

Possibilities and Limitations of Vitamin B12 Fortification in Fermented Plant-Based Foods – a postgraduate student literature review

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Abstract

People who follow a vegetarian or vegan diet could develop a vitamin B12 deficiency. The microbial fermentation of plant-based foods may lead to an increase in vitamin B12 levels in these foods. This narrative review investigated to what extent and how the fermentation of suitable foods can produce amounts of vitamin B12 that are bioavailable to humans. A literature search in PubMed and Google Scholar identified 15 articles on this topic that were used for this review. The studies confirm that the fermentation-based enrichment of bioavailable active vitamin B12 in plant-based foods represents a potential strategy to achieve the quantities deemed necessary. In most cases, cereal- or soybean-based foods were fermented using *Propionibacterium (P.) freudenreichii*. Vitamin B12 levels up to 11.1 µg/100 g dry matter were determined in plant-based foods. Future research should address the sensory acceptance of fortified foods as well as the stability of vitamin B12 during storage and thermal processing and ensure that the analytical methods reliably identify bioavailable vitamin B12.

Keywords: bioavailability, cobalamins,

Introduction

Vitamin B12 (cobalamins) are various water-soluble compounds that play diverse metabolic roles within human organism, including cellular metabolism, amino acid and fat metabolism, and DNA synthesis [1]. The German Nutrition Society (DGE) recommends a daily intake of 4.0 µg of vitamin B12 for adolescents and adults [2].

Vitamin B12 is exclusively produced by microorganisms. Plants do not contain relevant amounts of Vitamin B12. The necessary amount required by humans must therefore be obtained through the consumption of foods of animal origin. Various microorganisms used in food fermentation are capable of synthesizing vitamin B12 [3]. For this reason, vitamin B12 deficiencies occur particularly in strict vegetarian and vegan diets, but also in certain medical conditions. A deficiency increases the risk of neurological disorders. Excessive intake has no adverse health effects [4].

This narrative review examines the possibilities and limitations of enriching plant-based fermented foods with vitamin B12. In particular, the study

examines whether, to what extent, and in what ways vitamin B12 can be produced in a form that is bioavailable to humans through the fermentation of suitable foods

Material and Methods

Search procedure

The search for literature was performed according to the PRISMA Checklist and SANRA Scale for reviews. The literature search was limited to studies published in peer-reviewed journals. Based on current research and increased public interest, the time frame was limited to a 10-year period. Relevant publications were identified through systematic searches in PubMed and Google Scholar on March 31st, 2026 (Fig. 1). In both databases, the search terms “Vitamin B12 AND Fermentation AND Food” were used. Additionally, a filter was applied to include only articles published within the last ten years. First, all titles were screened for relevance to the topic. Subsequently, the abstracts were evaluated with a particular focus on how they described the possibilities and limitations of vitamin B12 production through fermentation of plant-based food. The full texts of the remaining studies were then thoroughly assessed by the whole study group of seven students, and final papers were selected. Finally, all identified articles were systematically assessed against predefined inclusion criteria to determine their eligibility for this review. These criteria specified that each paper had to address the production or enrichment of Vitamin B12 in plant-based foods. To be included, studies also had to be experimental trials investigating the enrichment of food through fermentation or reviews about the same topic and had to be published in either German or English. Studies were excluded if access to the full text was restricted or if they were not relevant to the scope of this review.

Vitamin B12 – Functions, Forms, and Analysis

Vitamin B12 (B12) plays an essential role in human metabolism. Most B12 is stored in the liver and transported in blood by the binding protein transcobalamin II [3; 5]. The biologically active forms, methylcobalamin and 5'-Deoxyadenosylcobalamin, act as coenzymes. Methylcobalamin serves as a cofactor for methionine synthase, involved in the remethylation of homocysteine to methionine, and playing a central role in DNA synthesis.

