

Review

Vitamin B₁₂-Containing Plant Food Sources for Vegetarians

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Abstract: The usual dietary sources of Vitamin B₁₂ are animal-derived foods, although a few plant-based foods contain substantial amounts of Vitamin B₁₂. To prevent Vitamin B₁₂ deficiency in high-risk populations such as vegetarians, it is necessary to identify plant-derived foods that contain high levels of Vitamin B₁₂. A survey of naturally occurring plant-derived food sources with high Vitamin B₁₂ contents suggested that dried purple laver (nori) is the most suitable Vitamin B₁₂ source presently available for vegetarians. Furthermore, dried purple laver also contains high levels of other nutrients that are lacking in vegetarian diets, such as iron and *n*-3 polyunsaturated fatty acids. Dried purple laver is a natural plant product and it is suitable for most people in various vegetarian groups.

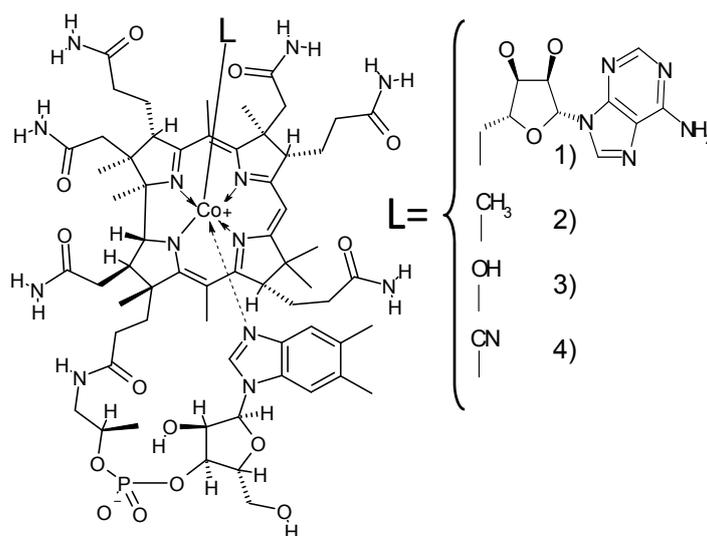
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1. Introduction

Vitamin B₁₂ (molecular weight = 1355.4) belongs to the “corrinoids” group, which comprises compounds that contain a corrin macrocycle. The term “Vitamin B₁₂” is usually restricted to cyanocobalamin, which is the most chemically stable and unnatural form of cobalamin [1], but Vitamin B₁₂ refers to all potentially biologically active cobalamins in the present review. Cyanocobalamin is included in most human dietary supplements, and it is readily converted into the coenzyme forms of cobalamin, *i.e.*, methylcobalamin functions as a coenzyme for methionine synthase (EC 2.1.1.13; involved in methionine biosynthesis), and 5'-deoxyadenosylcobalamin functions as a

coenzyme for methylmalonyl-CoA mutase (EC 5.4.99.2; involved in amino acid and odd-chain fatty acid metabolism in mammalian cells) [2,3] (Figure 1). Corrinoids with a base other than 5,6-dimethylbenzimidazole as the lower ligand (cobalt-coordinated nucleotide) were recently found in certain foods and they are inactive in humans [4].

Figure 1. Structural formula of Vitamin B₁₂ and partial structures of Vitamin B₁₂ compounds. The partial structures of the Vitamin B₁₂ compounds only show the regions of the molecule that differ from Vitamin B₁₂. (1) 5'-Deoxyadenosylcobalamin; (2) methylcobalamin; (3) hydroxocobalamin; and (4) cyanocobalamin or Vitamin B₁₂.



Vitamin B₁₂ is synthesized only by certain bacteria, and it is primarily concentrated in the bodies of predators located higher in the food chain [5]. Vitamin B₁₂ is well-known to be the sole vitamin that is absent from plant-derived food sources. Foods (meat, milk, eggs, fish, and shellfish) derived from animals are the major dietary sources of Vitamin B₁₂ [4]. The recommended dietary allowance (RDA) of Vitamin B₁₂ for adults is set at 2.4 µg/day in the United States (and Japan) [6,7]. The major signs of Vitamin B₁₂ deficiency are megaloblastic anemia and neuropathy [6]. Vegetarians are at a higher risk of Vitamin B₁₂ deficiency than non-vegetarians [8]. The frequencies of the deficiency among vegetarians were estimated as 62%, 25%–86%, 21%–41%, and 11%–90% in pregnant women, children, adolescents, and elderly subjects, respectively, by review of the 18 reports evaluating Vitamin B₁₂ status of vegetarians [9]. The objective of this review is to present up-to-date information on Vitamin B₁₂-containing plant-derived food sources to prevent vegetarians from developing Vitamin B₁₂ deficiency.

