

Review

Fermented vegetables and fruits as vitamin B₁₂ sources: An overview

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Article history

Received:

15 July 2022

Received in revised form:

12 November 2022

Accepted:

8 May 2023

Keywords

cobalamin,
cyanocobalamin,
fermented plants,
vegetarianism,
health

Abstract

There is a trend towards the consumption of plant foods, especially from the public that aims to reduce meat consumption. Plant-based food diets may have low source of vitamin B₁₂ as plants do not produce them. A possible alternative to mitigate this is the consumption of fermented vegetables and fruits. Therefore, we aimed to provide an overview of the work being done with fermented vegetables and fruits, and show evidence that it is possible to obtain the necessary daily amount of vitamin B₁₂ for human health and maintenance. Vitamin B₁₂, also known as cobalamin, acts as a cofactor for the enzyme methionine synthase and methylmalonylCoA mutase in eukaryotes. The dietary reference values for adult men and women range between 2 and 4 µg/day; however, the requirement may increase depending on special recommendations. The main causes for vitamin B₁₂ deficiency are autoimmune conditions (*e.g.*, pernicious anaemia), malabsorption, and dietary insufficiency. One of the commonly adopted measures to deal with vitamin deficiency is supplementation. It is also possible to obtain food enriched with vitamin B₁₂ through fermentation. Different plant materials and microorganisms can be used to produce fermented products and enhance traditional products, such as tempeh, to increase vitamin B₁₂ concentration in the final product. The bioaccessibility and bioavailability of vitamin B₁₂ in fermented vegetables and fruits are important factors to be considered, and demand more studies. The intake of soy fermented foods, such as tempeh, tofu, and cheonggukjang was associated with cognitive enhancement and neuroprotective effects. In addition to fermented vegetables and fruits, other non-animal sources of vitamin B₁₂ that deserve great attention are algae and mushrooms. Since fermentation can produce considerable amounts of vitamin B₁₂, fermented vegetables and fruits are feasible alternative sources for the intake of this vitamin.

DOI

<https://doi.org/10.47836/ifrj.30.5.02>

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Introduction

The awareness that the planet will not support our lifestyle for much longer has increased in our society. It is known that food, or in other words, the way food is produced, promotes several damages to the environment. In this scenario, the meat production chain stands out, especially ruminant meat (Salami *et al.*, 2019; González *et al.*, 2020; van Vliet *et al.*, 2020). Livestock production and industry are responsible for up to 12% of total greenhouse gas emissions. Also, it negatively affects the water

footprint, pollution, and scarcity (Farchi *et al.*, 2017; Allen and Hof, 2019; González *et al.*, 2020).

In addition to environmental concerns, people have other motivations to reduce or stop consuming animal products, such as health-related issues, animal suffering, and personal or religious beliefs. Vegetarian diets are increasingly being associated with several health benefits such as reduced risk of cardiovascular diseases, diabetes mellitus, and chronic kidney diseases (Kahleova *et al.*, 2017; Olfert and Wattick, 2018; Rocha *et al.*, 2019). Nonetheless, it is important to mention that the way vegetables are

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grown must also be considered. For example, chronic exposure to pesticides has been implicated in several human diseases including cardiovascular diseases (Adeyemi *et al.*, 2021).

Vitamin B₁₂ (cyanocobalamin) is not produced by plants since they do not need this vitamin for their survival. Animal and fermented products are the main sources of this vitamin, and the *de novo* biosynthesis of vitamin B₁₂ is carried out by microorganisms. Its biological relevance is due to its role as a cofactor of two important enzymes for anabolism and catabolism in the human body such as haematopoiesis and tissue expansion. Vitamin B₁₂ is also important for neuronal function, participating in the maintenance of the myelin sheath (Rizzo and Laganà, 2020). Currently, it is known that fermented foods can contain considerable amounts of vitamin B₁₂. In addition, considerable concentrations of folate (vitamin B₉) and riboflavin (vitamin B₂) are also being quantified in fermented foods.