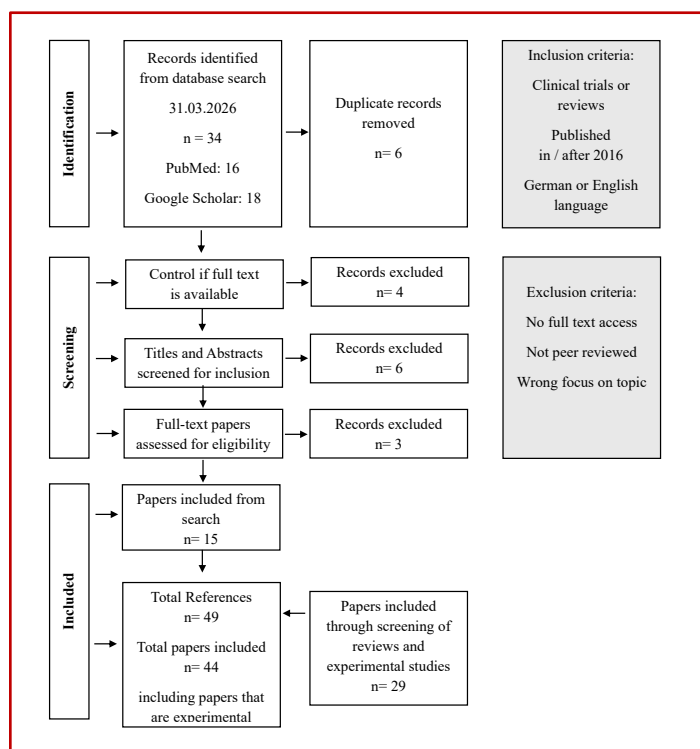


Fig. 1: Research tree for possibilities and limitations of Vitamin B12 fortification in fermented plant-based foods.

5'-Deoxyadenosylcobalamin is a co-factor of methylmalonyl-CoA mutase and is thus crucial for the breakdown of certain fatty acids and amino acids. B12 deficiency leads to elevated homocysteine and methylmalonic acid [1]. Bioactive cobalamins and their precursors are referred to as B12. Cobalamins are corrinoids containing a central cobalt ion [6]. Different ligands yield properties crucial for bioactivity in human metabolism. The cobalamins relevant to human metabolism feature 5,6-dimethylbenzimidazole (DMBI) as an α -axial ligand [7]. These include methylcobalamin and 5'-deoxyadenosylcobalamin, as well as the precursors cyanocobalamin and hydroxocobalamin, which act as provitamins in human metabolism [8; 9]. Other inactive forms cannot be utilized by human metabolism. A well-known inactive form is adenylobamide, also known as pseudovitamin B12 [10].

Cyanocobalamin is the most stable form and can be produced synthetically; it is commonly analyzed to determine total B12 content [3; 11]. B12 availability is reduced by up to 50% when exposed to heat (pasteurization, cooking) [12]. In contrast to the directly bioactive forms, its biological activity remains largely intact despite light sensitivity; agents such as ascorbic acid, sulfites, and iron (II) salts degrade hydroxocobalamin [13; 14]. B12 availability in foods varies greatly. B12 is synthesized by microorganisms and is typically ingested through animal-origin foods, which can contain up to 83.1 μg of B12 per 100 g of food. Absorption occurs in the ileum and depends on the amount ingested and the food matrix (absorption is relatively efficient from meat and dairy) [3; 15]. The absorption rate decreases as availability increases and can range from 5% to 50%, with average bioavailability around 40% [16; 17]. Low B12 levels have been detected in some plant-based foods and mushrooms, attributed to microbial activity, contamination or soil uptake [18]. However, many earlier studies used analytical methods that could not distinguish B12 from other corrinoids. Microalgae such as spirulina are often promoted as B12 sources, but they contain only inactive forms unsuitable for human use [19].

B12 in foods has traditionally been measured using a microbiological assay with *Lactobacillus delbrueckii* ATCC 7830 as the test organism. Under

standardized conditions, bacterial growth is proportional to B12 concentration. This method is the reference method [20]. It has high sensitivity but low specificity, often overestimating B12 levels by 5–30% because pseudovitamin B12 or nucleic acids can also support growth. The method cannot distinguish between B12 and pseudovitamin B12 [19]. Consequently, studies using this method cannot inform on B12.

In recent years, new methods have been developed to analyze and quantify B12. An ultra-high-performance liquid chromatography (UHPLC/UV) method was developed and validated to sensitively and selectively determine B12 in cell extracts of *P. freudenreichii* subsp. *shermanii* and in cereals fermented by *P. freudenreichii*. The method demonstrated excellent separation, high linearity, and a low limit of detection (0.075 ng/5 μL injection). B12 could be clearly distinguished from pseudovitamin B12 using mass spectrometry (UHPLC–MS). Recovery from purified cereal matrices was >90%. Within 24 hours at 28 °C, *P. freudenreichii* produced up to 1.9 μg /100 g whole product of B12 in cereal malt matrices [19].

Table 1 lists the analytical methods used to determine cobalamin levels in the studies reviewed. Most recent studies use the HPLC method described by Chamlagain et al. (2015) [19]. However, there are also studies that do not clearly specify the methodology used, leaving it unclear whether only B12 was measured or whether pseudovitamin B12 was also included (e.g., Palachum et al., 2020 [21]).

