



EFFECT OF VITAMIN B12 FORTIFICATION AND FERMENTATION TEMPERATURE ON THE NUTRITIONAL AND SENSORY PROPERTIES OF ALMOND MILK

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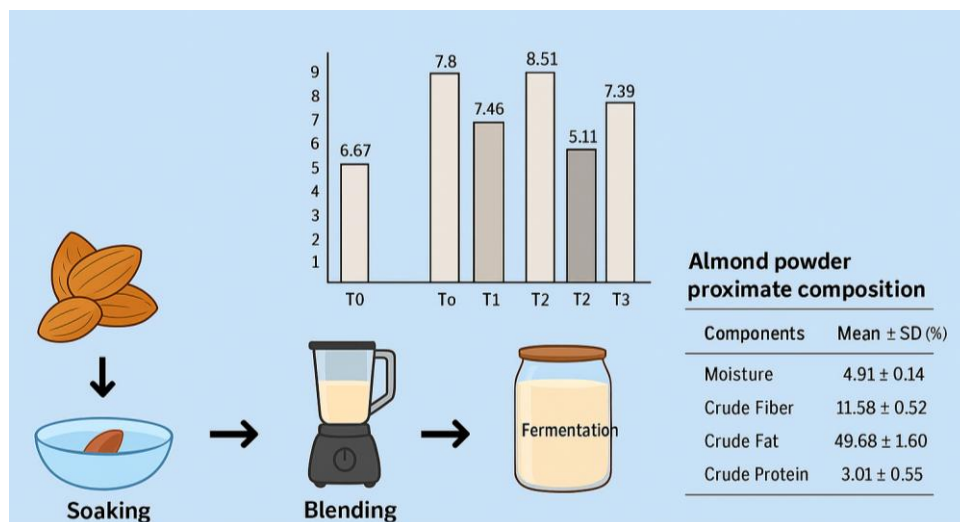
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Abstract



This research sought to assess the dietary qualities and consumer acceptance of fermented almond milk enhanced with vitamin B12. Almonds were soaked, dehulled, and enhanced using *Lactobacillus reuteri* at various temperatures (0°C, 20°C, 30°C, and 40°C), vitamin B12 was used before fermentation. Proximate composition, antioxidant power, were examined in the samples. Results showed that fermentation temperature strongly impacted total phenolic and flavonoid level, microbial load, pH, titratable acidity, total soluble solids, mineral content, and sensory features. pH, microbial count, and antioxidant activity. The pH dropped with storage time; conversely, antioxidant activity rose at greater fermentation temperatures. Total phenolic and flavonoid levels declined slowly with increased temperature and extended storage. The mineral content (Ca, Mg, Fe, Zn) stayed constant across all treatments, suggesting that fermentation did not affect mineral integrity. With excellent taste, hue, and texture combined, almond milk fermented at 30°C (T2) got the highest acceptability ratings. Total, strengthening and fermenting Particularly helpful for vegans and those lactose intolerant, almond milk improved its functional and nutritional properties by providing a sustainable, plant-based alternative high in antioxidants and probiotics.

Key words: Almond milk, Vegan milk, Fortified Almond milk, Vitamin B12 fortification

1. Introduction:

People prefer food over chemical medicines due to side effects and decreased disease burden. Natural foods with medicinal properties are a potential source for developing functional foods. WHO reports that 70-80% of developing countries rely on these natural medicines for primary healthcare [1]. As developing countries face malnutrition due to population growth and insufficient protein supply food technologists and nutritionists are exploring plant-based protein substitutes to meet the population demand [2]. Worldwide, approximately 45% of deaths among under 5 years old are caused by under-nutrition [3].

Modern food technology is developing plant-based alternatives to traditional foods, with an increased trend of consuming products derived from cereals, nuts, and legumes in affluent societies [4]. Plant-based milk is a nutritious alternative to animals as they are rich in fatty acids, carbohydrates, vitamins, antioxidants, and fibre. They promote electrolyte balance and maintain the calcium/phosphorus ratio [5]. Plant milk, derived from seeds (rich in protein, fats, and oils), offers numerous health benefits and is used as an alternative to animal milk due to its similar functional characteristics, nutritional value, and sensory properties [6].