In most cases, people use supplementation of pharmaceutical origin to remedy vitamin B₁₂ deficiency. Many studies indicate adequate and possible concentrations to be ingested daily from fermented vegetables and fruits (Xu *et al.*, 2016; Yongsmith *et al.*, 2016; Xie *et al.*, 2018; Thompson *et al.*, 2020). Based on this evidence, the present review aimed to provide an overview of the work being done with plant fermented foods, and show evidence that it is possible to obtain the daily vitamin B₁₂ necessary for human health and maintenance.

Methodology

Information sources

To compile the necessary information, data, and literature, electronic scientific databases such as Google Scholar, PubMed, SciELO, Science Direct, SCOPUS, and Web of Science were used.

Search terms

The strategy for the selection of studies used the following keywords and a combination of terms: B-vitamins, B₁₂ bioenrichment, cobalamin, cyanocobalamin, fermented foods, fermented plants, fermented plant extract, plant-based foods, vegetarianism, and health. The selected works were published mainly between 2016 and 2021.

Selection criteria

The selected studies were screened based on

titles, abstracts, and keywords. In order to refine the works found, it was (i) limited to research articles and reviews, (ii) not published before 1988, (iii) published in English, and (iv) the removal of duplicate works. Studies selected based on the established criteria were independently reviewed by two authors. Data synthesis was performed by separating the articles into two categories: those which provided examples of fermented products, and those necessary for the theoretical basis of the items discussed. Afterward, exclusion criteria were used such as (i) no access to the full text, (ii) fermented products with added vitamin B₁₂, and (iii) fermented products with components of animal origin. Then, the material was organised and stored in Mendeley[®] for further data extraction.

Vitamin B₁₂

Vitamin B₁₂ (α -(5, 6-dimethylbenzimidazolyl) cobamidcyanide), or cobalamin, is an important water-soluble nutrient that contains cobalt in the core of its molecular structure (Smith *et al.*, 2018). This vitamin plays an important role in several physiological processes in the human body, especially in maintaining neuronal health and haematopoiesis (Green *et al.*, 2017). Chemically, cobalamin has a corrin ring (tetrapyrrole macrocycle) with cobalt in the centre, coordinated *via* the four pyrrole nitrogen atoms. Attached to the cobalt-coordinated corrin ring, through a side chain linked to ring D of the macrocycle, is a lower nucleotide loop that contains a base called dimethylbenzimidazole (DMB) (Smith *et al.*, 2018; Watanabe and Bito, 2018). In biological systems, when the upper cyano (beta) ligand is replaced, hydroxocobalamin (hydroxyl), methylcobalamin (methyl), and adenosylcobalamin (adenosyl) can be formed (Figure 1). There are pseudovitamins, which can be formed by bases other than DMB. This is the case of pseudovitamin B₁₂, formed from adenine, which is produced by anaerobic microorganisms, and commonly found in foods (Watanabe *et al.*, 2014; Watanabe and Bito, 2018).

Vitamin B₁₂ *de novo* biosynthesis is restricted to prokaryotes, and occurs through aerobic or anaerobic pathways, in bacteria and archaea, respectively. Some differences between these pathways are related to cobalt chelation and oxygen requirements (the aerobic pathway requires oxygen to promote ring-contraction, while the anaerobic

