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Original Research

Determination of Vitamin B_{12} and Folate Compounds in Commercially Available Edible Seaweed Products

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Abstract

Background: Information on the contents of both vitamin B_{12} and folate in edible seaweeds is limited, of which deficiencies disrupt methionine biosynthesis to accumulate homocysteine as a risk factor of cardiovascular diseases. **Methods**: Both vitamins were determined in commercially available edible seaweed products using high-performance liquid chromatography. **Results**: Dried purple laver (*Neopyropia yezoensis*) products contain higher levels of vitamin B_{12} (approximately 30–60 μg/100 g dry weight) and folate compounds (approximately 880–1300 μg/100 g dry weight) than other seaweed products, such as kombu (*Saccharina japonica*), hijiki (*Sargassum fusiformis*), and wakame (*Undaria pinnatifida*). 5-methyltetrahydrofolate was the major folate compound in purple laver products. 5-formyltetrahydrofolate was found at a moderate level, whereas tetrahydrofolate, 5,10-metenyltetrahydrofolate, 10-formyltetrahydrofolate, and folic acid were found to be minor folate compounds. **Conclusions**: These findings suggest that dried purple laver (nori) products are suitable sources of vitamin B_{12} and folate compounds for humans, especially vegetarians.

Keywords: edible seaweeds; folates; nutrients; purple laver; vitamin B_{12}

1. Introduction

Seaweed is a group of marine macroalgae species and is rich in dietary fiber, which suppresses the rapid rise in postprandial blood sugar [1]. Edible seaweed is an important ingredient in Asian counties, especially Japan [2]. For example, kombu (*Saccharina japonica*) is used in soup stock, and hijiki (*Sargassum fusiformis*) is mainly served as a boiled dish. Edible seaweeds are important in Japanese cuisine. Recently, interest in healthy foods in Europe and the United States has been growing [2]. Thus, the use of edible seaweed as a food ingredient is increasing and is often called "sea vegetable" [3]. The red alga *Neopyropia* spp. (formerly *Porphyra* spp.) is one of the most commercially available edible seaweeds because it is farmed in Japan, Korea, and China. Commercially available dried *Neopyropia* products are called purple laver (Europe and the United States), nori (Japan), zicai (China), and kim (Korea) [4].

According to our previous studies on plant-based foods with high vitamin B_{12} (B_{12}) contents (Fig. 1), among commercially available edible seaweed products, only dried purple laver (nori) products contain substantial amounts of B_{12}, which is the sole vitamin not found in plant-based food sources [5]. This finding suggests that nori is the B_{12} source suitable for vegetarians. B_{12} and folate are involved in the biosynthesis of methionine and nucleic acid in mammals [6] (Fig. 2). B_{12} and folate deficiencies disrupt this metabolic pathway to accumulate homocysteine [7], which is known as a risk factor for cardiovascular and cerebrovascular diseases such as Alzheimer's disease, because the accumulated homocysteine induces the formation of reactive oxygen species [8].

Folate compounds found in naturally occurring foods are present in reduced forms such as tetrahydrofolate (THF) (Fig. 3) and methyl, methylene, methenyl, formyl, or formimino-group that binds to the *N*5 or *N*10 (or both) of the molecules [9]. Moreover, the length of the poly-γ-glutamate chain of THF varies depending on the food [10]. Accurate quantification of folate compounds in foods is important in determining the nutritional value of folate. The poly-γ-glutamate chain of THF must be treated with folate conjugase to be converted into the mono-γ-glutamate form before being analyzed using both microbiological assay and high-performance liquid chromatography (HPLC) [11].

HPLC has also been widely used to determine folate compounds in foods [12]. However, purification is required when using HPLC to determine food extracts. Various solid-phase extraction procedures with commercially available disposable cartridges such as strong anion exchange cartridges have been reported [12,13]. Affinity chromatography with folate-binding protein (FBP; from cow's milk) attached to agarose gel removes impurities more effectively [12,14]. Affinity columns are not available commercially; thus, they must be prepared by the investigator. Because