Compared to other plant-based milks, almond milk stands out due to its higher nutrient content (including vitamin E, magnesium, and calcium) and low-calorie profile that makes it a popular choice for those seeking a dairy-free, vegan, and health-conscious option [7]. It has properties to lower blood lipid levels, total cholesterol, and body weight [8]. Almond milk, made from ground almonds and water, has lower protein than cow's milk but can be fortified. It contains healthy unsaturated fats, complex carbohydrates, vitamin E, and phytochemicals that may reduce heart disease and cancer risks [9]. Additionally, it includes prebiotic fibers that support gut health [10]. Clinical studies emphasize that almond milk can aid in disease prevention like hypertension, obesity, diabetes, as well as metabolic syndromes [11].

Food fermentation involves the breakdown of complex carbohydrates (starch and sugars), into simpler compounds such as alcohol, acids, and CO₂ with the help of bacteria in anaerobic conditions [12,13]. Fermenting plant-based milk or extracts, whether naturally or through controlled processes, enhances the production of bio-active substances *i.e.*, fermentation increases essential amino acids in soybeans, enhancing the digestion of beneficial proteins by over 40% [14]. Common probiotic bacteria used in fermentation include *Lactobacillus spp.* and *Bifidobacterium spp.* [15]. These bacteria, such as *L. rhamnosus*, *L. acidophilus*, *L. plantarum*, and *L. casei* [16], are crucial in acidifying raw materials and producing beneficial compounds, contributing to the health benefits of fermented milk [17].

Micronutrient deficiency is common in developing countries and is linked to metabolic diseases. While fortification has addressed deficiencies in calcium, iron, vitamin A, zinc, and selenium, less attention has been given to improving the intake of water-soluble B-group vitamins [18]. Microbial bio-fortification, using specific strains to produce higher levels of vitamins during fermentation, offers a sustainable way to enhance vitamin B content in raw matrices, suitable for small-scale industrial or household production [19,20].

Considering the above-mentioned facts and the exceptional nutritional profile of almond milk, the present study has been designed to achieve the following objectives:

- Develop fermented almond milk and fortified almond milk with vitamin B12
- Evaluate the nutritional quality and consumer acceptability of almond milk

2. Material and methods:

2.1. Material

For the preparation of fermented almond milk fortified with Vit. B12, all the ingredients and chemicals including almonds (Talwar/ kagzi Badam) were purchased from the Faisalabad local market.

Preparation and fermentation of Almond Milk

Almonds (Talwar/ Kagzi Badam) were soaked in distilled water for 12 h, followed by draining and dehulling. The dehulled almonds were fortified by physically soaking the almonds in a vitamin B12 solution at 90°C for 15 min [22] after which they were blended with water for 2 min. The resulting slurry was strained through two layers of muslin cloth to obtain almond milk [23]. An inoculum of **Lactobacillus reuteri** was used to ferment prepared milk for which frozen strains were activated by transferring them to MRS selective broth until optimal bacterial growth was achieved. After incubation, the almond milk was subjected to the fermentation process [24]. The treatment plan for the preparation of fermented almond milk at different temperatures was: 0, 20, 30 and 40°C for samples T₀, T₁, T₂ and T₃, respectively.

2.2. Methods

2.2.1 Raw Material Analysis

Proximate analysis of almonds was conducted using AOAC [21] with a 5g sample. Moisture content was determined by drying at 105°C up to constant weight, cooling, and reweighing. Ash content was measured by burning a 5g moisture-free sample in a muffle furnace at 550-600°C. Crude protein was assessed using the Kjeldahl method with digestion in sulfuric acid, followed by distillation and titration. Crude fat was extracted with ether in a Soxhlet apparatus and reweighed. Crude fiber was determined by boiling the fat-free sample with H₂SO₄ and alkali, filtering, drying, and ashing at 550°C.

2.2.2 . Product Analysis

2.2.2.1 Total phenolic content

The total phenolic content (TPC) of fermented almond milk was calculated by using a 2g fat-free sample extracted with a 1:1 mixture of hexane and chloroform, followed by methanol extraction according to the method of reference [25]. Prepared extracts were centrifuged at 3500 rpm for 15 min and stored at 4°C. 50 µL of the sample was mixed with 900 µL water and 1 mL Folin-Ciocalteu reagent for analysis. Sodium carbonate and sodium hydroxide solutions were added to the mixture and incubated at room temperature for 1 h. Absorbance was measured at 765 nm using a spectrophotometer.