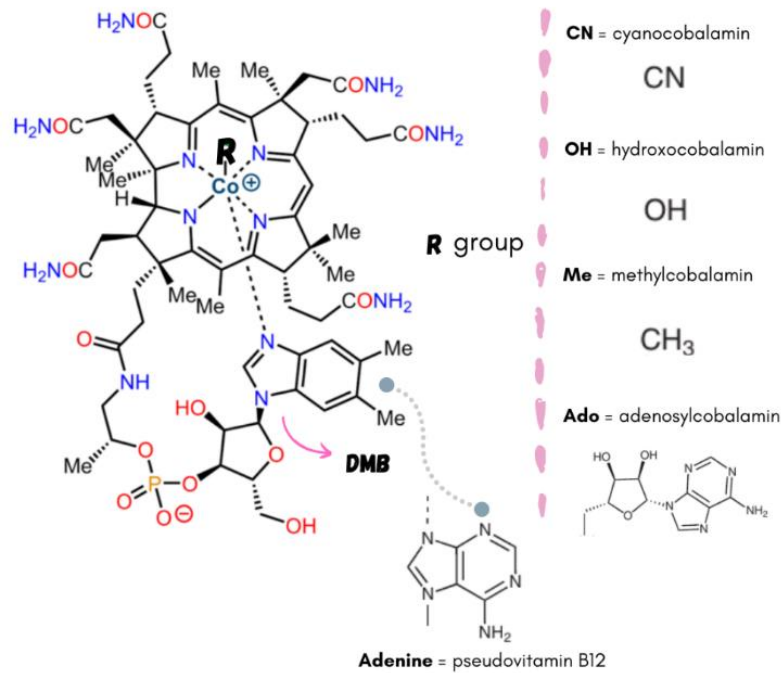


Figure 1. Structure of vitamin B₁₂ and partial B₁₂-related compounds. CN: cyanocobalamin (vitamin B₁₂); OH: hydroxocobalamin; Me: methylcobalamin; Ado: adenosylcobalamin; Adenine: pseudovitamin B₁₂; DMB: 5,6-dimethylbenzimidazole (Smith *et al.*, 2018; Watanabe and Bitto, 2018).

pathway does not require oxygen in this step) (Fang *et al.*, 2017). Both pathways for the synthesis of AdoCbl from the common tetrapyrrole primogenitor uroporphyrinogen III diverge at an intermediate called precorrin-2, and converge with the formation of adenosylcobyrinic acid. Vitamin B₁₂ molecule is extremely complex, and approximately 30 enzymes are involved in its biosynthesis (Smith *et al.*, 2018).

The function of vitamin B₁₂ in eukaryotes is acting as a cofactor for the enzymes methionine synthase (MetH) and methylmalonylCoA mutase (MCM) (Smith *et al.*, 2018). MetH is important for amino acid synthesis, while MCM acts in the amino acid breakdown. The formation of methionine, one of the amino acids formed with the participation of vitamin B₁₂, is essential for providing methyl groups for methylation (Smith *et al.*, 2018; Rowley and Kendall, 2019). Higher plants and fungi do not require B₁₂ for their biological functions. Besides its vital function in the human body, vitamin B₁₂ may also present antioxidant properties, such as acting directly in the reactive oxygen species (ROS) scavenging, particularly superoxide anion, or indirectly by stimulating ROS scavenging through preservation of glutathione (van de Lagemaat *et al.*, 2019).

During digestion, vitamin B₁₂ is absorbed in the ileum, the final part of the small intestine. With

help of the mammalian protein intrinsic factor (IF), which is produced in the stomach, the vitamins bind, and are thus able to be absorbed into the bloodstream (Stabler and Allen, 2004; Rowley and Kendall, 2019). The dietary reference values for adult men and women range between 2 and 4 µg/day; however, the requirement may increase depending on special recommendations, such as during pregnancy and lactation (Obeid *et al.*, 2019). According to Obeid *et al.* (2019), despite the evidence of malabsorption and deficiency in elderly people, there are no special intake recommendations.

The main causes for vitamin B₁₂ deficiency are autoimmune conditions (*e.g.*, pernicious anaemia), malabsorption, and dietary insufficiency (Green *et al.*, 2017). The classic clinical presentation of vitamin B₁₂ deficiency is the Addison-Biermer disease with megaloblastic anaemia. However, psychiatric disorders such as depression, mania, psychosis, and suicidal thoughts have been reported (Wolffenbuttel *et al.*, 2019). One of the commonly adopted measures to deal with vitamin deficiency is supplementation. The main microorganisms used for vitamin B₁₂ production are *Propionibacterium freudenreichii*, *Propionibacterium shermanii*, and *Pseudomonas denitrificans*. Several factors must be considered when supplementing. For example, high fibre diets can impair vitamin absorption, and joint intake of

Multivitamins containing vitamin C and copper can lead to the formation of inactive by-products. Furthermore, vitamin B₁₂ absorption is dose- and frequency-dependent (Rizzo *et al.*, 2016). Despite the undoubted importance, studies are suggesting that long-term supplementation and high concentrations of B₁₂ are associated with an increase in the risk of colorectal (Araghi *et al.*, 2019) and lung cancers (Fanidi *et al.*, 2018). The best alternative, whenever possible, is to maintain a balanced, healthy diet that provides all the necessary nutrients in their required amounts.