2. Main Types of Vegetarian Diets

There are several main types of vegetarian groups: (1) Lacto-ovo vegetarianism [10]: many people are familiar with this type of vegetarianism, which comprises most vegetarians. “Lacto” indicates that a person consumes milk and milk products (butter, yogurt, cheese, *etc.*), and “ovo” means that a person consumes eggs. In general, lacto-ovo vegetarians do not consume animal meats (including fish and shellfish). Some vegetarian groups are ovo only or lacto only, *i.e.*, they consume only eggs or only

milk and its products, respectively, as animal products; (2) Raw veganism [11]: this diet is mostly or entirely based on fresh fruits, vegetables, nuts, and seeds; (3) Fruitarianism [12]: this is generally a raw style of eating that primarily depends on fruits, nuts, and seeds; (4) Buddhist vegetarianism [13]: this is a vegan diet that excludes all animal products and Allium family vegetables (onion, garlic, leeks, and shallots) on ethical grounds; (5) Macrobiotic [14]: this diet is primarily focused on grains, beans, and similar staples, including some vegetables and other whole foods. Processed foods and most animal products are strongly avoided; and (6) Jain vegetarianism [15]: another religious dietary practice that includes dairy products, but excludes eggs and honey as well as root vegetables.

3. Nutritional Characterization of Vegetarian Diets

From a nutrient intake perspectives, vegetarian diets are usually rich in carbohydrates, *n*-6 polyunsaturated fatty acids, dietary fibers, carotenoids, folic acid, Vitamin C, Vitamin E, and magnesium (Mg), but these diets are relatively low in proteins, saturated fatty acids, *n*-3 polyunsaturated fatty acids (particularly eicosapentaenoic and docosahexaenoic acids), Vitamin A (retinol), Vitamin B₁₂, Vitamin D₃ (cholecalciferol), zinc, iron, and calcium [16–18] (Table 1). In particular, Vitamins A, B₁₂, and D₃ are found only in animal-derived foods, whereas Vitamin D₂ (ergocalciferol) and provitamin A (β -carotene) are found in mushrooms and vegetables, respectively [19,20]. Furthermore, Vitamin D₃ can be synthesized in the human skin under sunlight [21]. A vegetarian diet usually provides a low intake of saturated fatty acids and cholesterol but a high intake of dietary fibers and health-promoting phytochemicals (e.g., various polyphenol compounds) due to an increased consumption of fruits, vegetables, whole-grains, legumes, nuts, and various soy products. As a result, vegetarians typically have lower body mass index, serum cholesterol levels, and blood pressure [18]. Compared with non-vegetarians, vegetarians also have reduced rates of mortality due to ischemic heart disease, probably because of lower blood cholesterol. However, there are no clear differences with respect to other major causes of death such as stroke and cancers [17]. Craig [17] reported that, compared with non-vegetarians, vegetarians have lower incidences of hypertension, stroke, type 2 diabetes, and certain cancers. Pawlak *et al.* [9] showed that vegetarians can develop Vitamin B₁₂ depletion or deficiency regardless of their demographic characteristics, place of residency, age, or type of vegetarian diets. The Vitamin B₁₂ content is not high in whole eggs (approximately 0.9–1.4 μ g/100 g), most of which is located in the egg yolk [22]. The average bioavailability of Vitamin B₁₂ from cooked eggs is 3.7%–9.2% [23]. Thus, the Vitamin B₁₂ in eggs is generally poorly absorbed compared with that in other animal-derived products [24]. The Vitamin B₁₂ content of various types of milk is very low (approximately 0.3–0.4 μ g/100 g) [4], and appreciable losses of Vitamin B₁₂ occur during the processing of milk [25,26]. Approximately 20%–60% of the Vitamin B₁₂ that is initially present in milk is recovered in cottage cheese, hard cheese, and blue cheese [27]. The Vitamin B₁₂ content in the whey is considerably reduced during lactic acid fermentation [28]. These observations explain why Vitamin B₁₂ deficiency is relatively common in lacto-ovo-vegetarians. Furthermore, food-bound Vitamin B₁₂ malabsorption occurs with certain gastric dysfunctions, particularly atrophic gastritis with low stomach acid secretion [29]. The body storage level of Vitamin B₁₂ is significantly depleted by a persistent vegetarian diet; thus Vitamin B₁₂ deficiency may readily develop in elderly vegetarians. However, Vitamin B₁₂ deficiency may go