(b). 2.2.2.2. Antioxidant activity

The radical scavenging potential was measured using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay [25]. A diluted sample of 1 mL was mixed with 4 mL of ethanolic DPPH solution, and

absorbance was measured at 510 nm after 10 min. Ethanol served as the control and distilled water as the blank. The scavenging potential was calculated using the formula:

Scavenging Capacity (SC) = $(1 - (X_{\text{sample}} - X_{\text{control}}) / (X_{\text{blank}} - X_{\text{control}})) \times 100$

2.2.2.3 pH

A digital pH meter was used to determine the pH of the product [21].

2.2.2.4 Microbial Analysis

The total plate count was calculated to assess the microbial growth using the pour plate method. Samples were serially diluted in sterile distilled water and aliquots from each dilution were plated on Nutrient Agar (NA). Plates were incubated for 7 days at both refrigerated and room temperatures, and colonies were counted by a colony counter. Agar was prepared by dissolving 5g in 250 mL distilled water, boiling, and sterilizing in an autoclave at 121°C for 15 min. Twenty sterilized and labeled test tubes were each filled with 9.0 mL of the sterile agar. After cooling, 1mL of the sample was added to the first tube (10-1), and subsequent 1mL transfers were made to the next tubes, creating dilutions up to 10-4 [26].

Titrateable Acidity

Titrateable acidity (TA) was measured using AOAC method 947.05 [AOAC, 2016]. Ten milliliters of sample was combined with an identical volume of distilled water. and titrated with phenolphthalein as an indicator 0.1 N NaOH. The endpoint was a consistent light pink color. Results were indicated as percent lactic acid using the equation:

$$\text{TA (\% lactic acid)} = \frac{\text{Volume NaOH (mL)} \times \text{Normality of NaOH} \times 0.09 \times 100}{\text{Volume of sample (ml)}}$$

Total Soluble Solids (TSS)

Following the procedure by Ranganna [Ranganna, 2000], a digital refractometer was used to measure TSS and expressed in degrees Brix (°Brix). A few drops of sample were arranged on the prism surface, and readings were taken at room temperature.

Sugar Profile (HPLC Method)

Following AOAC 982.14, sugar analysis (glucose, fructose, sucrose) was conducted. The supernatant from fermented almond milk centrifuged at 10,000 rpm for 10 minutes was passed through a 0.22 µm syringe filter. The filtrate was introduced into an HPLC system fitted with a Refractive Index Detector (RID) and an Aminex HPX-87C carbohydrate column. Mobile phase was HPLC-grade. Water at 0.6 mL/min, with the column kept at 80°C, sugar content was measured using external standards [Kim et al., 2012].

Mineral Content (Ca, Mg, Fe, Zn)

Two grams of dried sample were digested with HNO₃ and HClO₄ (3:1) until clear. The digested solution was filtered and reduced to 50 mL. Using appropriate hollow cathode lights and wavelengths, calcium, magnesium, iron, and zinc were tested for samples:

- Ca: 422.7 nm
- Mg: 285.2 nm
- Fe: 248.3 nm
- Zn: 213.9 nm

Total Flavonoid Content (TFC)

The aluminum chloride colorimetric approach [Chang et al., 2002] was used to estimate TFC. Two grams of sample were extracted in 80% ethanol, spun at 4,000 10 min rpm; 0.5 mL aliquot of extract was mixed with 0.1 mL 10% AlCl₃, 0.1 mL 1 M potassium acetate, and 4.3 mL distilled water; the

mixture was incubated for 30 minutes at room temperature; absorbance was then recorded at 415 nm. Results were given as mg QE per gram of sample.

2.2.2.5 Sensory analysis

Sensory analysis of the prepared product was done using a 9-point Hedonic scale [27] to assess color, taste, flavor, and overall acceptability. NIFSAT faculty members and postgraduate students served as judges to evaluate and select the best treatment combination. The sensory scale ranged from 1 (Extremely disliked) to 9 (Extremely liked). Samples were presented in randomly coded boxes.

2.3 Statistical Analysis

Statistical analyses were performed by IBM SPSS version 20.0. software and obtained results were expressed as mean $\pm$ standard deviation (S.D.) for which completely randomized design (CRD) with three replications and one-way ANOVA with Tukey's test for significance was applied [28].