It was previously mentioned that higher plants do not produce vitamin B₁₂; however, it is possible to obtain food enriched with this vitamin through fermentation.

Fermented vegetables and fruits

Several studies are demonstrating that fermented vegetables and fruits are rich sources of vitamin B₁₂ (Table 1). Different plant materials are being used to produce fermented products, such as soybeans, guava, cashew, wheat, and amaranth (Xu *et al.*, 2016; Palachum *et al.*, 2020; Thompson *et al.*, 2020; Xie *et al.*, 2021). In addition to vitamin B₁₂, fermentation also produces other vitamins (B₂ and B₉), proteins, essential amino acids, and essential fatty acids. Fermentation also extends shelf life, improves food safety, and enhances sensory food quality (Feng *et al.*, 2017; Tangyu *et al.*, 2019). It is worth mentioning that improved digestibility of carbohydrates in plant foods through fermentation occurs as fermentation eliminates plant compounds with anti-nutritive properties, such as phytates, cyanogenic glycosides, and fermentable oligosaccharides. Gänzle (2020) refers to fermentation used by humans as an *ex vivo* digestion step. This is an interesting perspective since humans use their cognitive capacity to find an external method for improving the nutritional value of plant crops.

Yongsmith *et al.* (2016) demonstrated procedures to obtain fermented plant foods bioenriched with vitamin B₁₂, such as tempeh and soy sauce. An important microorganism used for this purpose is *Propionibacterium* spp. as they synthesise relatively large amounts of vitamin B₁₂. In addition, there was no decrease in vitamin B₁₂ production in tempeh when *Propionibacterium shermanii* was used with *Lactobacillus casei* and *Rhizopus oligosporus*

(Krusong *et al.*, 1991; Yongsmith *et al.*, 2016). An important feature that Yongsmith *et al.* (2016) mentioned is related to the flavour. The traditional tempeh has low acceptance by Westerners due to the strong ammonia smell; however, the use of *Propionibacterium* (largely used in the cheese industry) provides a flavour that is suitable to non-Asians.

Traditional processes must be increasingly targeted by studies aiming to increase the vitamin concentration in the final product. An example is tempeh, traditionally made with soybean, developed with lupin (*Lupinus angustifolius*). A study showed that it is an innovative alternative that possesses high amounts of vitamin B₁₂ as compared to the traditional version. Synergic effects of *Rhizopus* and *Propionibacterium* in increasing vitamin B₁₂ up to 1,230 ng/g were observed (Signorini *et al.*, 2017). Another important topic is the degradation of vitamin B₁₂ during cooking. Castanheira *et al.* (2020) found that sautéed tempeh had the highest vitamin B₁₂ content as compared to the stewed tempeh. Also, sautéed tempeh contributed to 32% of the daily requirements of vitamin B₁₂. Cyanocobalamins are sensitive to prolonged heat processing, at or close to neutral pH, as well as to other food processing conditions, such as exposure to oxidising agents, light, and acid/basic pH, among others (Mazzocato *et al.*, 2019).

The development of new products following plant-based diet trends is growing across Western countries (McClements, 2020; Alcorta *et al.*, 2021). Vegetable/plant-based drinks, also known as vegetable/plant-based milks, are not a novelty, and there is a great diversity of this type of product on the market. Nonetheless, two big problems of vegetable drinks are the nutritional unbalance and the flavour profile that limit their acceptance. Fermentation thus appears to be an excellent alternative to improve sensory profiles, nutritional properties, textural properties, and microbial safety, as well as to diversify the market (Tangyu *et al.*, 2019). Another example is the development of candies with fermented plant materials, thus making the product more nutritious and reachable to different consumer profiles (Palachum *et al.*, 2020; Vergara *et al.*, 2020). Studies with sensory acceptance have shown the potential of fermented plant products, showing good acceptance by consumers (Tangyu *et al.*, 2019; Palachum *et al.*, 2020). A gummy jelly product

Table 1. Studies regarding fermented plants and their findings on vitamin B₁₂ content and bioaccessibility/bioavailability.