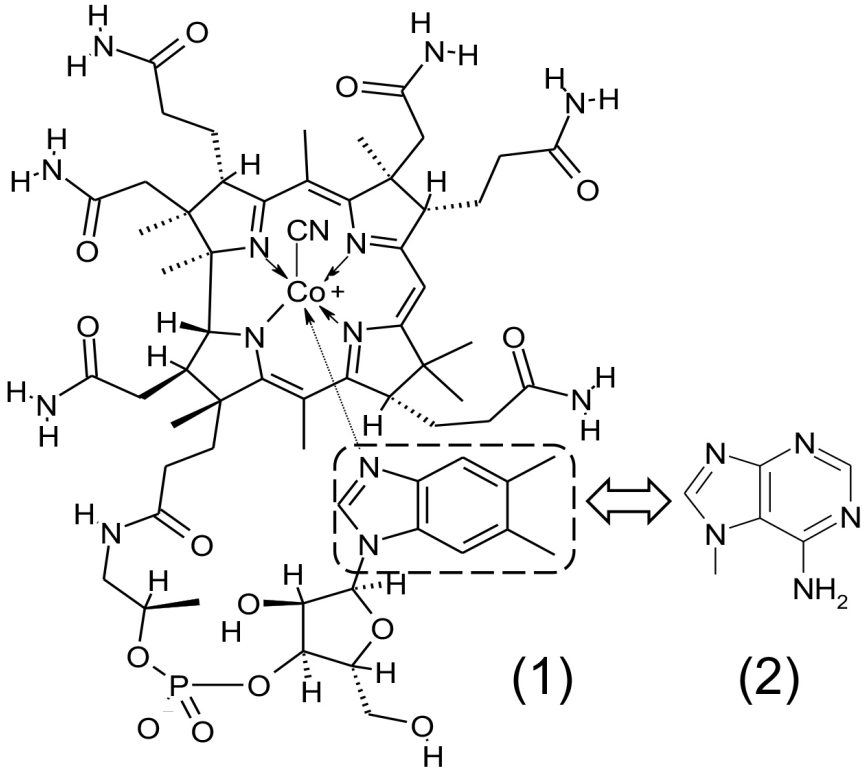


Fig. 1. Structural formula of vitamin B_{12} and partial structures of pseudovitamin B_{12}. (1) Vitamin B_{12} and (2) pseudovitamin B_{12}.

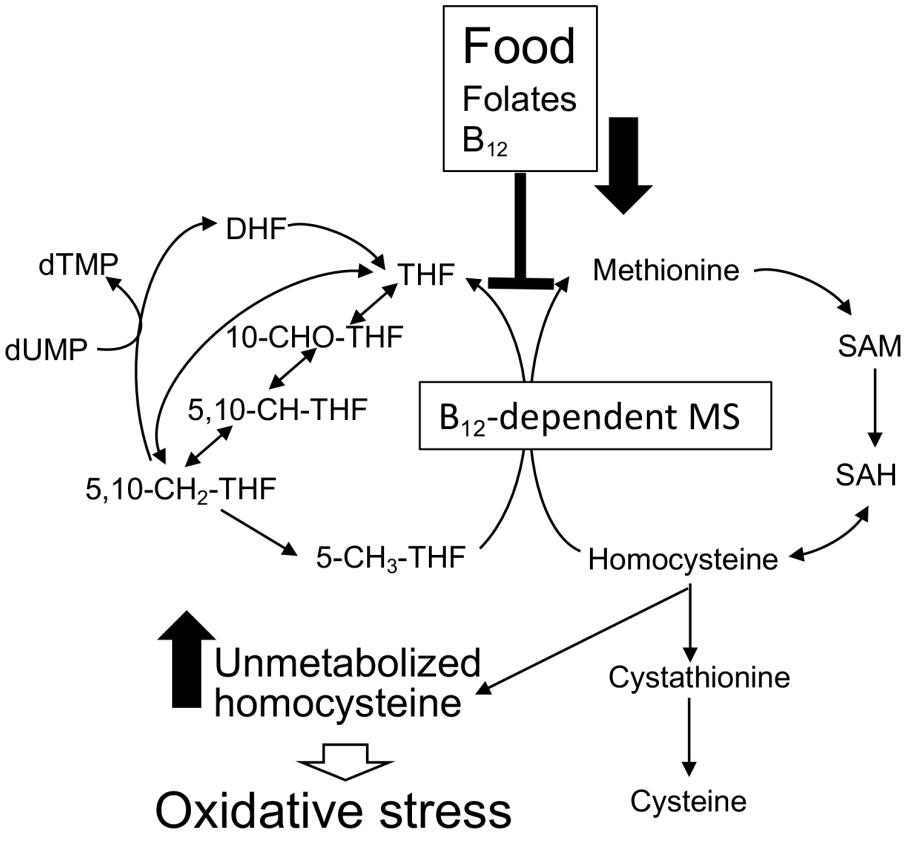
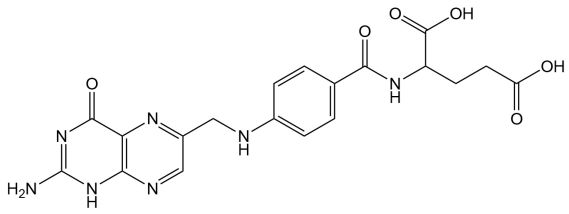


Fig. 2. Physiological effects of folate and B_{12} on methionine metabolism in humans. DHF, dihydrofolate; dTMP, deoxythymidine monophosphate; dUMP, deoxyuridine monophosphate; MS, methionine synthase; SAH, *S*-adenosylhomosysteine; SAM, *S*-adenosylmethionine; THF, tetrahydrofolate.

(1)



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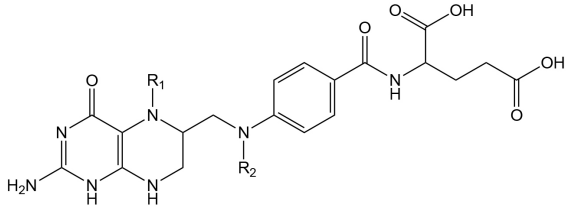

R_1	R_2	Folate species
H	H	THF
CH_3	H	5-CH_3-THF
CHO	H	5-CHO-THF
H	CHO	10-CHO-THF
-CH_2-		5,10-CH_2-THF
-CH=		5,10-CH=THF

Fig. 3. Structure of folate compounds. (1) Folic acid or pteroyl glutamate and (2) reduced folate compounds.

such process of food folate assay is laborious, little information is available on the folate contents of edible seaweeds, especially purple laver products. If commercially available dried purple laver products contain substantial amounts of folate compounds, they would be good sources of both B_{12} and folate compounds in humans. Thus, we developed a simple method for purifying folate compounds from food extracts using FBP and a commercially available centrifugal ultrafiltration unit. Accordingly, this study aimed to determine and characterize folate compounds from commercially available dried purple laver products using HPLC with FBP purification.