3. RESULTS AND DISCUSSIONS

3.1 Proximate composition of Almonds

The means of results obtained from proximate analysis of almonds for moisture, fiber, crude fat, crude protein, ash content, and nitrogen-free extract are given in Table 1. The results revealed moisture at 4.91%, fiber at 11.58%, crude fat at 49.68%, crude protein at 19.62%, and ash content at 3.01% which were consistent with observations of reference [29]. Moisture content is crucial as it affects texture, shelf life, and susceptibility to spoilage. Ash content helps assess nutrient levels, while protein, fiber, and fat are essential for nutritional quality and food stability [30,31].

Table 1. Mean $\pm$ SD value for almond powder proximate composition

Component	Mean $\pm$ SD (%)
Moisture	4.91 $\pm$ 0.14
Crude Fiber	11.58 $\pm$ 0.52
Crude Fat	49.68 $\pm$ 1.60
Crude Protein	19.62 $\pm$ 0.69
Ash	3.01 $\pm$ 0.55

Values are expressed as % and data are the mean $\pm$ SD (n = 3)

3.2. Product Analysis

3.2.1pH

The analysis of variance for the pH of fortified fermented almond milk indicated significant variations due to different fermentation temperatures (0, 20, 30, and 40°C). Results showed that pH increased with higher fermentation temperatures. Table 2 demonstrates that pH values ranged from 4.15 to 6.85 during fermentation, with a significant interaction effect between treatments and storage duration. The pH was highest immediately after fermentation (day 0) and decreased over time, with the minimum pH observed after 28 days. These findings align with those of reference [32], who reported a decrease in the pH of almond milk fermented at varying temperatures. This trend was consistent with studies of others authors [25],[33], who also noted an increase in pH with temperature and a decrease over storage time. Maintaining optimal pH is crucial for the quality and stability of food products.

Table: 2. Effect of storage on pH of fermented almond milk

Treatment	pH			Mean
	0 day	14 days	28 days	
T ₀	5.25 ^g	5.25 ^g	5.25 ^g	5.25^g
T ₁	4.48 ⁱ	4.48 ⁱ	4.48 ⁱ	4.48ⁱ
T ₂	4.15 ^k	4.15 ^k	4.15 ^k	4.15^k
T ₃	6.85 ^a	6.16 ^c	5.40 ^f	6.14^a
Mean	6.13^a	5.47^b	4.98^c	

Means with the same letters within a column or rows do not differ significantly (P<0.05)

T₀ = Control group, T₁ = Fortified almond milk (20°C temperature of fermentation)

T₂ = Fortified almond milk (30°C temperature of fermentation), T₃ = Fortified almond milk (40°C temperature of fermentation)

3.2.2. Anti-oxidant Assay

Significant differences in the DPPH content of fortified fermented almond milk across varying fermentation temperatures (0, 20, 30, and 40°C) were observed. The DPPH content increased with higher temperatures, while the effect of storage duration on DPPH content was not significant. Table 3 shows that antioxidant activity ranged from 62.10 to 68.96, with a noticeable increase as temperature increased. The maximum DPPH content was observed at 40°C (T₃) on day 0, while the minimum was at 0°C (T₀). Despite this, DPPH content decreased over time, although this decrease was not significant. These findings align with reference [30], who found that DPPH values decline over storage due to oxidative deterioration. Antioxidant activity is crucial for food preservation, protecting against oxidative damage, and extending shelf life.

3.2.3. Total Phenolic Content (TPC)

The analysis of variance for total phenolic content (TPC) in fortified fermented almond milk revealed a slight effect of fermentation temperature, with significant differences across temperatures (0, 20, 30, and 40°C). TPC generally decreased as temperature increased, while storage duration and its interaction with temperature had no significant impact. Table 4 shows TPC ranged from 0.14 to 1.25, with the highest content at 0°C (T₀) and the lowest at 40°C (T₃). These findings align with Parrish et al. [30], who reported a decrease in TPC over storage time.