Table 1: Quantification methods of cobalamins in the studies.

Studies	Methods for Determining Vitamin B12 Concentrations	Refers to
Zhang et al. (2026)	UHPLC ¹	Chamlagain et al. (2015); Zhang et al. (2025)
Ashagrie et al. (2025)	UHPLC	Chamlagain et al. (2015); Ashagrie et al. (2023) ³
Călinoiu et al. (2024)	HPLC ²	Chamlagain et al. (2015) ³
Rastogi et al. (2024)	HPLC	Xie et al. (2018) ³
Tindjau et al. (2024)	HPLC	Tindjau et al. (2023) based on Campos-Giménez et al. (2008); Giménez. (2014); Heudi et al. (2006)
Thompson et al. (2020)	Microbiological Methods according to AOAC Official Method 952.20	Ball GFM. (2006)
Signorini et al. (2018)	HPLC	Samadi-Maybodi et al. (2008)
Chamlagain (2016)	HPLC	
Kustyawati et al. (2020)	HPLC	Lawrance P. (2011)
Xie et al. (2021)	UHPLC	Chamlagain et al. (2015) ³
Palachum et al. (2020)	"adaptations of published methods" (not further described)	Chen et al. (2006)
Luthi et al. (2025)	HPLC	method AOAC (2014)
He & Howell (2022)	HPLC	Chamlagain et al. (2015); Okamoto et al. (2020)
Bernhardt (2019)	UHPLC	

¹UHPLC: ultra-high-performance liquid chromatography

²HPLC: high-performance liquid chromatography

³with modifications

Tab. 2: Overview of reviewed studies on the fortification of plant-based foods with cobalamin through microbial fermentation.

Substrate	Plant-Based Food	Microorganism Involved	Cobalamin Content Increase	Sensory Evaluation	References
Glutinous rice	Glutinous rice	<i>Propionibacterium freudenreichii</i> (J117, DSM 20271), <i>Rhizopus oryzae</i>	0.015 µg/100 mL	<i>R. oryzae</i> + <i>P. freudenreichii</i> (J117): less lactic acid, more floral, cereal, sweet, and dairy notes; the most aromatic <i>Rhizopus</i> + <i>P. freudenreichii</i> (DSM 20271): less aroma.	Zhang et al., 2026
Review- no testing	Soy milk, soy yogurt, tempeh, Injera, Stinky tofu & pickled vegetables	<i>Limosilactobacillus reuteri</i> , <i>Propionibacterium shermanii</i> <i>Propionibacterium freudenreichii</i> , <i>Levilactobacillus brevis</i> <i>Limosilactobacillus reuteri</i> <i>Rhizopus oryzae</i> , <i>Propionibacterium freudenreichii</i> <i>Rhizopus oligosporus</i> , <i>Klebsiella sp.</i> , <i>Saccharomyces cerevisiae</i> <i>Propionibacterium freudenreichii</i> (NCC 1177) <i>Loigolactobacillus coryniformis</i> Native microbial strains	Soy yogurt: 18 µg/100 mL (Gu et al., 2015) Injera: 2.1 µg/100 g (Ashagrie et al., 2023) Stinky Tofu Pickled Vegetables: >10 µg/100 g (Koseki et al., 2023)	-	Deokar et al., 2025
Teff flour	Injera (ethiopian flatbread)	<i>Propionibacterium freudenreichii</i> (ATCC 13673), <i>Lactiplantibacillus plantarum</i> (P2R3FA)	11.1 ± 1.4 µg/100 g dry weight	-	Ashagrie et al., 2025
Wheat & Oat Bran	Wheat & oat Bran	<i>Propionibacterium freudenreichii</i> , (DSM 20271)	0.6 µg/100 g dry weight (without supplementation)	-	Călinoiu et al., 2024
Pasteurized guava juice	Guava juice	<i>Levilactobacillus</i> (KU15152)	109.5 µg/ L	Sour. Better with added honey	Rastogi et al., 2024
Soybeans	Soy whey	<i>Bifidobacterium animalis</i> (B1-04), <i>Propionibacterium freudenreichii</i> (DSM 20271)	8.93 µg/ L	Reduction of soy volatile (beany note). Increasing of volatile organic acids	Tindjau et al., 2024
Cauliflower, white bean	Cauliflower & white beans mixture	<i>Lactobacillus plantarum</i> (299v, Lp900, 299, Heal19)	0.048 ± 0.013 µg/100 g	Salty and sour, like traditional cabbage + underlying umami taste	Thompson et al., 2020
Lupines	Tempeh	<i>Rhizopus oligosporus</i> (-), <i>Propionibacterium freudenreichii</i> (-)	123 µg/100 g dry weight	(Informal assessment only): pleasant	Signorini et al., 2018
Malted barley, barley flour, wheat aleurone	Barley & wheat aleurone	<i>Propionibacterium freudenreichii</i> , <i>Propionibacterium acidipropionici</i>	0.45 to 3.35 µg/100 mL	-	Chamlagain, 2016
Soybeans	Soybean tempeh	<i>Rhizopus oligosporus</i> (natural), <i>Saccharomyces cerevisiae</i> (natural), <i>Klebsiella sp.</i> (-)	0.81-3.15 µg/100 g	Yeasty aroma, production of alcohol, ester, styrene, caryophyllene, phenols, isoflavones maltol	Kustyawati et al., 2020
Cereals: (oat bran, rice bran, rye bran, millet flour, sorghum flours) Pseudocereals: (buckwheat bran, amaranth flour, quinoa flour) Legumes: (soybean flour, faba bean flour, lupine flour)	Dough	<i>Propionibacterium freudenreichii</i> (DSM 20271), <i>Levilactobacillus brevis</i> (ATCC 14869)	0.742 µg/100 g dry weight	-	Xie et al., 2021