undetected in vegetarians because their diets are rich in folic acid, which may mask vitamin B₁₂ deficiency until severe health problems occur [30]. Vitamin B₁₂ deficiency contributes to the development of hyperhomocysteinemia, which is recognized as a risk factor for atherothrombotic [31] and neuropsychiatric disorders [32], thereby negating the beneficial health effects of a vegetarian lifestyle. Thus, many investigators have suggested that vegetarians should maintain an adequate intake of Vitamin B₁₂ by consuming supplements that contain Vitamin B₁₂ or Vitamin B₁₂-fortified foods [29,33].

Table 1. Nutrient imbalance in vegetarian diets.

Rich	Low
Fiber	Vitamin A
Vitamin C	Vitamin D ₃
Vitamin E	Vitamin B ₁₂
Folate	Iron
Magnesium	Cholesterol
<i>n</i> -6 Polyunsaturated fatty acids	<i>n</i> -3 Polyunsaturated fatty acids
Carbohydrates	Saturated fatty acids

4. Vitamin B₁₂-Containing Plant-Derived Food Sources

In the United States, ready-to-eat cereals fortified with Vitamin B₁₂ comprise a high proportion of the dietary Vitamin B₁₂ intake [6]. Several research groups have suggested that eating a breakfast cereal fortified with folic acid, Vitamins B₁₂ and B₆ increases the blood concentrations of these vitamins and decreases the total homocysteine concentrations in the plasma of elderly subjects [34]. Thus, Vitamin B₁₂-fortified breakfast cereals may be a particularly valuable source of Vitamin B₁₂ for vegetarians. However, processed foods are strongly avoided by most vegetarians in addition to animal products. Thus, it is necessary to identify plant-derived food sources that naturally contain a large amount of Vitamin B₁₂ to prevent Vitamin B₁₂ deficiency in vegetarians.

4.1. Vitamin B₁₂-Enriched Beans and Vegetables Produced Using Organic Fertilizers or Hydroponics

Mozafar [35] demonstrated that adding an organic fertilizer such as cow manure significantly increased the Vitamin B₁₂ content of spinach leaves, *i.e.*, approximately 0.14 µg/100 g fresh weight. However, the consumption of several hundred grams of fresh spinach would be insufficient to meet the RDA of 2.4 µg/day for adult humans [6,7]. Furthermore, our recent [36] and unpublished research indicates that most organic fertilizers, particularly those made from animal manures, contain considerable amounts of inactive corrinoid compounds. These compounds are also present in human feces where they account for more than 98% of the total corrinoid content [37].

Some researchers attempted to prepare Vitamin B₁₂-enriched vegetables by treating them with a solution that contains high levels of Vitamin B₁₂ [38,39]. This resulted in significant increases in the plant Vitamin B₁₂ contents, thereby suggesting that Vitamin B₁₂-enriched vegetables may be particularly beneficial to vegetarians. However, artificially Vitamin B₁₂-enriched vegetables may not fit the philosophy of vegetarians.

4.2. Fermented Beans and Vegetables

The Vitamin B₁₂ contents of soybeans are low or undetectable. However, a fermented soybean-based food called tempe contains a considerable amount of Vitamin B₁₂ (0.7–8.0 µg/100 g) [40]. Bacterial contamination during tempe production may contribute to the increased Vitamin B₁₂ content of tempe [41]. Other fermented soybean products contain minute amounts of Vitamin B₁₂ [42,43]. Only trace amounts of Vitamin B₁₂ were found in broccoli, asparagus, Japanese butterbur, mung bean sprouts, tassa jute, and water shield [44]. Fermented Korean vegetables (kimuchi) contain traces (<0.1 µg/100 g) of Vitamin B₁₂ [43]. High Vitamin B₁₂ (approximately 10 µg/100 g)-enriched vegetable products tend to be produced by fermentation with certain lactic acid or propionic bacteria [45,46].