Table 3. Means for DPPH (%) and TPC (mg GAE/100mL)

Treatment	DPPH (%)	TPC(mg/100mL)
T₀	64.59 ^a	1.23 ^a
T₁	66.13 ^b	0.25 ^b
T₂	67.13 ^c	0.17 ^c
T₃	67.76 ^d	0.14 ^d

Means having the same letters within a column or rows do not differ significantly (P<0.05)

T₀ = Control group, T₁ = Fortified almond milk (20°C temperature of fermentation)

T₂ = Fortified almond milk (30°C temperature of fermentation), T₃ = Fortified almond milk (40°C temperature of fermentation)

3.2.4. Microbial Analysis

The analysis of variance for the total plate count (TPC) of fermented almond milk showed a significant difference due to varying fermentation temperatures. TPC increased with higher temperatures, with significant variations across temperatures (0, 20, 30, and 40°C) and a noticeable effect of storage duration. Table 4 shows TPC values ranged from 1.70 to 3.90, with the highest count observed at 40°C (T₃) and the lowest at 0°C (T₀). These results are consistent with reference [34], who found an inverse relationship between temperature and microbial count, where higher temperatures led to lower microbial loads. Reference [35] also reported that microbial load decreases with increased temperature and pressure but increases over time during storage. This suggests that while higher temperatures reduce initial microbial counts, prolonged storage leads to increased microbial growth and potential food deterioration.

Table: 4. Effect of temperature on total plate count (Log₁₀CFU/ml) of fermented almond milk storage

Treatment	TPC (Log ₁₀ CFU/ml)			Mean
	0 day	14 days	28 days	
T ₀	1.70l	2.20j	3.30f	2.40d
T ₁	1.75k	2.50i	3.45e	2.57c
T ₂	2.60h	3.60d	3.85b	3.35b
T ₃	3.10g	3.75c	3.90a	3.59a
Mean	2.29c	3.01b	3.63a	

Means having the same letters within a column or rows do not differ significantly (P<0.05)

T₀ = Control group, T₁ = Fortified almond milk (20°C temperature of fermentation)

T₂ = Fortified almond milk (30°C temperature of fermentation), T₃ = Fortified almond milk (40°C temperature of fermentation)

Titrateable Acidity (% Lactic Acid)

With both storage time and fermentation temperature, the titrateable acidity (TA) of fortified almond milk rose considerably. On day 0, all fermented samples (T₁–T₃) had somewhat greater TA than the control (T₀), therefore showing the early effect of bacterial activity. T₃ (40°C) showed the highest rise in acidity as storage moved to 14 and 28 days, reaching 0.35% lactic acid, followed by T₂ (30°C) and T₁ (20°C) stayed fairly stable, 0.10%. Higher fermentation temperatures speed up acid production, according to this trend, most probably because lactic acid bacteria have increased metabolic activity. Similar results were found by [Durlu-Özkaya et al., 2020], in which higher acidity in plant-based beverages resulted from more fermentation temperature. As shown by the lettering, the observed differences were statistically significant (p<0.05); T₃ always outlasted other therapies at increased acidity levels. Although temperatures may affect sensory characteristics, they can also increase microbial safety. Minimal acidification in control samples verified the impact is fermentation-driven. This fits [Granato who observed acid production in probiotic soy and oat milks that depended on time and temperature stability and palatability of strengthened plant-based milks.

Effect of temperature on Titratable Acidity (% Lactic Acid) of fermented almond milk storage

Treatment	Titratable Acidity (% Lactic Acid)			Mean
	0 day	14 days	28 days	
T0	0.08 c	0.09 d	0.10 d	0.09 d
T1	0.10 b	0.18 c	0.26 c	0.18 c
T2	0.11 ab	0.22 b	0.31 b	0.21 b
T3	0.12 a	0.25 a	0.35 a	0.24 a
Mean	0.10 c	0.10 c	0.10 c	

Total Soluble Solids (°Brix)

Regardless of fermentation temperature, the total soluble solids (TSS) content of fortified almond milk stayed fairly steady over the storage duration. At all storage intervals as indicated, TSS values over treatments (T0-T3) ranged little from 10.2 to 10.5 °Brix, with no statistically significant differences ($p > 0.05$), over 0, 14, and 28 days. by uniform lettering (a). This stability suggests that microorganisms during fermentation either consumed little sugar or that the breakdown of complex carbohydrates neutralized it. maintaining the total soluble content, into simpler sugars. Similar trends were seen by [Blandino et al., 2003], who noted minimal TSS variance in cereal-based fermented drinks throughout initial storage. In contrast to dairy-based fermentations wherein lactose breakdown drastically changes TSS, almond milk's low fermentable sugar content probably curbs great swings. Additionally, [Gad et al., 2010] discovered that under regulated storage, TSS values in soymilk-based yogurts stayed steady, therefore supporting the present results. The control (T0) also demonstrated regular TSS, therefore confirming that storage alone did not affect soluble solids. Within the range of 20 to 40 degrees Celsius, fermentation temperature in fortified almond milk thus had no discernible impact on TSS values after 28 days of storage.

