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Nutritional Assessment of Fermented Almond Milk Fortified with Vitamin B12

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ABSTRACT

Dietary patterns have been drastically changed by sedentary behaviors and changes in lifestyle, which has increased nutritional imbalances in a number of populations. The ability of functional meals to deliver vital nutrients with minimal adverse effects has led to their widespread acceptance as a natural substitute for traditional medications. Because of its rich nutritional profile and health-promoting qualities, almond milk has become a possible plant-based substitute for other functional beverages. Notwithstanding these advantages, vitamin B12, a necessary ingredient for DNA synthesis, neurological function, and red blood cell development, is absent from almond milk. Developing fermented almond milk supplemented with vitamin B12 and assessing its nutritional makeup, mineral profile, antioxidant activity, and consumer acceptability were the goals of the current study. Probiotic cultures were used for fermentation, and standardized quantities of vitamin B12 were added for fortification. Fortified samples outperformed unfortified controls in terms of nutrition, according to proximate composition and mineral analysis. As fermentation progressed, antioxidant activity rose dramatically, and sensory analysis verified that the enriched product was well-received in terms of taste, color, texture, and general palatability. According to the research, vitamin B12-fortified fermented almond milk may be a sustainable, health-promoting beverage that is especially advantageous for vegetarians, people with lactose intolerance, and groups at risk of micronutrient shortages.

INTRODUCTION

Globally, sedentary behavior, rising urbanization, and lifestyle changes are causing dietary habits to change. Due to these changes, undernutrition and over nutrition are more common and frequently show up as obesity, micronutrient deficiencies, and chronic illnesses (Asim & Nawaz, 2018). Food influence's identity, culture, and health in addition to being a source of energy. Natural and functional foods are becoming more popular among consumers, who favor them over artificial vitamins and pharmaceuticals (Landstrom et al., 2009).

When it comes to lowering the risk of chronic illnesses, functional meals are crucial. According to reports, between 70 and 80 percent of people in underdeveloped nations get their primary medical treatment from natural food-based solutions (UNICEF, 2020). Instead of these dietary changes, malnutrition is still prevalent. According to National nutrition survey 2018, Pakistani children who are under 5 had concerning rates of underweight prevalence (28.9%), wasting (17.7%), and stunting (40.2%) (GOP, 2018).

These figures highlight the urgent need for cost-effective, long-lasting food-based interventions that address nutrient deficiencies in at-risk populations. Malnutrition still exists in spite of these dietary changes. The high Nutritional content of Almonds has drawn attention. According to Sethi et al. (2016) and Catarino et al. (2018), it is a rich source of proteins, unsaturated fatty acids, vitamins E and B-complex, and minerals including calcium, magnesium, and phosphorus. Almond milk has become more and more well-liked as a plant-based, lactose- and cholesterol-free substitute especially for lactose intolerant individuals. It is especially appropriate for people who are lactose intolerant and those who are vegetarians or vegans (Demir et al., 2016; Prytulska et al., 2021). Almond milk has several minerals, however it is inherently

lacking in vitamin B12, a water-soluble vitamin that is necessary for metabolic processes, hemopoiesis, and nervous system function (Bernstein, 2017). Vitamin B12 deficiency is prevalent in populations with limited access to animal-based foods, contributing to anemia, neurological disorders, and impaired cognitive development. Fermentation of almond milk using probiotics can further enhance its bioactive potential, improve antioxidant activity, and provide additional health benefits (Bernart et al., 2015; Manzoor et al., 2021).
Lifestyle modification and sedentary behavior have become widespread, leading to major shifts in eating patterns that influence nearly all age groups. The increased consumption of calorie-dense and nutrient-poor diets has resulted in escalating health problems such as obesity, diabetes, hypertension, and cardiovascular diseases (Asim & Nawaz, 2018). The saying "we are what we eat" reflects the importance of food as not only a source of nutrients but also a determinant of identity, culture, and overall health. Consumers increasingly associate natural foods with positive health outcomes, while processed foods and synthetic additives are often viewed as harmful (Landstrom et al., 2009).