2. Materials and Methods

2.1 Materials

Folic acid and FBP (from bovine milk) were obtained from Sigma-Aldrich (St. Louis, MO, USA). (6*R*, *S*)-5,10-Methenyl-5,6,7,8-tetrahydrofolic acid (5,10-CH=THF) chloride, (6*R*, *S*)-5,6,7,8-tetrahydrofolic acid (THF) trihydrochloride, (6*R*, *S*)-5-formyl-5,6,7,8-tetrahydrofolic acid (5-CHO-THF) calcium salt, and (6*S*)-5-methyl-5,6,7,8-tetrahydrofolic acid (5-CH_3-THF) calcium salt were purchased from Schircks Laboratories (Zurich, Switzerland) and used as folate standard compounds. 10-CHO-THF was prepared according to the method published previously [15]. Type II porcine kidney acetone powder (Sigma-Aldorich) was used as folate conjugases. α-amylase (from *Aspergillus oryzae*), protease (type XIV, from *Streptomyces griseus*), and certified reference material BCR-485 (from mixed vegetables) were obtained from Sigma-Aldrich. Ultracel®-10K (Amicon® Ultra-2 mL) was purchased from Merck Millipore Ltd. (Tullagreen, Ireland) and used for centrifugal filter units for separation from FBP to folate compounds. Purple laver (*Neopyropia yezoensis*, previously *Porphyra yezoensis*) products (dried, toasted, and seasoned and toasted), dried kombu (*Saccharina japonica*) products, dried wakame (*Undaria pinnatifida*) products, and boiled and dried hijiki (*Sargassum fusiformis*) products were obtained from local markets in Tottori City, Japan, on October 25, 2022.

2.2 Preparation of Folate Conjugase

To remove the endogenous folate compounds, porcine kidney (0.12 g) powder was dissolved in 20 mL of 0.1 mol/L sodium phosphate buffer (pH 6.1), treated with activated charcoal powder (2.0 g), stirred for 1 h at 4 °C, and centrifuged at 900 $\times$ *g* for 10 min at 4 °C. The supernatant fraction was treated with a membrane filter (25AS020AS; ADVANTEC® Tokyo Roshi Kaisha Ltd., Tokyo, Japan) and used as the folate conjugase.

2.3 Extraction of Folate Compounds and Tri-Enzyme Treatments

Total folate compounds were extracted from commercially available dried purple laver products according to the tri-enzyme method [11]. Five grams of the laver products were homogenized with a mortar and pestle. An aliquot (0.2 g) of the homogenate was dissolved in 2.0 mL of 0.1 mol/L sodium phosphate buffer (pH 6.1) containing 2% (v/v) ascorbic acid and 0.2% (v/v) 2-mercaptoethanol and diluted with distilled water to a final volume of 5.0 mL. Octanol (100 μL) was then added, and the homogenates were autoclaved at 100 °C for 10 min. After cooling to room temperature (25 °C), 1 mL of 0.1 mol/L sodium phosphate buffer (pH 6.1) containing 2% (v/v) ascorbic acid and 0.2% (v/v) 2-mercaptoethanol and 100 μL of protease solution (7.0×10^{-4} U) were added to each homogenate, and the homogenates were then incubated at 37 °C for 3 h. To stop the protease enzyme reaction, the homogenates were heated at 100 °C for 3 min. After cooling with ice, they were treated with 100 μL of α-amylase. solution (0.3 U) for 2 h at 37 °C. Thereafter the homogenates were further treated with 400 μL of porcine kidney folate conjugase for 16 h at 37 °C. To stop the enzyme reactions, the treated homogenates were heated at 100 °C for 3 min and then cooled to room temperature (25 °C). The homogenate was diluted with distilled water to a final volume of 10 mL, filtered through filler paper (type 2, 90 mm, ADVANTEC®), and used as a food folate extract.

2.4 Microbiological Assay of Total Folate Compounds

Folate assays were performed using a polypropylene tube (13 × 100 mm, Bio-Rad Laboratories, Hercules, CA, USA), which contain the food extract (50 μL), 0.1 mol/L sodium phosphate buffer (pH 7.0, 200 μL), and *Lactobacillus rhamnosus* folate assay medium (1 mL). The prepared assay mixture was diluted with distilled water to give a final volume of 2.0 mL and then autoclaved at 121 °C for 5 min. After cooling to room temperature (25 °C), the tube was inoculated aseptically with 50 μL of *L. rhamnosus* ATCC 7469 pre-cultured in Lactobacilli inoculum broth as described above, washed three times with 5 mL of saline buffer, and dissolved in saline buffer to achieve 92% light transmittance at 660 nm. After incubating the tube at 37 °C for 22 h, its optical density at 660 nm was determined using a UV-2550 Spectrophotometer (Shimadzu Corporation, Kyoto, Japan). Each food sample was assayed for folate contents in triplicates, and this was repeated at least thrice.