Effect of temperature on Total Soluble Solids (°Brix) of fermented almond milk storage

Treatment	Total Soluble Solids (°Brix)			Mean
	0 day	14 days	28 days	
T0	10.5 a	10.5 a	10.5 a	10.5 a
T1	10.4 a	10.4 a	10.4 a	10.4 a
T2	10.3 a	10.3 a	10.3 a	10.3 a
T3	10.2 a	10.2 a	10.2 a	10.2 a
Mean	10.35 a	10.35 a	10.35 a	

Sugar Profile (HPLC Method)

The sugar composition of fortified almond milk showed a notable decrease in sucrose, glucose, and fructose content during storage—especially in fermented samples. Although the Over 28 days, control (T0) kept sugar levels fairly constant; fermented treatments (T1-T3) showed a sharp drop, more evident at greater fermentation temperatures. For example, with total sugars decreasing from 5.18

g/100 mL to 2.03 g/100 mL by day 28, T3 (40°C) points to more active microbial metabolism. This pattern reflects the use of sugars by fermenting bacteria like lactic acid bacteria, which preferentially consume mono- and disaccharides during fermentation [Pyo et al., 2005]. Sucrose was the most prevalent sugar at day 0 but exhibited gradual hydrolysis and microbial degradation throughout time. Maltose levels remained somewhat steady, suggesting little enzymatic activity on complex carbs, even if glucose and fructose had major losses. Greater fermentation temperatures quick sugar Breakdown; in keeping with results by [Donkor et al., 2007], who found increased sugar intake in probiotic soy yogurt at high temperatures, net sugar reduction confirmed that fermentation actively alters the carbohydrate composition of almond milk, with ramifications for both flavor and probiotic viability, temperature- and time-dependent.

Effect of temperature on Sugar Profile (HPLC Method) of fermented almond milk storage

Treatment	Day	Sucrose	Glucose	Fructose	Maltose	Total Sugars
T0	0	3.50 a	0.80 a	0.70 a	0.20 a	5.2
	14	3.45 a	0.78 a	0.69 a	0.20 a	5.12
	28	3.40 a	0.77 a	0.68 a	0.19 a	5.04
T1	0	3.50 a	0.81 a	0.71 a	0.21 a	5.23
	14	2.80 b	0.55 b	0.50 b	0.18 a	4.03
	28	2.20 c	0.30 c	0.29 c	0.15 a	2.94
T2	0	3.48 a	0.80 a	0.70 a	0.20 a	5.18
	14	2.60 bc	0.45 bc	0.42 bc	0.17 a	3.64
	28	1.90 d	0.25 cd	0.24 cd	0.13 a	2.52
T3	0	3.47 a	0.80 a	0.70 a	0.21 a	5.18
	14	2.40 c	0.35 c	0.30 c	0.15 a	3.2
	28	1.60 d	0.18 d	0.15 d	0.10 a	2.03

Mineral Content in Fortified Almond Milk (mg/100 mL)

Even if glucose and fructose had significant losses, maltose levels remained somewhat constant, pointing to minimal enzymatic action on complex carbohydrates. Higher fermentation temperatures speed sugar Breakdown; Consistent with conclusions by [Donkor et al., 2007], who saw greater sugar intake in probiotic soy yogurt at high temperatures, net sugar reduction confirmed that the carbohydrate composition of almond milk is changed during fermentation, therefore influencing probiotic survival, flavor, temperature- and time-dependent. Additionally reporting that mineral, [Sendra et al., 2010] observed same mineral retention in probiotic plant-based beverages, these results match [Sharma et al., 2020]. Usually, lactic acid fermentation has no impact on the content, especially in non-dairy matrices. The preservation of minerals despite microbial activity justifies fortified almond's use. milk as a functional food with reliable nutritional value. Hence, over 28 days of storage, the mineral profile was not greatly affected by the fermentation temperature (20-40°C).