Functional foods, which are derived from natural sources and provide additional health benefits beyond basic nutrition, are gaining attention as an effective approach to reducing disease burden. According to the World Health Organization, nearly 70–80% of the population in developing countries relies on natural food-based solutions as part of their primary healthcare system (UNICEF, 2020).

These functional foods contribute to prevention and management of non-communicable diseases, which are currently on the rise worldwide. Malnutrition, however, continues to be a pressing challenge in many developing nations, including Pakistan. The National Nutrition Survey (2018) reported that 40.2% of children under five years of age in Pakistan were stunted, 17.7% were wasted, and 28.9% were underweight (GOP, 2018). Micronutrient deficiencies are also highly prevalent, particularly deficiencies of iron, iodine, zinc, vitamin A, and vitamin B12. The lack of access to animal-based protein sources has worsened the nutritional status of vulnerable populations, making it essential to identify cost-effective and sustainable dietary interventions (Reyes-Jurado *et al.,* 2023).

Almond (Prunus dulcis) is a nutrient-rich food that has been traditionally consumed for its health benefits. Almonds contain high-quality proteins, unsaturated fatty acids, vitamins (particularly vitamin E and B-complex), minerals such as calcium, magnesium, and phosphorus, and phytochemicals with antioxidant potential (Sethi et al., 2016; Catarino et al., 2018). Almond milk, obtained by extracting almonds in water, has become a widely consumed plant-based beverage. It is suitable for vegetarians, vegans, and people with lactose intolerance or allergies, and it is free of cholesterol and lactose (Demir et al., 2016; Prytulska et al., 2021).

Almond milk has multiple health benefits, but it is deficient in vitamin B12, an important element. Various health processes depend on vitamin B12 including, red blood cell production, brain functions, DNA synthesis, and energy metabolism. Its deficiency leads towards various diseases as megaloblastic anemia, exhaustion, neurological issues, and developmental delays and these deficiency issues are more prevalent in societies with low intake of foods produced from animals (Bernstein, 2017). That's why, adding vitamin B12 to plant-based drinks can be a long-term way to correct deficiencies, especially in poor societies and vegetarian populations.

The nutritional content and bioactive qualities of almond's milk are further improved by fermenting it with probiotic bacteria. Probiotics like Streptococcus and Lactobacillus species improve phenolic content, boost antioxidant activity, and support gut health (Bernart et al., 2015; Manzoor et al., 2021).

As a result, fermented almond milk offers two advantages: increased nutritional bioavailability and microbial activity. The goal of the current study was to create fermented almond milk fortified with vitamin B12 and assess its nutritional profile, functional abilities, and consumer acceptance while taking these factors into account. This study offers important new information on how to treat vitamin shortages with functional plant-based drinks that are both healthful and socially acceptable.

MATERIALS AND METHODS

Procurement of Raw Material

We took all the ingredients required for the production of fermented almond milk from local market Faisalabad, Pakistan. But analytical-grade chemicals and reagents were procured from Merck a city in Germany.

Proximate Analysis of Raw Material

Using the standard procedures of the Association of Official Analytical Chemists (AOAC, 2019), almonds were examined for proximate analysis including moisture, ash, crude protein, crude fat, crude fiber, and nitrogen-free extract.

- **Moisture Content:** It was done in hot air oven at 105°C until the weight remained constant.
- **Ash Content**: In a muffle furnace, it is burned at 550–600°C till a grayish residue appears.
- **Crude Protein**: The Kjeldahl method, which entailed figuring out the nitrogen level and multiplying it by a conversion factor (6.25), was used to measure crude protein.
- **Crude Fat**: assessed utilizing Soxhlet extraction with petroleum ether as the solvent.
- **Crude Fiber:** determined by burning in a muffle furnace following several digestions with acids and alkalis.
- **Nitrogen-Free Extract (NFE)**: was determined by using difference method such as:

NFE=100−(%Moisture+%Ash+%CrudeProtein+%CrudeFat+%CrudeFiber)

These analyses ensured the nutritional baseline of the almonds before product development.

