Effects of Microwave Heating on the Loss of Vitamin B_{12} in Foods

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To clarify the effects of microwave heating on the loss of vitamin B_{12} in foods, raw beef, pork, and milk were treated by microwave heating and then their vitamin B_{12} contents were determined according to a chemiluminescent vitamin B_{12} assay with hog intrinsic factor. Appreciable loss (∼30−40%) of vitamin B_{12} occurred in the foods during microwave heating due to the degradation of vitamin B_{12} molecule by microwave heating. When hydroxo vitamin B_{12}, which predominates in foods, was treated by microwave heating and then analyzed by silica gel 60 thin layer chromatography, two vitamin B_{12} degradation products were found. One of the compounds with a R_f of 0.16 was purified and partially characterized. The vitamin B_{12} degradation product did not show any biological activity in the growth of a vitamin B_{12} requiring microorganism, *Euglena gracilis* Z, and was not bound to hog intrinsic factor, a mammalian vitamin B_{12} binding protein. Intravenous administration of the compound (1 μg/day) for 7 days to rats showed that the compound neither has toxicity nor acts as a vitamin B_{12} antagonist in mammals. These results indicate that the conversion of vitamin B_{12} to the inactive vitamin B_{12} degradation products occurs in foods during microwave heating.

Keywords: *Vitamin B_{12}; degradation; microwave heating; intrinsic factor; rat*

INTRODUCTION

A deficiency of vitamin B_{12} (B_{12}) can lead to the human disease pernicious anemia. Foods contain various B_{12} analogues with different β-ligands; especially, methyl vitamin B_{12} (MeB_{12}) and 5′-deoxyadenosyl vitamin B_{12} ($AdoB_{12}$), which function as coenzymes of methionine synthase (EC 2.1.1.13) involved in methionine biosynthesis, and methylmalonyl-CoA mutase (EC 5.4.99.2), which is involved in amino acid and odd-chain fatty acid metabolisms in mammals, respectively (Schneider and Stroiñski, 1987).

Cyano vitamin B_{12} ($CN\text{-}B_{12}$), used in medicine, does not occur in raw foods, which contain predominantly hydroxo vitamin B_{12} ($OH\text{-}B_{12}$). Even if the coenzyme B_{12} form occurs, it would be photolyzed to form $OH\text{-}B_{12}$ by light (Schneider, 1987). Bennink and Ono (1982) have reported appreciable loss of B_{12} during cooking of raw beef, but it is unclear why such loss occurs.

Microwave ovens are widely used for cooking and food processing. Extensive studies (Cross and Fung, 1982; Hoffman and Zabik, 1985) have shown equal or better retention of some vitamins (B_1, B_2, B_6, C, and folic acid) after microwave heating compared with conventional heating. There is, however, little information on how much loss of B_{12} occurs in foods during microwave heating. Here the effects of microwave heating on the loss of B_{12} in foods are described, and the loss of B_{12} due to its conversion to biologically inactive B_{12} degradation products by microwave heating is demonstrated.

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MATERIALS AND METHODS

Materials. $OH\text{-}B_{12}$, $CN\text{-}B_{12}$, $AdoB_{12}$, and MeB_{12} were obtained from Sigma Chemical Co. (St. Louis, MO). Thin layer chromatography (TLC) plates (silica gel 60) were obtained from Merck (Darmstadt, Germany). A reversed-phase high-pressure liquid chromatography (HPLC) column (Wakosil-II 5C18RS, ∅4.6 × 150 mm; particle size 5 μm) was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Raw beef and pork and pasteurized cow's milk were obtained from a local market in Kochi, Japan.

Microwave Heating of Foods. Ten grams of raw beef (or pork) was homogenized in 50 mL of distilled water at room temperature using a universal homogenizer (Nihon Seiki Seisakusho, Co., Tokyo, Japan). Each of the homogenates and pasteurized cow's milk (100 g) were placed into a glass dish suitable for microwave cooking and treated for 6 min by microwave heating. The microwave oven used was a Funai (Tokyo, Japan) Model MO57-6A. The full power was 500 W, and the frequency of radiation was 2450 MHz. Beef and pork homogenates and cow's milk without microwave treatment were used as controls.