2.5 Purification of Folate Compounds Using FBP and Ultracel®-10K Centrifugal Filter Unit

An Ultracel®-10K centrifugal filter unit was treated with 1 mL of 25% (v/v) methanol solution and centrifuged at 7000 × *g* for 10 min to wash its membrane. Then, 1 mL of 50 mmol/L potassium phosphate buffer containing 2% (v/v) ascorbic acid and 0.2% (v/v) 2-mercaptoethanol was added to the filter unit, which was centrifuged under the same conditions. Aliquots (100 μg) of lyophilized FBP was dissolved in 0.5 mL of 50 mmol/L potassium phosphate buffer containing 2% (v/v) ascorbic acid and 0.2% (v/v) 2-mercaptoethanol. The FBP solution was added to each solution (0.5 mL) of standard folate compounds (100 ng/mL) in a microtube, mixed well, and incubated on ice for 30 min. Then, the mixture was transferred into the washed Ultracel®-10K centrifugal filter unit and centrifuged at 7000 × *g* for 10 min at 4 °C (**Supplementary Fig. 1**). The centrifugal filter unit was washed twice with 1 mL of 50 mmol/L potassium phosphate buffer containing 2% (v/v) ascorbic acid and 0.2% (v/v) 2-mercaptoethanol to purify the formed FBP–folate complex. Folate compounds were liberated from the FBP complex by the denaturation of FBP and treated with 300 μL of 40 mmol/L trifluoroacetic acid containing 2% (v/v) ascorbic acid and 0.2% (v/v) 2-mercaptoethanol, removed by centrifugation at 7000 × *g* for 10 min at 4 °C. The remaining folate compounds on the membrane of the filter unit was recovered to wash twice with 400 μL of 25% (v/v) methanol solution containing 2% (%) ascorbic acid and 0.2% (v/v) 2-mercaptoethanol. The filtrate fractions were combined and used as HPLC samples.

After the denatured FBP was recovered with 50 mmol/L potassium phosphate buffer from the filter unit, it was treated in 50 mmol/L potassium phosphate buffer for 24 h at 4 °C until the denatured FBP was completely converted to the renatured form. FBP could be reused approximately 10 times to purify folate compounds in this system.

2.6 Determination of Folate Compounds Using HPLC

After the pH of the purple laver extracts prepared as described above was adjusted to pH 7.4 by the addition of 1 mol/L NaOH, the treated extracts were diluted 1.5 times with distilled water. An aliquot (1.5 mL) of the diluted purple laver extracts was mixed with 1.0 mL of 200 μg/mL FBP solution at 4 °C for 20 min to purify folate compounds. Then, the solution was then transferred to an Ultracel®-10K centrifugal filter unit and subjected to centrifugation at 7000 × *g* at 4 °C for 15 min. Subsequently, the FBP–folate complex was washed twice with 1 mL of 50 mmol/L potassium phosphate buffer (pH 7.4) containing 2% (%) ascorbic acid and 0.2% (v/v) 2-mercaptoethanol.

After the washed FBP–folate complex was treated with 500 μL of 40 mmol/L trifluoroacetic acid solution containing 2% (%) ascorbic acid and 0.2% (v/v) 2-mercaptoethanol to denature FBP, folate compounds were recovered by centrifugation under the same conditions. After the Ultracel®-10K unit was washed with 400 μL of 25% (v/v) methanol solution containing 2% (%) ascorbic acid and 0.2% (v/v) 2-mercaptoethanol, the filtrate and washing fraction were combined and used as an HPLC sample.

The prepared sample was analyzed by HPLC using a Shimazu HPLC apparatus (SCL10A system controller, LC-10Ai pump, CT-20A column oven, fluorescence detector Shimazu RF-530, and electrochemical detector GL Science ED 723) and CDS version 5 chromato-data processing system (LAsoft, Ltd., Chiba, Japan) (**Supplementary Fig. 2**). A 500-μL aliquot of each sample was placed on an Intert-Susuain AQ-C18 HPLC column (5 μm, 4.6 × 100 mm, GL Sciences) at 40 °C and eluted equilibrated with 50 mmol/L potassium dihydrogen phosphate solution (pH 2.0) containing 7% (*v/v*) acetonitrile at a flow rate of 1.0 mL/min. Folate compounds were detected at 292-nm excitation/362-nm emission (fluorescence detector) and at 1000 mV versus Ag/AgCl (electrochemical detector). Authentic THF, 5-CH_3-THF, and 5-CHO-THF were detected during monitoring at fluorescence detection, and the retention times were 6.6 min, 7.7 min, and 17.5 min, respectively (**Supplementary Fig. 3**). Authentic 5,10-CH=THF (with a retention time of 10.9 min) and folic acid (with a retention time of 21.4 min) were completely separated from other compounds contained in the sample such as ascorbic acid and 2-mercaptoethanol during monitoring at electrochemical detection; however, THF, 5-CH_3-THF, and 5-CHO-THF could not. Since 10-CHO-THF and 5,10-CH=THF could not be separated under these conditions, the peak fraction with a retention time of 10.9 min represents the sum values of 10-CHO-THF and 5,10-CH=THF.