Fermented plant food	Microorganism	Vitamin B ₁₂ content	Bioaccessibility/Bioavailability	Reference
Cauliflower (<i>Brassica oleracea</i> L.) and white beans (<i>Phaseolus vulgaris</i> L.)	<i>Lactobacillus plantarum</i> 299	66% higher than control (0.029 ± 0.002 to 0.048 ± 0.013 µg/100 g FW); however, it is a small fraction of recommended daily intake.	No analyses were performed.	Thompson <i>et al.</i> (2020)
Rye (<i>Secale cereale</i> L.), oat (<i>Avena sativa</i> L.), buckwheat (<i>Fagopyrum esculentum</i> Moench), and rice (<i>Oryza sativa</i> L.) bran; sorghum (<i>Sorghum bicolor</i> (L.) Moench), millet (<i>Panicum miliaceum</i> L.), quinoa (<i>Chenopodium quinoa</i> Willd.), amaranth (<i>Amaranth</i> spp.), faba (<i>Vicia faba</i> L.) bean, soybean (<i>Glycine max</i> (L.) Merr.), and lupin (<i>Lupinus angustifolius</i> L.) flour	<i>Propionibacterium freudenreichii</i> DSM 20271 and <i>Levilactobacillus brevis</i> (formerly <i>Lactobacillus brevis</i>) ATCC 14869	More than 300 ng/g dw of vitamin B ₁₂ (daily requirement: 2.4 µg) were produced during fermentation in most of the studied brans and legumes.	No analyses were performed.	Xie <i>et al.</i> (2021)
Fermented soy (<i>Glycine max</i> (L.) Merr.) products marketed in China (douchi, natto, miso, sufu, black bean sauce, fried yellow soybean sauce, and tofu)	Not specified for each product. The authors cite that for the types of products evaluated, yeasts, <i>Aspergillus</i> , <i>Mucoraceae</i> , <i>Rhizopus</i> , and <i>Lactobacillus</i> are generally used.	“Liubiju” dry yellow soybean paste (732.94 ng/g), “Shanfu” doenjang (531.29 ng/g), “Yangfan” black bean douchi (524.93 ng/g), tempeh (508.76 ng/g), and rice miso (484.44 ng/g) had noticeable vitamin B ₁₂ values.	No analyses were performed.	Xu <i>et al.</i> (2016)
Guava (<i>Psidium guajava</i> L.) gummy jelly product	<i>Lactobacillus plantarum</i> WU-P19	1.07 mg/100g dw.	No analyses were performed.	Palachum <i>et al.</i> (2020)
Durum (<i>Triticum durum</i>) flour, whole wheat (<i>Triticum aestivum</i>), and wheat bran	<i>Propionibacterium freudenreichii</i>	Durum flour (33 ng/g dw), wholewheat (87 ng/g dw), and wheat bran (155 ng/g dw).	No analyses were performed.	Xie <i>et al.</i> (2018)
Cashew (<i>Anacardium occidentale</i> L.) apple juice	<i>Lactobacillus acidophilus</i> TISTR 1338, <i>Lactobacillus casei</i> TISTR 390, <i>Lactobacillus plantarum</i> TISTR 543, <i>Bifidobacterium longum</i> TISTR 2195, and <i>Leuconostoc mesenteroides</i> TISTR 053	Cashew apple juice fermented for 24 h with <i>Lb. plantarum</i> (41.55 mg/L), <i>Lb. casei</i> at 48 h (36.08 mg/L), and <i>Lb. acidophilus</i> at 24 h (35.71 mg/L).	No analyses were performed.	Kaprasob <i>et al.</i> (2018)