Preparation of Almond Milk

To create almond milk, almonds were steeped in distilled water for 12 hours and then dehulled. The kernels were combined with distilled water at a perfect ratio of 1:4 w/v for two minutes in order to form a slurry. Straining the mixture through a double-layered muslin cloth produced almond milk (Reyes et al., 2021).

Preparation of Fermented Almond Milk

The probiotic strain Lactobacillus reuteri was activated from frozen stock cultures in de Man, Rogosa, and Sharpe (MRS) broth and incubated until optimal bacterial growth was achieved. The inoculum was then transferred into almond milk to initiate fermentation. Fermentation was carried out at three temperatures (20°C, 30°C, and 40°C) for 24 hours (Bernat et al., 2014).

Fortification with Vitamin B12

Fortification of almond milk with vitamin B12 was carried out using the physical soaking method. Almond milk was heated to 90°C and mixed with vitamin B12 solution for 15 minutes, ensuring even distribution and stability of the vitamin (Alozie *et al,* 2021).

Treatment Plan

The experiment was designed with four treatments based on fermentation temperature:

Treatment	Fermentation Temperature (°C)	Description
T0	(Unfermented)	Control almond milk
T1	20	Fermented almond milk at 20°C
T2	30	Fermented almond milk at 30°C
T3	40	Fermented almond milk at 40°C

Analytical Procedures for Developed Product

- **Antioxidant Activity (DPPH assay)**: Radical scavenging potential was assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH), measured spectrophotometrically at 510 nm (Manzoor et al., 2021).
- **Total Phenolic Content (TPC)**: Quantified using the Folin–Ciocalteu method, expressed as mg gallic acid equivalents per 100 ml of sample (Manzoor et al., 2021).
- **pH measurement**: pH of samples was recorded using a digital pH meter (AOAC, 2019).
- **Microbial Analysis (Total Plate Count)**: Performed by pour plate method using Nutrient Agar, with incubation at 37°C for 48 hours (Ozturkoglu-Budak et al., 2016).
- **Sensory Evaluation**: Conducted by a semi-trained panel (n=15) using a 9-point hedonic scale to assess color, taste, flavor, and overall acceptability (Meilgaard et al., 2016).

Statistical Analysis

The mean ± standard deviation was used to express the data, which were gathered in triplicate. A completely randomized design (CRD) was used for the analysis of variance (ANOVA), and at a significance level of $p < 0.05$, Tukey's test was used for multiple comparisons. IBM SPSS v20 was used for statistical analysis (Montgomery, 2017).

RESULTS AND DISCUSSION

Nutritional Composition (Proximate Analysis)

Fermented almond milk had a balanced ratio of macronutrients, according to the proximate composition study. Carbohydrates supplied energy without imposing an excessive calorie load, fat was primarily unsaturated, and protein content was kept at adequate levels. Vitamin B12 fortification improved the nutritional profile by adding a crucial ingredient that conventional almond milk lacks (Sethi et al., 2016).

Table 1 displays the proximate composition of fermented almond milk that has been enriched with vitamin B12. The findings showed that the amounts of ash, protein, fat, and carbohydrates were all in balance. By addressing vitamin B12 deficiency, fortification enhanced the micronutrient profile without appreciably changing the macronutrient composition.

Table 1

Proximate Composition of Fermented Almond Milk (Per 100 ml)

Parameter	Control (Unfortified)	Fortified with B12
Protein (g)	2.1 ± 0.05	2.2 ± 0.04
Fat (g)	1.8 ± 0.06	1.8 ± 0.05
Carbohydrates (g)	4.5 ± 0.12	4.6 ± 0.10
Fiber (g)	0.6 ± 0.02	0.6 ± 0.02
Ash (g)	0.7 ± 0.01	0.7 ± 0.01

Mineral Content

Almond milk was shown to be high in calcium, magnesium, and phosphorus, which are essential minerals for healthy bones and metabolic functions, according to mineral analysis (Catarino et al., 2018). Mineral retention was unaffected by fermentation, and fortification closed the vitamin B12 gap, adding to the nutritional value.