2.7 Extraction and Determination of B_{12}

Each sample (2 g) of the commercially available dried purple laver products was homogenized using a mortar and

Table 1. Total B_{12} and folate contents of commercially available edible seaweed products.

	Total folate compounds (μg/100 g)	Total B_{12} (μg/100 g)
Dried purple laver (*N. yezoensis*)	1309.0 ± 53.4	59.7 ± 18.2
Toasted purple laver (*N. yezoensis*)	1259.6 ± 46.2	58.4 ± 18.0
Seasoned and toasted purple laver (*N. yezoensis*)	876.8 ± 136.4	28.9 ± 11.6
Dried kombu (*S. japonica*)	230.3 ± 23.4	0.1 ± 0.1
Boiled and dried hijiki (*S. fusiformis*)	149.0 ± 30.0	ND
Dried wakame (*U. pinnatifida*)	66.5 ± 27.0	0.5 ± 0.1

After each of the commercially available edible seaweed products described in the table was extracted and treated with tri-enzymes, total folate compounds were determined using the microbiological method. B_{12} was extracted from seaweed products, purified with a B_{12}-immunoaffinity column, and determined using HPLC. Data are represented as mean ± SD (n = 3). ND, not detected.

pestle. Total B_{12} compounds were extracted from each homogenate by boiling with 40 mL of distilled water and 10 mL of 0.57 mol/L acetate buffer (pH 4.5) containing 0.05% (w/v) KCN for 30 min in the dark to convert B_{12} compounds into the CN forms. The extraction procedures were performed in a draft chamber (Dalton Co., Tokyo, Japan). An aliquot (20 mL) of each extract (100 mL) prepared above was placed on a Sep-Pak ® plus C18 cartridge (Waters Corp., Milford, MA, USA) that was activated with 5 mL of 100% ethanol and equilibrated with 10 mL of distilled water. B_{12} compounds were eluted from the C18 cartridge with 2 mL of 75% (v/v) ethanol. After the eluate was filtered with a DISMIC-25JP membrane filter (Toyo Roshi Kaisya, Ltd, Tokyo, Japan), the remaining B_{12} compounds on the filter were recovered with 1 mL of 75% (v/v) ethanol. The filtrates were combined and evaporated to dryness under reduced pressure. The residual fraction was dissolved in 1.0 mL of distilled water. B_{12} compounds were purified from the solution using an immunoaffinity column (EASI-EXTRACT B_{12} P80; φ 8.0 × 60 mm, R-Biopharm, Darmstadt, Germany) according to the manufacturer's protocol. The elute was evaporated to dryness under pressure is reduced, dissolved in 100 μL of Milli-Q water, and used as an HPLC sample, as described previously [16]. The HPLC apparatus (SPD-10AV UV-Vis detector, SCL-10A VP system controller, DGU-20A_3 degasser, LC-10Ai liquid chromatograph, CTO-20A column oven) and a reversed-phase HPLC column (Wakosil-II 5C18RS, φ4.6 × 150 mm; FUJIFILM Wako Pure Chemical Corp., Osaka, Japan) were used. B_{12} compounds were isocratically eluted with 20% (v/v) methanol containing 1% (v/v) acetic acid at a flow rate of 1.0 mL/min at 40 °C and monitored by measuring the absorbance at 361 nm.

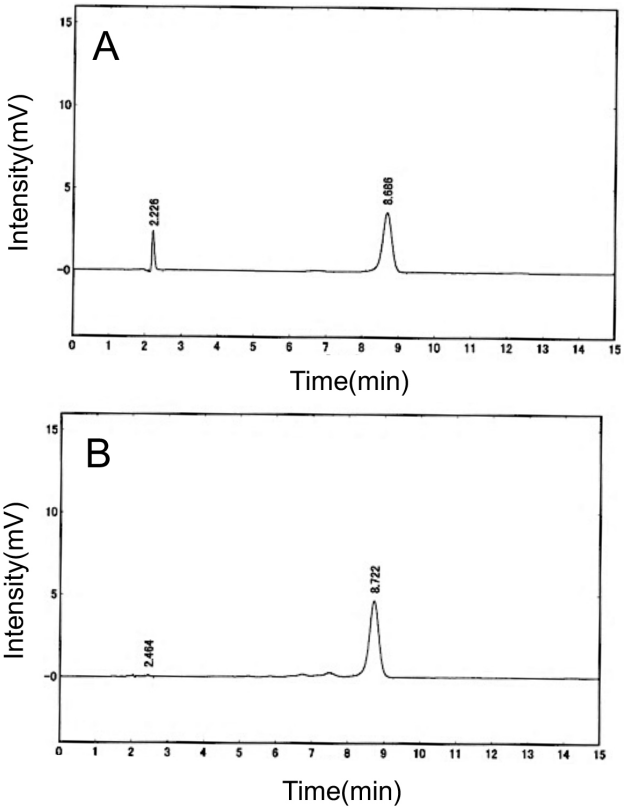

Fig. 4. HPLC chromatograms for authentic B_{12} and corrinoid compounds present in dried purple laver products. (A) Authentic B_{12}. (B) Corrinoid compounds present in dried purple laver products. These are typical HPLC chromatograms of authentic B_{12} and corrinoid compounds present in dried purple laver products for three independent experiments.