Cow's milk (100 g) was also treated for 30 min in a boiling water bath and used as a boiled sample. Temperatures of the boiled and microwave-treated milk were measured during the experimental time course by a Asea Fiber 1110 thermometer (Vasteras, Sweden).

Extraction and Assay of B_{12}. Total B_{12} was extracted from the control, boiled, or microwave-treated foods by boiling with KCN in acidic pH (Frenkel et al., 1980). The extraction procedures were done in a Dalton (Tokyo, Japan) drauft chamber. Ten milliliters of 0.5 mol/L acetate buffer, pH 4.8, and 20 mg of KCN were added to the beef and pork homogenates, which were boiled for 30 min at 98 °C in the dark. These homogenates were centrifuged at 10000*g* for 10 min. The supernatant was used as the sample for the B_{12} assay.

In the case of milk, 100 mL of 0.2 mol/L acetate buffer, pH 4.8, and 20 mg of KCN were added to the control, boiled, or microwave-treated milk, boiled for 30 min at 98 °C in the dark,

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and then centrifuged at 10000*g* for 10 min. The supernatant was used as the sample for the B_{12} assay.

B_{12} was assayed by a fully automated chemiluminescent ACS-180 B_{12} analyzer (Chiron Diagnostics, East Walpole, MA) according to the manufacturer's instruction. The B_{12} extract was directly applied to the analyzer and the B_{12} concentration was automatically determined.

Microwave Heating of Authentic OH-B_{12}. Authentic OH-B_{12} used in the experiments was purified by silica gel 60 TLC with 1-butanol/2-propanol/water (10:7:10) as a solvent, since the OH-B_{12} reagent (≈98% pure) contained a small amount of an impurity (R_f value = 0.27). The red compound with R_f of 0.03 (OH-B_{12} fraction) was collected, dissolved in an appropriate amount of the solvent, and extracted three times from the silica gels. The combined extracts were evaporated to dryness and dissolved in a small amount of distilled water. The concentration of the purified OH-B_{12} was determined on the basis of a molecular extinction coefficient for aqua vitamin B_{12} of 8.5×10^3 $(mol/L)^{-1}$ cm^{-1} at 527 nm in water. Aqua vitamin B_{12} is the conjugated acid form of OH-B_{12}. Aqua and hydroxo vitamin B_{12} are interconvertible in solution depending on pH (below pH 8.0, aqua vitamin B_{12} tends to predominate over OH-B_{12} at equilibrium) (Smith et al., 1962). Two milliliters of 0.1 mmol/L purified OH-B_{12} solution was treated for 6 min in the dark by microwave heating with the Funai microwave oven at 500 W. The OH-B_{12} solution was also treated for 30 min in a boiling water bath in the dark. Each treated solution was evaporated to dryness and dissolved in a small amount of distilled water. The concentrated solution was spotted quantitatively several times in an identical site on the silica gel 60 TLC plate and developed with 1-butanol/2-propanol/water (10:7:10) as a solvent in the dark. Authentic OH-B_{12}, CN-B_{12}, AdoB$_{12}$, and MeB$_{12}$ (all at 10 mmol/L) were used as standards in TLC analysis.

The TLC plates developed were dried at room temperature in the dark and analyzed spectrophotometrically with a Shimadzu (Kyoto, Japan) dual-wavelength TLC scanner (CS-930) and data recorder (DR-2). B_{12} was monitored by measurement of absorbance at 527 nm. The amounts of the degradation products formed from OH-B_{12} were expressed as percentage against total B_{12} peak area detected in a lane of the TLC plate.

Isolation of B_{12} Degradation Product. When the purified OH-B_{12} was treated by microwave heating and analyzed by silica gel 60 TLC, two B_{12} degradation products with R_f of 0.16 (18.2 ± 0.6% of total B_{12}) and R_f of 0.27 (4.2 ± 0.3%) were found. The B_{12} degradation product with R_f of 0.16 was isolated as follows. The purified OH-B_{12} solution was treated by microwave heating for 6 min in the dark. The treated solution was evaporated to dryness and dissolved in a small amount of distilled water. The concentrated solution was put on the silica gel 60 TLC plates and developed with 1-butanol/2-propanol/water (10:7:10) as a solvent in the dark. The plates were dried at a room temperature, and the red spot with R_f of 0.16 was collected in the dark. The collected TLC silica gels were dissolved in an appropriate amount of the solvent, and the B_{12} degradation product was extracted from the silica gels three times. The combined extracts were evaporated to dryness and dissolved in a small amount of distilled water. The compound obtained was further purified by the silica gel 60 TLC under the same conditions.