Functional beverage from mature coconut (<i>Cocos nucifera</i> L.) water	<i>L. plantarum</i> DW12	15.93 µg/mL	No analyses were performed.	Kantachote et al. (2017)
Pickled parsley (<i>Petroselinum crispum</i> (Mill.) Fuss) juice and sea-buckthorn (<i>Hippophae rhamnoides</i> L.) jam (acquired at local market in Poland)	Not specified	50.5 µg/L (pickled parsley juice) and 224.5 µg/kg (sea-buckthorn jam).	No analyses were performed.	Jedut et al. (2021)
Fermented Japanese black tea (batabata-cha)	<i>Lactobacillus delbrueckii</i> subsp. <i>Lactis</i> (formerly <i>Lactobacillus leichmannii</i>) ATCC 7830	456 ng/100 g dry tea leaves and 2.0 ng/100 mL of tea drink.	The hepatic vitamin B ₁₂ contents were about 2-fold greater in the tea-supplemented rats (1473 pg/g wet tissue) than in both control and cyano-B ₁₂ -supplemented rats (768 pg/g wet tissue), demonstrating bioavailability in mammals.	Kittaka-Katsura et al. (2004)
Kombucha	“Tea fungus Kombucha” - symbiotic culture of acetic acid bacteria and yeast (tea fungus)	0.84 mg/mL.	No analyses were performed.	Villarreal-Soto et al. (2018), Bauer-Petrovska and Petrushevska-Tozi (2000)
Tempeh made with lupin beans (<i>Lupinus angustifolius</i> L.)	<i>Rhizopus oryzae</i> CBS 285.55 and <i>Propionibacterium freudenreichii</i> subsp. <i>freudenreichii</i> DSM 20271	Significant increase of vitamin B ₁₂ content (up to 0.97 µg/100 g) was achieved by fermenting lupin using a mixed starter of <i>R. oryzae</i> and <i>P. freudenreichii</i> .	No analyses were performed.	Wolkers-Rooijackers et al. (2018)
Furu (fermented tofu)	<i>Lactobacillus reuteri</i>	Vitamin B ₁₂ content in furu inoculated with <i>L. reuteri</i> gradually increased up to 141.7 ng/g (wet weight), which was higher than the control (36.0 ng/g).	No analyses were performed.	Xuan et al. (2018)

developed with the fermented pulp of guava showed great acceptance in the sensory test (Palachum *et al.*, 2020).

As mentioned earlier, some fermented foods consumed in Asia and Eastern Europe are not sensorily pleasing to Western countries (Yongsmith *et al.*, 2016). Also, we have different cultural habits, and this is reflected in our food habits and behaviour (Bezerra *et al.*, 2017; Tamang *et al.*, 2020). In Brazil, it is common to consume fermented alcoholic beverages made of plants; however, fermented vegetables and fruits are not frequently observed in daily consumption. There are several fermented vegetables and fruits of indigenous origin with immense nutritional potential. Calugi, cauim, tarubá, and yakupa are examples of these foods, and they can even be sources of B-complex vitamins (Ramos and Schwan, 2017). Introducing fermented vegetables and fruits as a source of vitamin B₁₂ in Brazil would be a major challenge. The use of fermented pulps or flours, for example, in the preparation of products, would be a more viable alternative.

Another important study objective is the identification of new potential sources of vitamin B₁₂ in different fermented vegetables and fruits, and in different countries. A study with different fermented products available in the Poland market discovered two new rich sources of vitamin B₁₂ (pickled parsley juice and sea-buckthorn jam; Table 1), evidencing the importance of this type of exploratory analysis (Jedut *et al.*, 2021). The studies in Table 1 show that fermented plant products have satisfactory amounts of vitamin B₁₂ and that they would probably be sufficient to reach the RDA (Recommended Dietary Allowance). However, is this vitamin accessible? Does it play its biological role in the human body after consumption?

Bioaccessibility and bioavailability: Is it possible to achieve the dietary intake through consumption of fermented vegetables and fruits?

Regarding nutrients and bioactive compounds, two especially important factors must be taken into consideration in research: bioaccessibility and bioavailability (Thakur *et al.*, 2020). Bioaccessibility is related to the part of ingested compound released from its food matrix that becomes available for absorption in the small intestine (Heaney, 2001; Thakur *et al.*, 2020; Yaman *et al.*, 2021). According to Wood (2005), bioavailability is defined as part of the ingested nutrient that reaches the systemic

circulation, and is available for utilisation in normal physiological functions. The bioaccessibility/bioavailability of water-soluble vitamins is affected by different factors such as food matrix, gastrointestinal pH, temperature, antioxidants, dietary fibres, vitamin-binding proteins, and metal ions (Yaman *et al.*, 2021).