3. Results and Discussion

3.1 Content of Total B_{12} and Folate Compounds in Commercially Available Edible Seaweed Products

Total B_{12} was extracted from various edible seaweed products and determined using HPLC. Dried purple laver (nori) products contained substantial amounts (approximately 30–60 μg B_{12}/100 g dry weight) of B_{12}. These values were much higher than those in other edible seaweeds (<0.5 μg/100 g dry weight) (Table 1). Moreover, nori products did not contain pseudovitamin B_{12} (Fig. 1) mostly found in edible cyanobacteria because only a single peak of B_{12} was detected during HPLC (Fig. 4) (**Supplementary Fig. 4**). These results coincide with previously reported

values determined using a microbiological assay method [17].

Miyamoto *et al.* [18] reported that the B_{12} contents of the seasoned and toasted purple laver products were reportedly about half of the values of the dried purple laver products. Similar results were obtained in this study (Table 1). No B_{12} content was reduced in dried purple laver products during the toasting process [18], suggesting that the decreased B_{12} contents in the seasoned and toasted laver products may be due to B_{12} destruction caused by the interaction of various seasonings rather than the toasting process.

Total folate compounds were extracted, treated with the tri-enzyme (proteinase, α-amylase, and conjugase) method, and determined using microbiological assay. The total folate content was much higher in dried purple laver (nori) products (approximately 880–1300 μg/100 g dry weight) than in other edible seaweeds (<230 μg/100 g dry weight). These values determined from the tri-enzyme-treated extracts of seaweed products were similar to those from the extracts treated with di-enzymes (proteinase and conjugase) that were adopted as an official method of food composition analysis in Japan [19]. These results indicated that dried purple laver (nori) products contain high levels of B_{12} and folate compounds compared with other seaweed products tested.

3.2 Determination of Folate Compounds Found in Some Purple Laver (Nori) Products Using HPLC after FBP Purification

Before the HPLC analysis, folate compounds from food extracts should be purified. Affinity chromatography with FBP attached to agarose gel has been used to remove impurities more effectively. The preparation of affinity columns is time-consuming, and the folate-binding capacity of FBP is slightly lost during the preparation. Thus, we developed a simple method for purifying folate compounds from food extracts using FBP and Ultracel®-10K centrifugal filter unit (**Supplementary Fig. 1**). To evaluate the recovery rates of folate compounds during purification, various standard compounds such as THF, 5-CHO-THF, 5,10-CH=THF, and 5-CHO-THF were used. As shown in **Supplementary Table 1**, more than 85% of the recovery rate were obtained at each folate compound. To evaluate whether this method can be applied into food folate analysis, certified reference material BCR-485 (from mixed vegetables) was used as a sample. Our HPLC analysis indicated that 5-CH_3-THF (229 μg/100 g) is the predominant folate compound in BCR-485, which contained 244 μg of total folate compounds per 100 g weight (**Supplementary Table 2**). These values coincided those reported previously, indicating that this method can be applicable to food folate analysis.

This HPLC method was performed to determine folate compounds found in some purple laver (nori) products.

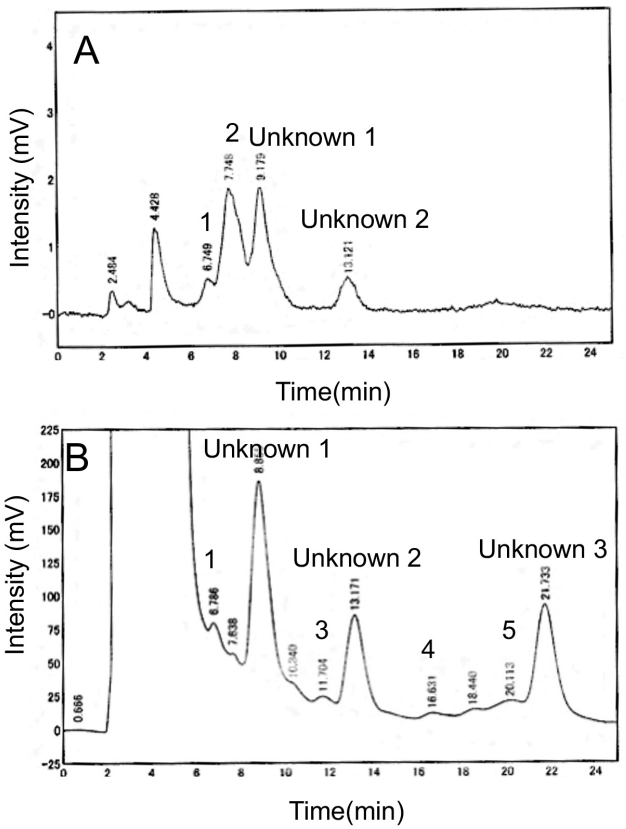

Fig. 5. HPLC chromatograms for folate compounds present in dried purple laver products. (A) Fluorescence detector; and (B) electrochemical detector. The retention times of peaks 1, 2, 3, 4, and 5 were identical to those of authentic THF, 5-CH_3-THF, and 5,10-CH = THF or 10-CHO-THF, 5-CHO-THF, and folic acid (FA), respectively.