The B_{12} degradation product was further purified by HPLC using a Shimadzu HPLC apparatus (LC-6A pump, SPD-6A spectrophotometer, C-R6A Chromatopac). The sample was put on a reversed-phase HPLC column (Wakosil-II 5C18RS, Ø4.6 × 150 mm; particle size 5 μm) equilibrated with 5% (v/v) methanol solution containing 1% (v/v) acetic acid at 35 °C. The flow rate was 1 mL/min. The B_{12} degradation product was eluted at 40 mL of a linear gradient (5−99%, v/v) of methanol in the same solution, monitored by measuring absorbance at 275 nm, and collected at 1.0 mL with a Bio-Rad (Hercules, CA) fraction collector (Model 2110). The B_{12} degradation product was separated in two peaks with retention times of 15.0 (identical retention time to authentic OH-B_{12}) and 19.0 min. The peak with retention time of 19.0 min was pooled, evaporated to dryness, and dissolved in a small amount of distilled water. The purified compound was assayed by using the same HPLC system with OH-B_{12} as a standard.

The purified B_{12} degradation product gave a single red spot with R_f values of 0.01, 0.14, 0.09, 0.04, 0.14, and 0.60 on analytical silica gel 60 TLC with various solvent systems [1-butanol/2-propanol/water (10:7:10); 2-butanol/acetic acid (99:1); 2-butanol/acetic acid/water (127:1:50); 2-butanol/28% NH_4OH/water (50:7:18); 2-propanol/28% NH_4OH/water (7:1:2), respectively].

Biological Activity of the Purified B_{12} Degradation Product in *Euglena gracilis* Z. *E. gracilis* Z was normally cultured for 16 days at 25 °C with illumination (50 μE m^{-2} s^{-1}) in a propionate-supplemented (6.6 g/L medium) Cramer−Myers medium (CN-B_{12}, 10 μg/L medium) (Watanabe et al., 1996). Authentic OH-B_{12} (10 μg/L) or the purified B_{12} degradation product (10 μg/L) with R_f of 0.16 or both were added aseptically to a culture (150 mL) of *E. gracilis* Z in the Cramer−Myers medium without CN-B_{12}. *Euglena* culture (3.0 mL) was sampled every 3 days, and cell growth was estimated by measuring absorbance at 660 nm with a Shimadzu UV-1600 spectrophotometer.

Intravenous Administration of the Purified B_{12} Degradation Product in Rats. Eight male Wistar rats (5 weeks old) were obtained from Kiwa Laboratory Animals Co. Ltd. (Wakayama, Japan). B_{12}-deprived diet contained 400 g (per kg of diet) of soybean protein (Fuji Oil Ltd., Osaka, Japan), 438 g of glucose anhydrous (Nacalai Tesque, Ltd., Kyoto, Japan), 100 g of soybean oil (Nacalai), 50 g of mineral mixture, 5 g of *dl*-methionine (Nacalai), 5 g of B_{12}-free vitamin mixture, and 2 g of choline chloride (Nacalai) as described previously (Watanabe et al., 1991). The rats were fed the B_{12}-deprived diet a week before experiments. The 6-week-old rats were housed in individual metabolic cages at 24 °C in a room with a 12 h light/dark cycle. They had free access to the B_{12} deprived diet and tap water during experiments. The 6-week-old rats (188.4 ± 12.5 g) were injected intravenously with 1.0 mL (1 μg) of the B_{12} degradation product−saline (1 μg/mL) every 24 h for 7 days. Rats injected with only 1.0 mL of saline under the same conditions were used as controls.