Vitamin B₁₂ is found in various types of tea leaves (approximately 0.1–1.2 µg Vitamin B₁₂ per 100 g dry weight) [47]. For example, Vitamin B₁₂-deficient rats were fed a Japanese fermented black tea (Batabata-cha) drink (50 mL/day, equivalent to a daily dose of 1 ng Vitamin B₁₂) for 6 weeks, and the urinary methylmalonic acid excretion (an index of Vitamin B₁₂ deficiency) levels in the tea drink-supplemented rats was significantly lower than in those of the deficient rats [48]. These results indicate that Vitamin B₁₂ found in fermented black tea is bioavailable in rats. However, the consumption of 1–2 L of the fermented tea drink (typical regular consumption in Japan), which is equivalent to 20–40 ng of Vitamin B₁₂, is not sufficient to meet the RDA of 2.4 µg/day for adult humans.

4.3. Edible Mushrooms

Several wild edible mushroom species are popular among vegetarians in European countries. Zero or trace levels (approximately 0.09 µg/100 g dry weight) of Vitamin B₁₂ were measured in the dried fruiting bodies of porcini mushrooms (*Boletus* sp.), parasol mushrooms (*Macrolepiota procera*), oyster mushrooms (*Pleurotus ostreatus*), and black morels (*Morchella conica*). In contrast, the fruiting bodies of black trumpet (*Craterellus cornucopioides*) and golden chanterelle (*Cantharellus cibarius*) contained higher levels of Vitamin B₁₂ (1.09–2.65 µg/100 g dry weight) than the abovementioned mushrooms [49]. To determine whether the fruiting bodies of dried black trumpet and golden chanterelle contain Vitamin B₁₂ or other corrinoid compounds that are inactive in humans, we purified the corrinoid compound using an immunoaffinity column and identified it as Vitamin B₁₂ by liquid chromatography-electrospray ionization tandem mass spectrometry [49]. In addition, high levels of Vitamin B₁₂ were detected in the commercially available dried shiitake mushroom fruiting bodies (*Lentinula edodes*), which are used in various vegetarian dishes. The Vitamin B₁₂ contents of dried shiitake mushroom fruiting bodies (100 g dry weight) significantly varied and the average Vitamin B₁₂ value was approximately 5.61 µg [50]. Dried shiitake mushroom fruiting bodies rarely contained the inactive corrinoid, Vitamin B₁₂[*c*-lactone] as well as Vitamin B₁₂ [50]. Lion's mane mushroom (*Hericium erinaceus*) fruiting bodies also contain considerable amounts of Vitamin B₁₂[*c*-lactone] [51]. Stabler *et al.* [52] demonstrated that Vitamin B₁₂[*c*-lactone] binds very weakly to the most specific Vitamin B₁₂-binding protein, *i.e.*, the intrinsic factor involved in the gastrointestinal absorption of Vitamin B₁₂, and it strongly inhibits Vitamin B₁₂-dependent enzymes, methylmalonyl-CoA mutase and methionine synthase.

The consumption of approximately 50 g of dried shiitake mushroom fruiting bodies could meet the RDA for adults (2.4 µg/day), although the ingestion of such large amounts of these mushroom fruiting bodies would not be possible on a daily basis.

4.4. Edible Algae

Various types of edible algae are consumed worldwide as food sources. Dried green laver (*Enteromorpha* sp.) and purple laver (*Porphyra* sp.) are the most widely consumed edible algae, and they contain substantial amounts of Vitamin B₁₂ (approximately 63.6 µg/100 g dry weight and 32.3 µg/100 g dry weight, respectively) [53] (Figure 2). However, excluding these two genera, other edible algae contain zero or only traces of Vitamin B₁₂ [54]. To determine whether dried purple and green lavers contain Vitamin B₁₂ or inactive corrinoids, the algal corrinoid compounds were purified and confirmed as Vitamin B₁₂ [55,56]. A substantial amount (133.8 µg/100 g dry weight) of Vitamin B₁₂ was found in dried Korean purple laver (*Porphyra* sp.), but seasoned and toasted laver products contain lower amounts of Vitamin B₁₂ (approximately 51.7 µg/100 g dry weight) [57]. In particular, when the dried purple laver was treated by toasting until the laver's color changed from purple to green, the decreases in the Vitamin B₁₂ contents of the seasoned and toasted laver products were not due to the loss or destruction of Vitamin B₁₂ during the toasting process [57]. *In vitro* gastrointestinal digestion experiments indicated that the estimated digestion rate of Vitamin B₁₂ from dried purple laver was approximately 50% at pH 2.0 (as a model of normal gastric function). The release of free Vitamin B₁₂ from the purple laver significantly decreased to approximately 2.5% at pH 7.0 (as a model of severe atrophic gastritis) [57]. Edible purple laver predominantly contains coenzyme forms (5'-deoxyadenosylcobalamin and methylcobalamin) of Vitamin B₁₂ or hydroxocobalamin (or both) [57–59].