Fig. 5A,B showed the elution profiles of the FBP-purified compounds of a dried purple laver product in fluorescence and electrochemical detectors, respectively. The retention times of peaks 1 (6.7 min) and 2 (7.7 min) were identical to those of authentic THF and 5-CH_3-THF, respectively, in fluorescence detection. Whereas in electrochemical detection, the retention times of peaks 1 (6.7 min), 3 (11.7 min), 4 (16.6 min), and 5 (20.1 min) were identical to those of authentic THF, 5,10-CH=THF (or 10-CHO-THF or both), 5-CHO-THF, and folic acid (FA), respectively. Similar elution patterns of FBP-purified compounds were found in all purple laver products. Table 2 summarizes the levels of folate compounds determined from commercially available purple laver products. 5-CH_3-THF (163–253 μg/100 g dry weight) was the major folate compound in all purple laver products. 5-CHO-THF was found in the similar level (approximately 73–175 μg/100 g dry weight). 5,10-CH=THF and 10-CHO-THF (approximately 57–123 μg/100 g dry weight), THF (approximately 17–28 μg/100 g dry weight), and FA (9.8–52 μg/100 g dry weight) were the minor folate compounds found in purple laver products. FA would be due to the oxidation of the reduced folate compounds such

Table 2. Levels of folate compounds of commercially available dried purple laver products.

	Dried purple laver	Toasted purple laver	Seasoned and toasted purple laver
	(μg/100 g dry weight)		
THF	27.2 ± 1.9	27.8 ± 7.4	16.8 ± 1.6
5-CH_3-THF	253.7 ± 24.4	202.7 ± 45.0	163.3 ± 19.5
5,10-CH=THF + 10-CHO-THF	123.1 ± 26.0	82.2 ± 11.2	56.6 ± 8.3
5-CHO-THF	127.1 ± 3.5	175.6 ± 93.0	73.3 ± 9.7
FA	52.0 ± 17.1	25.7 ± 11.7	9.8 ± 8.0
Total folate compounds	581.1 ± 51.2	514.0 ± 130.5	319.8 ± 26.6

Folate compounds were purified from the tri-enzyme-treated extract of purple laver products using FBP and then determined using HPLC. Data are represented as mean ± SD (n = 3).

as 5-CH_3-THF during food processing and storage. The sum of these folate compounds identified in HPLC was approximately 40% of total folate compounds determined by the microbiological assay in each purple laver product, suggesting that some of the unidentified peaks may be derived from certain folate compounds. However, no information is available on whether these unknown 1–3 fractions contain some folate compounds because the antioxidant reagent 2-mercaptoethanol was eluted in the fraction of unknown peak 1. Although information on the levels of reduced folate compounds in edible seaweeds is very limited, *Porphyra* spp. (100 g, dry weight) reportedly contained approximately 61 μg of folate compounds, which consist of 5-CH_3-THF (approximately 34 μg/100 g, dry weight) and FA (approximately 36 μg/100 g, dry weight) [20].

Because the weight of one sheet (20 × 20 cm) of dried purple laver products is approximately 3 g [21], two sheets (approximately 6 g) of dried purple laver products would be sufficient to meet the recommended dietary allowance (RAD) of adults for B_{12} (2.4 μg/day). In RAD for folates (400 μg/day), ingestion of two sheets of the products would provide approximately 80 μg of folates per day [22].

It is concerning that the excessive consumption of edible seaweed products may lead to the ingestion of harmful amounts of iodine because substantial amounts of iodine (approximately 200 mg/100 g dry weight) have been found in dried kombu products [23,24]. However, dried purple laver products reportedly contained less iodine (approximately 4–8 mg/100 g dry weight) [21]. Therefore, consuming two sheets (approximately 6 g/day) of dried purple laver products would not lead to an excessive intake of iodine (approximately 0.2–0.5 mg/day).

4. Conclusions

This study indicated that dried purple laver (*N. yezoensis*) products contained higher levels of B_{12} (approximately 30–60 μg/100 g dry weight) and folate compounds (approximately 880–1300 μg/100 g dry weight) than other seaweed products. Moreover, considerably low levels of dietary iodine were reportedly found in purple laver products. These results suggest that dried purple laver products are suitable sources of B_{12} and folate compounds for humans, especially vegetarians. However, the bioavailability of B_{12} and folate compounds found in dried purple laver products in humans remains to be determined.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

KKoseki, RY, KI, and KKatsuura performed most of the experiments. KKoseki and RY analyzed the folate compounds using HPLC and interpreted the results. KI analyzed the total folate compounds using the microbiological assay. KKatsuura analyzed the vitamin B_{12} using HPLC. KKoseki, TB, and FW designed the experiments, interpreted the results, and wrote the manuscript. All authors reviewed and commented on the manuscript and approved the final version.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/j.fbe1502010.

References

[1] Zaharudin N, Tullin M, Pekmez CT, Sloth JJ, Rasmussen RR, Dragsted LO. Effects of brown seaweeds on postprandial glucose, insulin and appetite in humans - A randomized, 3-way, blinded, cross-over meal study. Clinical Nutrition (Edinburgh, Scotland). 2021; 40: 830–838.