Milk and dairy products in human nutrition

Guava pulp	Gummy jelly	<i>Lactobacillus plantarum</i> (WU-P19)	1,070 µg/100 g	Natural taste of guava: sweet, sour fermented end product (jelly): good taste especially with citric acid added, helps against odor because of fatty acid oxidation	Palachum et al., 2020
Water of sauerkraut fermentation (cabbage)	Sauerkraut water	<i>Propionibacterium freudenreichii</i> subsp. <i>globosum</i> (-), naturally occurring bacteria	1.62 µg/100 mL	Increase of acetic acid, propionic acid	Lüthi, Serventi 2025
Soybeans	Tempeh	<i>Propionibacterium freudenreichii</i> (ATCC 9617), <i>Rhizopus oligosporus</i> (ATCC 22959)	0.425 µg/100 g	-	He, Howell 2022
Tofu made with plant materials/extracts	Tofu	<i>Acetobacter pasteurianus</i> (DSM 3509), <i>Lactobacillus reuteri</i> (DSM 20016), <i>Propionibacterium freudenreichii</i> (DSM 20271)	0.171 µg/100 mL	-	Bernhardt, 2019

Suitable Foods for Fermentation

Cobalt content

Legumes, grains, pseudograins, and cruciferous vegetables are particularly suitable as substrates for the production of a fermentatively enriched food containing B12 [34; 40; 42]. These food groups naturally contain cobalt, which is essential for the formation of B12. While oat and rye bran have cobalt levels of up to 20 ng/g dry matter (DM), significantly higher cobalt levels have been detected in rice bran, amaranth flour, and buckwheat bran, which can exceed 150 ng/g DM. Field bean flour stands out in particular with a cobalt content of 696 ng/g DM [40].

Starting materials and end products

The substrates listed in Table 2 were fermented. The products include typical fermented foods such as tempeh, soy yogurt, injera (Ethiopian flatbread made from millet flour), and pickled vegetables. Particularly noteworthy in this context is sauerkraut brine, a byproduct of sauerkraut production that can be enhanced through targeted post-fermentation to enrich it with B12 [42; 47]. Many sources also make use of substrates that are not usually fermented. These include, for example, soy milk, tofu, glutinous rice, wheat and oat bran, whole grain barley, wheat, and cauliflower. Another unusual substrate examined in two studies is guava juice and its processing into gummy candies [21; 27].

Foods such as tofu, which are already consumed very regularly by vegans, can help meet daily requirements after being fortified with B12. Fermentative fortification of this product with B12 could easily counteract a deficiency in this population group without the need to incorporate an additional product into the diet. For example, a B12 content of 52 µg was detected in 100 g of traditionally fermented Chinese tofu ("stinky tofu") [48].

Practical application – Tempeh

Tempeh production offers a way to provide B12. For example, through co-fermentation with *P. freudenreichii* and *Rhizopus* (*R.*) *oligosporus*, it is possible to produce tempeh with a B12 content of 8.26 µg (± 0.13 µg) per 100 g of product. Given the high and consistent B12 content, a single serving (100 g) of this tempeh could satisfy the daily requirement for B12 [44].

The achievable B12 levels can vary significantly depending on the choice of raw material. In the production of tempeh from lupins, even higher B12 levels of up to 123 µg B12 per 100 g of DM were achieved [35].