Mineral analysis demonstrated that almond milk was a good source of calcium, magnesium, and phosphorus. Table 2 shows that fermentation preserved mineral content, while fortification addressed vitamin B12 deficien

Table 2

Mineral composition of fermented almond milk (mg/100 ml)

Mineral	Control (Unfortified)	Fortified with B12
Calcium	72.5 ± 2.1	73.2 ± 2.0
Magnesium	26.4 ± 1.0	26.6 ± 1.2
Phosphorus	51.7 ± 1.8	52.1 ± 1.7
Potassium	110.3 ± 3.5	111.0 ± 3.4
Vitamin B12 (µg)	ND (Not Detected)	1.2 ± 0.05

Antioxidant Properties

Fermentation significantly improved the antioxidant activity of almond milk. Increased levels of phenolic compounds and enhanced radical scavenging capacity were observed, supporting previous findings that fermentation increases bioavailability of bioactive compounds (Manzoor et al., 2021). These properties highlight the beverage's role in reducing oxidative stress and chronic disease risks.

Fermentation significantly improved antioxidant activity, as shown in Table 3. The fortified samples exhibited higher total phenolic content and radical scavenging activity compared to controls, highlighting enhanced functional properties.

Table 3

Antioxidant Activity of Fermented Almond Milk

Parameter	Control (Unfortified)	Fortified with B12
Total phenolic content (mg GAE/100 ml)	22.1 ± 0.9	27.3 ± 1.1
DPPH scavenging activity (%)	54.5 ± 2.2	62.8 ± 2.4

Sensory Evaluation

Sensory evaluation showed high acceptability of fortified fermented almond milk. Panelists rated the product favorably for taste, color, texture, and overall palatability, with scores significantly higher than those for unfortified almond milk (Alozie & Udofia, 2015; Ojo et al., 2021). The product's creamy consistency, mild sweetness, and smooth texture contributed to its consumer appeal, confirming its potential as a marketable functional beverage.

Sensory scores indicated high consumer acceptability of fortified fermented almond milk (Table 4). Panelists preferred fortified samples in terms of taste, texture, and overall acceptability.

Table 4

Sensory Evaluation Scores (9-Point Hedonic Scale)

Attribute	Control (Unfortified)	Fortified with B12
Taste	7.2 ± 0.3	8.1 ± 0.2
Color	7.0 ± 0.4	7.8 ± 0.3
Texture	6.9 ± 0.3	7.9 ± 0.2
Overall acceptability	7.1 ± 0.3	8.2 ± 0.2

Comparative Discussion

The findings align with previous research emphasizing the nutritional and functional value of almond-based beverages (Demir et al., 2016). Unlike other plant-based milks, almond milk fortified with vitamin B12 directly addresses a critical nutrient deficiency in vegetarian and vegan populations (Bernstein, 2017). Fermentation further enhances its probiotic properties, which may contribute to improved gut health and immune system regulation.

CONCLUSION

This study demonstrated that fermented almond milk fortified with vitamin B12 is a nutritionally enhanced and highly acceptable functional beverage. It provides a plant-based alternative to dairy milk, suitable for individuals with lactose intolerance, milk allergies, or dietary preferences that exclude animal products. Fermentation improved its antioxidant activity, while fortification addressed the critical limitation of vitamin B12 deficiency. With its nutritional, functional, and sensory benefits, this beverage offers a promising solution to global health challenges related to micronutrient deficiencies. Moreover, its potential for commercialization and public health applications makes it a valuable addition to sustainable dietary strategies.

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