To measure the biological activity of Vitamin B₁₂ in lyophilized purple laver (*Porphyra yezoensis*), the effects of laver feeding were investigated in Vitamin B₁₂-deficient rats [58]. Urinary methylmalonic acid excretion was undetectable within 20 days of initiating a diet supplemented with dried purple laver (10 µg of Vitamin B₁₂/kg diet), and the hepatic Vitamin B₁₂ (especially coenzyme Vitamin B₁₂) levels significantly increased. These results indicate that Vitamin B₁₂ obtained from purple laver is bioavailable in rats. A nutritional analysis of six vegan children who had consumed vegan diets including brown rice and dried purple laver (nori) for 4–10 years suggested that the consumption of nori may prevent Vitamin B₁₂ deficiency in vegans [60]. Our preliminary study indicated that similar dried purple laver products that are available in local markets in Taiwan (Hong-mao-tai, *Bangia atropurpurea*) and New Zealand (Karengo, a mixture of *P. cinnamonea* and *P. virididentata*) contained 28.5 ± 3.9 and 12.3 ± 1.9 µg of Vitamin B₁₂ per 100 g weight, respectively (Figure 2).

For a long time, it was unclear whether algae have an absolute requirement for Vitamin B₁₂ for growth, and why algae that lack a requirement of Vitamin B₁₂ for growth contain substantial amounts of Vitamin B₁₂. However, recent biochemical and bioinformatics studies have accurately defined the Vitamin B₁₂ requirements of various algae (half of all algal species absolutely require Vitamin B₁₂ for their growth), and they have suggested possible physiological functions for Vitamin B₁₂ in algae [61,62].

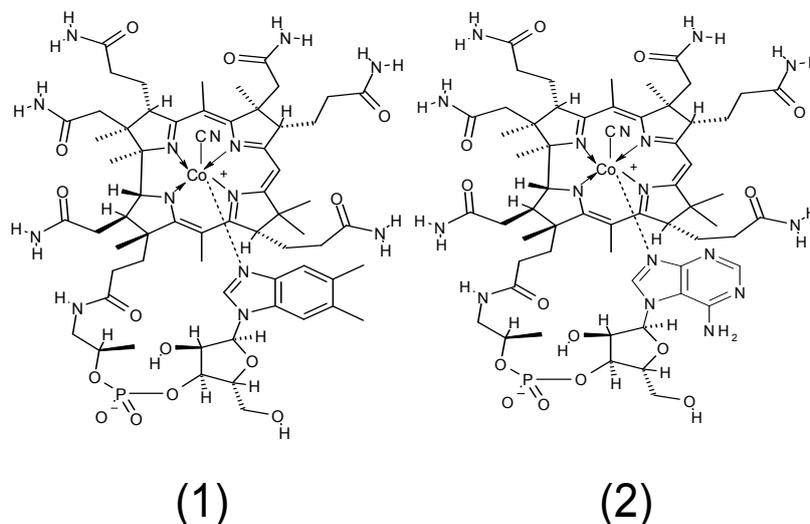
Figure 2. Various types of dried green and purple lavers are Vitamin B₁₂ sources: (1) a Japanese green laver, (Suji-aonori, *Enteromorpha prolifera*); (2) ordinary purple lavers (*Porphyra* sp.; nori, which has been formed into a sheet and dried); (3) Taiwan purple laver (Hong-mao-tai, *Bangia atropurpurea*); and (4) New Zealand purple laver (Karengo, a mixture of *Porphyra cinnamomea* and *Porphyra virididentata*).



Furthermore, the standard tables of food composition in Japan [63] indicate that dried purple laver (per 100 g) contains various other nutrients that are lacking in vegetarian diets, such as Vitamin A (3600 µg of Vitamin A equivalent as provitamin A), iron (10.7 mg), and *n*-3 polyunsaturated fatty acids (1.19 g), as well as Vitamin B₁₂ (77.6 µg). Purple laver also contains a large amount of a pigment protein, phycoerythrin, which is digested in the intestine to release the covalently linked chromophore moiety, a phycoerthrobilin compound (a potent antioxidant) [64].

