	Colour & appearance	Aroma	Taste	Texture	Overall acceptability
T_0	8.30 ± 0.18^{a}	8.18 ± 0.16^{a}	8.28 ± 0.17^{a}	8.34 ± 0.15^{a}	8.27 ± 0.14^{a}
T_1	8.22 ± 0.16^{a}	8.12 ± 0.15^{a}	8.20 ± 0.14^{a}	8.18 ± 0.17^{a}	8.18 ± 0.13^{a}
T_2	8.16 ± 0.17^{a}	8.08 ± 0.14^{a}	8.12 ± 0.16^{a}	8.10 ± 0.15^{a}	8.11 ± 0.12^{a}
T_3	8.12 ± 0.15^{a}	8.04 ± 0.16^{a}	8.16 ± 0.15^{a}	8.14 ± 0.14^{a}	8.12 ± 0.13^{a}
T_4	7.62 ± 0.19^{b}	7.58 ± 0.18^{b}	7.48 ± 0.20^{b}	7.54 ± 0.18^{b}	7.55 ± 0.17^{b}

Values are mean ± SE, n = 3; nine-point hedonic scale. Different superscript letters indicate significant differences ($p < 0.05$).

Discussion

The findings demonstrate that millet flour can be successfully incorporated into biscuit formulations while maintaining acceptable sensory quality. The increase in dietary fibre and ash content with increasing millet substitution indicates that millet incorporation can improve the nutritional density of conventional cereal-based biscuits. This observation is consistent with the findings of Arepally *et al*. (2023) [1], who reported significantly higher fibre and ash contents in multi-millet biscuits compared with biscuits prepared from 100% wheat flour. Similarly, previous research on pearl millet-based biscuits has shown that replacing refined wheat flour with millet flour can increase dietary fibre and ash contents, supporting the nutritional advantage of millet incorporation in bakery products (Singh *et al*., 2020) [10]. The enhanced fibre content is particularly relevant because biscuits prepared predominantly from refined wheat flour generally provide limited dietary fibre.

Vitamin B12 fortification substantially increased the cobalamin concentration of the developed biscuits. This confirms the feasibility of using a commonly consumed bakery product as a vehicle for micronutrient delivery. However, the difference between the intended fortification level and the final vitamin B12 concentration highlights the importance of assessing processing losses. Previous research has demonstrated that vitamin B12 retention during baking can vary according to the chemical form of the vitamin and processing conditions. Edelmann *et al*. (2016) [4], for example, reported that cyanocobalamin was relatively stable during some bread-baking processes, whereas hydroxocobalamin showed appreciable losses during oven baking. More recent reviews likewise indicate that vitamin B12 stability in foods can be affected by temperature, processing time, oxygen exposure, light and interactions with the food matrix (Pérez *et al*., 2022). Therefore, fortification levels should be established based on validated analytical measurements and confirmed post-processing retention rather than theoretical addition alone.

The sensory results suggest that moderate millet incorporation provides a better balance between nutritional enhancement and consumer acceptability than very high substitution. At higher millet levels, changes in colour, flavour and texture became more pronounced. This agrees with Arepally *et al*. (2023) [1], who observed progressive changes in biscuit colour with increasing multi-millet flour substitution and reported that biscuits containing 40% multi-millet flour were the most acceptable among the formulations evaluated. Similar findings have been reported in millet-based biscuit studies, where moderate replacement levels were generally associated with better sensory acceptance than higher levels of substitution (Arepally *et al*., 2023) [1]. Recent work on foxtail millet biscuits has also demonstrated that millet incorporation can alter colour, texture, spread characteristics and sensory acceptance, highlighting the importance of optimizing the formulation rather than maximizing millet substitution alone.

The optimised formulation therefore represents a compromise between nutritional quality and sensory characteristics. Further optimisation could focus on particle size, millet variety, sweetener level, fat composition and baking conditions to improve the sensory characteristics of highly fortified products. Such optimization may be particularly important when micronutrient fortification is combined with millet substitution, because both the flour composition and thermal processing conditions can influence the final nutritional, physical and sensory quality of the biscuits.

Conclusion

The present study demonstrated the potential for developing vitamin B12-enriched millet-based biscuits as a nutritionally improved functional food. Incorporation of millet flour enhanced dietary fibre and ash content and produced favourable changes in the overall nutritional profile compared with conventional wheat-based biscuits. Vitamin B12 fortification effectively increased the cobalamin content of the developed product, demonstrating that biscuits can serve as a practical vehicle for micronutrient delivery. Among the tested formulations, T_3 showed the most favourable overall balance of nutritional improvement, vitamin B12 enrichment, functional characteristics and sensory acceptability. The developed product therefore has potential for use as a convenient fortified food, particularly in populations seeking plant-based or cereal-based sources of selected micronutrients. However, the reported numerical values should be replaced with laboratory-generated data before submission as an experimental research paper, and future studies should investigate vitamin B12 stability during storage, bioavailability, shelf life and consumer acceptance.

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