There are different methods to assess the bioaccessibility/bioavailability of vitamin B₁₂ in fermented vegetables and fruits, which can be performed *in vitro* and *in vivo*. The *in vitro* methods are the most used, although *in vivo* tests have shown to provide more accurate information due to their shorter time and low cost. Another factor that makes *in vivo* testing difficult is ethical restrictions (Thakur *et al.*, 2020; Yaman *et al.*, 2021). Thakur *et al.* (2020) explain that few tests are conducted, and bioavailability is usually described as the fraction of a given compound in circulation. *In vivo* digestion and dialysis methods for simulating gastrointestinal conditions are examples of widely used tests to access the bioaccessibility of phytonutrients (You *et al.*, 2010; Yaman *et al.*, 2021).

A study with fermented Japanese black tea (batabata-cha) showed the bioavailability of its vitamin B₁₂ in mammals. Vitamin B₁₂-deficient rats were used to test methylmalonic acid in the urine and hepatic vitamin B₁₂ content, so as to compare the bioavailability of fermented black tea drink and cyano-B₁₂ supplement. The hepatic vitamin B₁₂ contents were about 2-fold greater in the tea drink-supplemented rats than in cyano-B₁₂-supplemented rats (Kittaka-Katsura *et al.*, 2004). A more recent study showed that it was possible to use B₁₂-enriched flour to produce bread with high vitamin bioavailability. High-purity crystalline 14C-B₁₂ (labelled vitamin) was dissolved in water and added to flour (2 µg B₁₂/100 g flour) in a bread maker, and made into rolls. The excess of 14C first appeared in plasma 4 h after ingestion of the 14C-fortified bread, and plasma levels returned almost to the background by 72 h. This study showed that when vitamin B₁₂ was added as a fortifier to flour, it survived the fermentation and baking, thus retaining ~50% bioavailability when fed in small doses to healthy elderly subjects. It is important to highlight that vitamin B₁₂ in this study was added to the flour, not naturally obtained through fermentation.

Even though fermented vegetables and fruits are potential sources of vitamin B₁₂, there is still a need for more studies related to bioaccessibility and

bioavailability to guarantee them as the main source of this vitamin. While more studies are not available, the ideal is to keep them in the diet along with supplementation and/or a balanced diet.

Health impacts: What do we know about the consumption of fermented vegetables and fruits?

The consumption of fermented vegetables and fruits is constantly associated with health benefits (Di Cagno *et al.*, 2016; Torres *et al.*, 2020). The phytochemicals present in plants, combined with the products obtained from fermentation, have brought several benefits to human health. Phytochemicals, such as bioactive peptides, may be released during LAB fermentation of soy, and when consumed, they are capable of inhibiting angiotensin-converting enzymes that are related to the desired anti-hypertensive effect (Hou *et al.*, 2000).

Chronic inflammatory disease is the immune system's biological response against different infectious or non-infectious stimuli. Some examples of chronic inflammatory disorders are cancers, obesity, diabetes, rheumatoid arthritis, and atherosclerosis (Shahbazi *et al.*, 2021). Fermented vegetables and fruits are extremely recommended for people with these conditions, as they present anti-inflammatory and immunomodulatory activity (Sikora *et al.*, 2020; Shahbazi *et al.*, 2021). In addition, probiotic consumption in the form of fermented vegetables and fruits can improve gut barrier integrity and immunity, and maintain gut homeostasis, improving anti-inflammatory activity and immunomodulatory effects (Park *et al.*, 2014; Sikora *et al.*, 2020; Shahbazi *et al.*, 2021). *Lactobacillus brevis* KU15006 isolated from kimchi showed anti-adhesion activity against foodborne pathogens and anti-diabetic properties (Son *et al.*, 2017). Another study showed that the consumption of kimchi caused changes in the intestinal microbiota due to the LAB content in the mature product. After consuming kimchi for two weeks (300 g/day), the abundance of *Lactobacillus* spp. and *Leuconostoc* spp. in the colon increased.