[2] Blouin NA, Brodie JA, Grossman AC, Xu P, Brawley SH. Porphyra: a marine crop shaped by stress. Trends in Plant Science. 2011; 16: 29–37.

[3] Kim SK, Li YX. Medicinal benefits of sulfated polysaccharides from sea vegetables. Advances in Food and Nutrition Research. 2011; 64: 391–402.

[4] Niu JF, Wang GC, Zhou BC, Lin XZ, Chen CS. Purification of R-phycoerythrin from Porphyra haitanesis (Bangiales, Rhodophyta) using expanded-bed absorption. Journal of Phycology. 2007; 43: 1339–1347.

[5] Watanabe F, Yabuta Y, Bito T, Teng F. Vitamin B_{12}-containing plant food sources for vegetarians. Nutrients. 2014; 6: 1861–1873.

[6] Froese DS, Fowler B, Baumgartner MR. Vitamin B_12 , folate, and the methionine remethylation cycle-biochemistry, pathways, and regulation. Journal of Inherited Metabolic Disease. 2019; 42: 673–685.

[7] Reynolds E. Vitamin B12, folic acid, and the nervous system. The Lancet. Neurology. 2006; 5: 949–960.

[8] Esse R, Barroso M, Tavares de Almeida I, Castro R. The Contribution of Homocysteine Metabolism Disruption to Endothelial Dysfunction: State-of-the-Art. International Journal of Molecular Sciences. 2019; 20: 867.

[9] Ducker GS, Rabinowitz JD. One-Carbon Metabolism in Health and Disease. Cell Metabolism. 2017; 25: 27–42.

[10] Melse-Boonstra A, Verhoef P, Konings EJM, Van Dusseldorp M, Matser A, Hollman PCH, *et al*. Influence of processing on total, monoglutamate and polyglutamate folate contents of leeks, cauliflower, and green beans. Journal of Agricultural and Food Chemistry. 2002; 50: 3473–3478.

[11] Hyun TH, Tamura T. Trienzyme extraction in combination with microbiologic assay in food folate analysis: an updated review. Experimental Biology and Medicine (Maywood, N.J.). 2005; 230: 444–454.

[12] Quinlivan EP, Hanson AD, Gregory JF. The analysis of folate and its metabolic precursors in biological samples. Analytical Biochemistry. 2006; 348: 163–184.

[13] Vahteristo L, Ollilanen V, Varo P. HPLC determination of folate in liver and liver products. Journal of Food Science. 1996; 61: 524–526.

[14] Edelmann M, Kariluoto S, Nystrom L, Piironen V. Folate in barley grain and fractions. Journal of cereal Science. 2013: 58; 37–44.

[15] Baggott JE, Johanning GL, Branham KE, Prince CW, Morgan SL, Eto I, *et al*. Cofactor role for 10-formyldihydrofolic acid. The Biochemical Journal. 1995; 308 (Pt 3): 1031–1036.

[16] Koseki K, Namura M, Bito T, Umebayashi Y, Watanabe F. Characterization of. vitamin B12 compounds in commercially available livestock livers used as foods. ACS Food Science & Technology. 2022; 2; 1369–1370.

[17] Watanabe F. Vitamin B12 sources and bioavailability. Experimental Biology and Medicine (Maywood, N.J.). 2007; 232: 1266–1274.

[18] Miyamoto E, Yabuta Y, Kwak CS, Enomoto T, Watanabe F. Characterization of vitamin B12 compounds from Korean purple laver (Porphyra sp.) products. Journal of Agricultural and Food Chemistry. 2009; 57: 2793–2796.

[19] Standard Tables of Food Composition in Japan-2015. The Council for Science and Technology Ministry of Education, Culture, Sports, Science and Technology, JAPAN (Ed.) Official Gazette Co-operation of Japan: Tokyo, Japan. 2015.

[20] Rodríguez-Bernaldo de Quirós A, Castro de Ron C, López-Hernández J, Lage-Yusty MA. Determination of folates in seaweeds by high-performance liquid chromatography. Journal of Chromatography. a. 2004; 1032: 135–139.

[21] Watanabe F, Takenaka S, Katsura H, Masumder SA, Abe K, Tamura Y, *et al*. Dried green and purple lavers (Nori) contain substantial amounts of biologically active vitamin B(12) but less of dietary iodine relative to other edible seaweeds. Journal of Agricultural and Food Chemistry. 1999; 47: 2341–2343.

[22] Institute of Medicine. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic, Acid, Biotin, and Choline (pp. 196–356). Institute of Medicine, National Academy Press: Washington, DC, USA. 1998.

[23] Yoshida M, Mukama A, Hosomi R, Fukunaga K, Nishiyama T. Serum and tissue iodine concentrations in rats fed diets supplemented with kombu powder or potassium iodide. Journal of Nutritional Science and Vitaminology. 2014; 60: 447–449.

[24] Miyai K, Tokushige T, Kondo M, Iodine Research Group. Suppression of thyroid function during ingestion of seaweed “Kombu” (Laminaria japonoca) in normal Japanese adults. Endocrine Journal. 2008; 55: 1103–1108.