Chlorella tablets (eukaryotic microalgae *Chlorella* sp.) used in human food supplements contain biologically active Vitamin B₁₂ [65]. However, our unpublished study indicates that the Vitamin B₁₂ contents significantly differ among various commercially available *Chlorella* tablets (from zero to several hundred µg of Vitamin B₁₂ per 100 g dry weight); we do not have any information on why such a huge variation occurs. Thus, vegetarians who consume *Chlorella* tablets as a source of Vitamin B₁₂ should check the nutrition labeling of *Chlorella* products to confirm their Vitamin B₁₂ contents. High levels of Vitamin B₁₂ are described in the nutritional labels of dietary supplements that contain edible cyanobacteria such as *Spirulina*, *Aphanizomenon*, and *Nostoc*. However, although substantial amounts of Vitamin B₁₂ were detected in these commercially available supplements using a microbiological Vitamin B₁₂ assay method, these supplements often contained large amounts of pseudovitamin B₁₂ [66–71] (Figure 3), which is biologically inactive in humans. Therefore, edible cyanobacteria and their products are not suitable for use as sources of Vitamin B₁₂ for vegetarians.

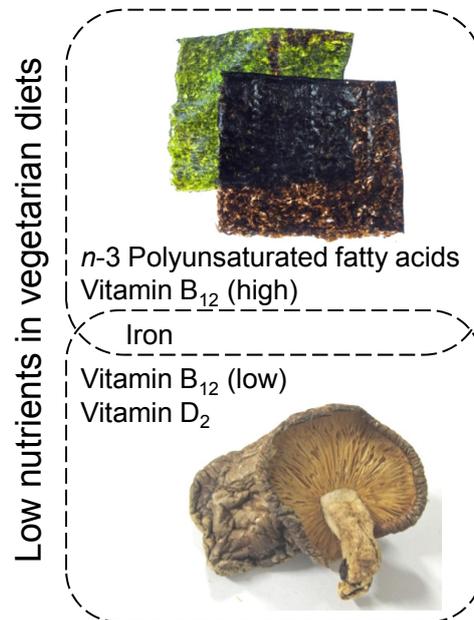
Figure 3 Structural formulae of Vitamin B₁₂ and pseudovitamin B₁₂. (1) Vitamin B₁₂ and (2) pseudovitamin B₁₂ (7-adeninyl cyanocobamide).



5. Conclusions

A survey of naturally occurring and high Vitamin B₁₂-containing plant-derived food sources showed that nori, which is formed into a sheet and dried, is the most suitable Vitamin B₁₂ source for vegetarians presently available. Consumption of approximately 4 g of dried purple laver (Vitamin B₁₂ content: 77.6 µg /100 g dry weight) supplies the RDA of 2.4 µg/day. In Japan, several sheets of nori (9 × 3 cm²; approximately 0.3 g each) are often served for breakfast. A large amount of nori can be consumed as certain forms of sushi (vinegared rice rolled in nori). In particular, hand-rolled sushi made by wrapping rice and fillings with nori is easy to prepare and facilitates the consumption of a large amount of nori. When dried purple laver was treated by toasting until the laver's color changed from purple to green, the toasting treatment did not affect the Vitamin B₁₂ contents [57]. Dried purple lavers could also be a suitable food item for integration in Italian, French, and other forms of western cuisine. Dried purple laver is also a rich source of iron and *n*-3 polyunsaturated fatty acids (Figure 4). Dried purple laver is a natural plant product; therefore, it is suitable for most vegetarian groups. Among edible mushrooms, relatively high levels of Vitamin B₁₂ were detected in the commercially available shiitake mushroom fruiting bodies, but the Vitamin B₁₂ content significantly varies (1.3–12.7 µg/100 g dry weight), which is significantly lower than that found in dried purple laver. However, the dried shiitake mushroom fruiting bodies (per 100 g) contain 18.9 mg of Vitamin D₂ (ergocalciferol) and 2.0 mg of iron [63], which are also nutrients that vegetarian diets tend to lack. Thus, the use of these plant-based food sources can significantly improve the nutrient imbalance in vegetarian diets to reduce the incidence of Vitamin B₁₂ deficiency in vegetarians.

Figure 4. Proposed method for improving nutrient imbalance in vegetarian diets using dried purple laver as a Vitamin B₁₂ source in addition to other plant-based food sources.



Author Contributions

All authors equally contributed to the preparation of the manuscript and have approved the final version.

Conflicts of Interest

The authors declare no conflict of interest.

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