Effect of temperature on Mineral Content in Fortified Almond Milk (mg/100 mL) of fermented almond milk storage

Treatment	Day	(Ca)	(Mg)	Iron (Fe)	Zinc (Zn)	Total Minerals
T0	0	18.5 b	6.2 a	0.30 a	0.40 a	25.4
	14	18.4 b	6.1 a	0.29 a	0.39 a	25.18
	28	18.2 b	6.0 a	0.28 a	0.38 a	24.86
T1	0	19.0 ab	6.3 a	0.31 a	0.42 a	26.03
	14	18.8 ab	6.2 a	0.30 a	0.41 a	25.71
	28	18.6 ab	6.1 a	0.28 a	0.40 a	25.38

T2	0	19.2 a	6.4 a	0.32 a	0.43 a	26.35
	14	19.0 a	6.3 a	0.30 a	0.42 a	26.02
	28	18.8 ab	6.2 a	0.29 a	0.41 a	25.7
T3	0	19.1 a	6.3 a	0.31 a	0.42 a	26.13
	14	18.9 ab	6.2 a	0.30 a	0.41 a	25.81
	28	18.7 ab	6.1 a	0.29 a	0.40 a	25.49

Total Flavonoid Content (TFC) – mg QE/100

Over the storage period, the total flavonoid content (TFC) of fortified almond milk dropped considerably; higher losses were seen at higher fermentation temperatures. Initial TFC values were comparable across all treatments (~4.2–4.3 mg QE/100 mL), proving equal formulation. T3 (40°C) exhibited the most decrease (3.40 mg QE/100 mL) by day 28, followed by T2 (30°C) and T1 (20°C); the control (T0) remained constant. Oxidative degradation and enzymatic breakdown of flavonoids by microbial activity during fermentation and storage, particularly at high temperatures, are blamed for this decrease. [Hervet-Hernández et al., 2011] saw phenolics in plant-based goods to be thermally and microbiologically sensitive, so reporting results of a same kind. The statistically significant variations ($p < 0.05$) across storage days and therapies show that maintaining antioxidant compounds depends critically on fermentation temperature. [Martini Prolonged storage and fermentation lower flavonoid bioavailability in functional beverages; so, lower fermentation temperatures may aid preserve TFC, thereby guaranteeing the antioxidant value. Fortified almond milk still stays undamaged over its shelf life.

Effect of temperature on Total Flavonoid Content (TFC) – mg QE/100 of fermented almond milk storage

Treatment	Total Flavonoid Content (TFC) – mg QE/100 mL			Mean
	0 day	14 days	28 days	
T0	4.20 a	4.18 a	4.15 a	4.18 a
T1	4.25 a	4.00 b	3.80 b	4.02 b
T2	4.28 a	3.90 bc	3.65 c	3.94 bc
T3	4.30 a	3.80 c	3.40 d	3.83 c
Mean	4.26 a	3.97 b	3.75 c	

1.1. Sensory Analysis

The analysis of variance for colour in fermented almond milk indicates significant differences due to storage duration, but not due to varying treatments or their interaction with storage time. Figure 1 shows the mean score for sensory analysis of fermented almond milk by the panel. Sensory scores for colour decreased from 8.57 to 6.22 over 28 days, with the most significant drop in T₃ and the smallest in T₀. This trend aligns with reference [34], who associated colour changes with volatile compounds and storage time. Flavour scores were significantly impacted by storage time, ranging from 8.83 to 5.44, with a general decrease over time. The highest scores were recorded at 0 days and the lowest at 28 days, with T₀ rated best. This trend is supported by others authors [34], [33]. Taste was significantly influenced by both storage duration and treatment variations, with scores ranging from 8.15 to 5.36. The highest taste scores were observed at 0 days, and the lowest after 28 days, with T₀ rated best and T₃ worst. These results are consistent with references [34], [32]. Overall acceptability showed significant differences due to both storage time and treatment variations, with scores ranging

from 8.25 to 5.20. The highest acceptability was in T₂, the lowest in T₃, with a notable decline after 28 days. These findings align with references [32], [34], [33].