There are several studies showing the association of fermented plant food intake with cognitive enhancement and neuroprotective effects (Go *et al.*, 2015; Sivamaruthi *et al.*, 2018). Tofu and tempeh were associated with improved memory. However, high consumption by elderly people has the opposite effect, worsening their memory (Hogervorst *et al.*, 2008). Cheonggukjang extract ameliorated

memory defects and neuronal cell death in TMT-treated mice (Go *et al.*, 2015). Tests in SH-SY5Y cells and SD rats showed that red mould rice has neuroprotective effects in the 6-OHDA induced-PD model *in vitro* and *in vivo* (Tseng *et al.*, 2016).

The fermentation of vegetable drinks can reduce drastically the level of anti-nutrients such as tannins, phytates, and cyanides (Tangyu *et al.*, 2019). Anti-nutrients are naturally synthesised in plants, and can interfere with the absorption of nutrients, thus reducing intake and digestion (Popova and Mihaylova, 2019). Fermentation of finger millet significantly reduced various undesired anti-nutrients, such as phytates and tannins, and simultaneously enhanced mineral extractability and digestibility (Antony and Chandra, 1998).

Other non-animal sources of B₁₂

Besides the great diversity of fermented plant foods, other non-fermented and non-animal sources of vitamin B₁₂ also deserve mention and attention. Algae and mushrooms are great examples (Watanabe *et al.*, 2014; Edelmann *et al.*, 2019). Regarding mushrooms, black trumpet (*Craterellus cornucopioides*), golden chanterelle (*Cantharellus cibarius*), and shiitake (*Lentinula edodes*) are good sources of vitamin B₁₂; however, a daily intake to achieve the RDA in adults might be insufficient, due to the large amount required (Watanabe *et al.*, 2014). Watanabe *et al.* (2014) suggest nori (a dried sheet made from seaweed) as a suitable vitamin B₁₂ source. Dried purple laver used in nori contains about 77.6 µg/100 g dry weight of B₁₂ vitamin.

A study conducted with commercial microalgae powders showed that pseudovitamin B₁₂ was predominant in *Spirulina* powders. Nonetheless, some *Chlorella* powders contained nutritionally marked amounts of active vitamin B₁₂. The product of Japanese origin, produced with a *Chlorella vulgaris* strain, had the highest concentration of active vitamin B₁₂, approximately 2.0 µg/g (Edelmann *et al.*, 2019). In another study, Castillejo *et al.* (2017) used different edible algae (sea lettuce, kombu, wakame, thongweed, dulse, Irish moss, nori, *Spirulina*, and *Chlorella*) to naturally supplement green smoothies with vitamin B₁₂ and fucose, assessing quality changes throughout 24 days at 5°C. They found that *Spirulina* supplementation of a 200 g smoothie portion met the recommended vitamin B₁₂ intake.

Hydroponic cultivation is an interesting technique that increases vitamin B₁₂ in foods. It was possible to obtain higher levels of B₁₂ in the leaves of soybean seedlings (9.8 µg/g fresh weight) through hydroponic cultivation with a solution containing 10 µmol/L of vitamin B₁₂ for 24 h (Alcorta *et al.*, 2021). Another study showed that the addition of cyanocobalamin (CNCBL) in a culture broth of spinach resulted in an increase in the content of the same compound in the leaves after 36 h, and even after boiling the leaves, the CNCBL content was 0.94 ± 0.11 µg/g, thus providing the daily requirement of vitamin B₁₂ (Zheng *et al.*, 2021). Furthermore, the fortification of processed plant-based foods with vitamin B₁₂ by directly adding it to the formulation is another important strategy to ensure daily intake (McClements, 2020).

Conclusion

Fermented vegetables and fruits are good alternative sources for vitamin B₁₂ intake. Considerable amounts of vitamin B₁₂ are produced through fermentation, and there are potential plant sources and microorganisms that can improve the production of vitamin B₁₂. With all its millenary history in different cultures, fermented plants continue to be reinvented, and are proving to be among the "foods of the future"; providing nutrients such as vitamin B₁₂ and improved sensory characteristics.

Acknowledgement

The present work was financially supported in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Brazil (CAPES), Finance Code 001. Authors are also grateful to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the financial support provided.

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