Practical application - Soy yogurt

Through fermentation with *Limosilactobacillus reuteri*, with the addition of 20 g/L glucose, 10 g/L glycerol, and 1 g/L fructose, a B12 content of 18 µg per 100 mL of product was achieved in soy yogurt. When only 20 g/L of glucose was added, a B12 content of approximately 6.8 µg per 100 mL could be achieved. Thus, the soy yogurt achieved a higher B12 concentration than other fermented soy products [49]. This demonstrates that fermentation can be significantly enhanced through supplementation. However, the B12 content should be interpreted with caution. In the detection method, several active and inactive forms of B12 are measured together and converted to bioavailable B12.

However various sources/articles do not always specify whether the final products contain B12 or pseudovitamin B12.

Microbial fermentation

Fermentative Enrichment of B12 in Plant-Based Foods

P. freudenreichii is predominantly used in the fermentation processes investigated for the production of B12 (Table 2). In addition, other microorganisms such as lactic acid bacteria (*Lactobacillus plantarum*, *Limosilactobacillus reuteri*, and *Levilactobacillus brevis*) are used. It should be noted that certain species (e.g. *L. reuteri* and *P. acidipropionici*) are capable of producing pseudovitamin B12 [37; 46]. During fermentation, by-products such as organic acids and gases are also produced, which influence the course of the process. As *P. freudenreichii* produces propionic acid, self-

inhibition of growth may occur as fermentation progresses.

A two-phase process is frequently used for B12 production with *P. freudenreichii*: first, anaerobic cultivation takes place over two to three days to promote cell growth. Fermentation is then continued under aerobic conditions, which stimulates B12 synthesis. *P. freudenreichii* achieves its optimal production rate at a neutral pH [35; 42]. The mould *R. oligosporus* is frequently used in co-fermentations [35; 44]. It produces amino acids that counteract the acidification caused by *P. freudenreichii*, resulting in a positive effect on the pH. *R. oligosporus* is also used to develop the structure of the food, for example in tempeh [35]. Consequently, in co-fermentations, not all strains are directly involved in the synthesis of B12, but they make a significant contribution to the overall process due to their metabolic and growth characteristics [22].

The cultivation conditions are largely comparable across the studies examined. In all cases, mesophilic temperatures in the range of 30–37°C are maintained. The microorganisms are typically pre-cultured on agar plates or in liquid media, such as Yeast Extract Lactate medium for *P. freudenreichii* and De Man, Rogosa and Sharpe medium for lactic acid bacteria [40; 42]. Subsequently, an inoculum of approximately 5–10% is used to initiate fermentation. The fermentation duration generally varies between two and eight days. In laboratory-based studies using optimised media, supplements such as riboflavin, nicotinamide or cobalt are also frequently added to increase the B12 yield [26]. However, these conditions can only be applied to food matrices to a limited extent, as the use of such additives is often restricted in that context.

Sensory profiles and fermentation effects

The fermentation of substrates using B12-producing microorganisms results not only in an increase in B12 content but also in the accumulation of other metabolites, such as organic acids, alcohols, and gases. This may affect the sensory properties (Table 2). Therefore, a sensory analysis and evaluation of the fermented food are important to assess the product's suitability as a food in terms of taste. The most observed change in taste is an increase in acidity of the food due to fermentation. Several sources report an increase in acids such as lactic, acetic, and/or propionic acid (see Table 2) [21; 27; 34; 42].

Furthermore, fermentation also leads to differences in flavor among various strains of the bacterium *P. freudenreichii*. Zhang (2026) identified significant sensory differences and variations in acid production among the *P. freudenreichii* strains during the co-fermentation of two different *P. freudenreichii* strains with *R. oryzae*. A strong positive correlation was also demonstrated between the presence of a grainy and a floral note, which was attributed to the formation of a pentene compound. Another observation was the correlation between the amount of acetic acid produced and a floral, fruity, milky flavor profile. According to Zhang, these correlations could therefore also be used in the future to tailor a desired aroma [22].

Conclusion

The present studies confirm that the fermentative enrichment of B12 in plant-based foods represents a viable strategy for meeting daily requirements. In consideration of biological activity, the investigation results for injera at 11.1 µg/100 g DM, wheat at 1.4 µg/100 g DM, dough at 0.7 µg/100 g DM, and tempeh at 0.4 µg/100 g represent potential product approaches. In these studies, *P. freudenreichii* (ATCC9617; DSM 20271) was used, partially supported by a co-culture. Future research should focus on the sensory acceptance of the enriched foods as well as the stability of B12 during storage and thermal treatment.

Disclosure of conflicts of interest

All authors declare no conflict of interest.

Compliance with Ethical Standards

This study has been conducted in compliance with ethical standards.

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