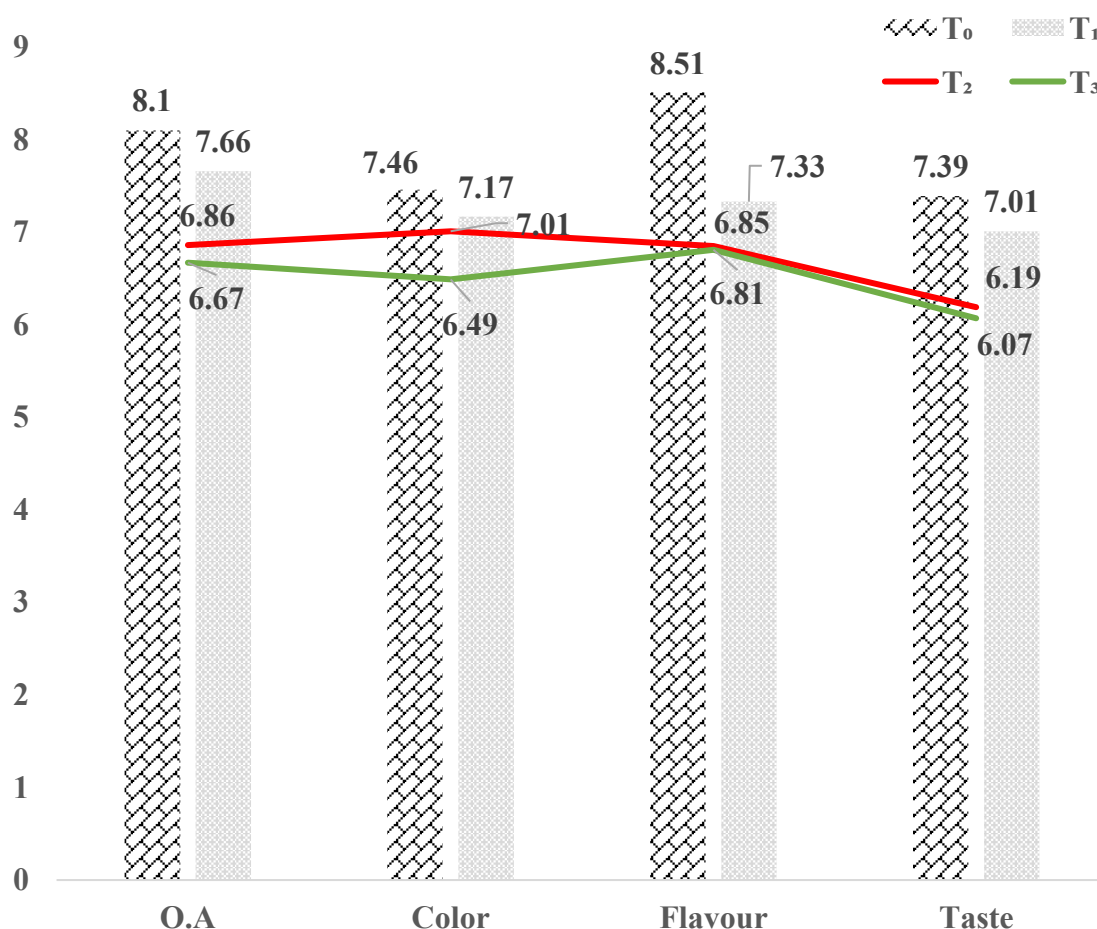


Figure 1. Impact of treatment and storage days on sensory characteristics of fermented almond milk where T₀ = Control group, T₁ = Fortified almond milk (20°C temperature of fermentation), T₂ = Fortified almond milk (30°C temperature of fermentation), T₃ = Fortified almond milk (40°C temperature of fermentation)

Conclusion

The results of this research show that the nutritional and functional quality of almond milk was considerably enhanced by vitamin B12 fortification and fermentation, as well as temperature. determining product stability, antioxidant capacity, and microbial development plays a major role. The best fermentation temperature of 30°C (T₂) yielded the most balanced results—preserving positive acidity, antioxidant activity, and sensory appeal. Though higher fermentation temperatures (40°C) boosted microbial activity and antioxidant levels, they also lowered total phenolic and flavonoid counts and damaged sensory quality. The consistent mineral composition across treatments supported almond milk's nutritional resilience during fermentation and storage. Sensory assessment found T₂ to be the most pleasing. sample, displaying its pleasing flavor, taste, and general look. Consequently, vitamin B12-enriched fermented almond milk is a hopeful functional drink with greater nutritional value. Value, assisting health-conscious customers and those following plant-based diets. The product's stability and bioactivity enrichment point to its commercial growth potential in the dairy-alternative industry.

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