The urine of the 7-day control and B_{12} degradation product treated rats in individual metabolic cages was sampled for 24 h. Livers and blood were obtained from the rats killed by decapitation under diethyl ether anesthesia. Livers were washed with saline and weighed. A portion (wet weight, 1 g) of the livers was immediately homogenized (using a glass homogenizer with a Teflon pestle) in ~4 volumes of 10 mmol/L acetate buffer, pH 4.8. Extraction and assay of total B_{12} from the liver homogenate and blood was done as described above.

Urinary methylmalonic acid was assayed by using HPLC as described previously (Toyoshima et al., 1994).

Statistics. Statistical analysis was performed using GB-STAT 5.4 (Dynamic Microsystems, Inc., Silver Spring, MD). One-way repeated-measures ANOVA was used, with post-hoc two-tailed Dunnett's test. In the case of body weight gains of the 7-day control and B_{12} degradation product-treated rats, a nonparametric test (Man−Whitney U-test) was used. Differences were considered significant if $P < 0.01$. Values in the text are mean ± SD.

RESULTS AND DISCUSSION

To determine how much loss of biologically active B_{12} occurred in foods during microwave heating, raw beef and pork and pasteurized cow's milk were treated for 6 min with a microwave oven. The total B_{12} was extracted and assayed with the chemiluminescent method using hog intrinsic factor, a mammalian B_{12} binding protein (Table 1). The control (without the microwave treatment) samples of beef, pork, and milk contained 2.56 ± 0.03, 0.64 ± 0.02, and 0.27 ± 0.01 μg of B_{12}/100 g of wet weight, respectively; these values are identical to previously published values (Schneider, 1987). The concen-

Table 1. Effect of Microwave Heating on Vitamin B_{12} Concentration in Some Foods

	B_{12} concn[a] (μg/100 g of wet wt)		
	beef	pork	milk
control	2.56 ± 0.03	0.64 ± 0.02	0.27 ± 0.01
microwave	2.13 ± 0.13*	0.55 ± 0.01*	0.14 ± 0.02*

[a] Beef and pork homogenates and cow's milk were treated with or without (control) microwave heating for 6 min. All values represent means ± SD ($n = 5$). *Significantly different from control (without the microwave heating), $P < 0.01$.

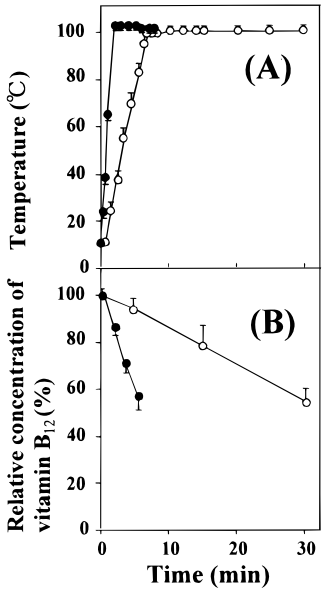

Figure 1. Effect of treatment times on concentration of cow's milk B_{12} by microwave heating. Cow's milk was treated by boiling at 100 °C (control) or by microwave heating. B_{12} was extracted from both treated milk samples and assayed as described in the text. Data represent mean ± SD ($n = 4$). (A) Temperature: control (○); microwave treatment (●). (B) Relative B_{12} concentration: control (○); microwave treatment (●). The relative B_{12} concentration was expressed as percentage against the B_{12} concentration of milk without the boiling and microwave heating.

tration of B_{12} in these foods was reduced significantly by the microwave heating. These results suggest that an appreciable loss (~30−40%) of B_{12} occurs in foods during the 6 min microwave heating, which is a lengthy heating period for reheating of foods but a likely time period for food processing and cooking.

Cow's milk is an excellent source of B_{12}, since 1.0 L of cow's milk (2 μg of B_{12}) is sufficient to satisfy the demands of daily intake for humans (Resources Council, Science and Technology Agency, 1995). Figure 1 shows the effect of microwave heating and boiling times on the loss of B_{12} in cow's milk. The loss of B_{12} increased with an increase in the treatment times for the boiled milk, as well as in the microwave-treated milk. The rate of the B_{12} loss in the microwave-treated milk was greater than that in the boiled milk after the temperature of milk reached ≈100 °C. The amount of B_{12} loss in the 6 min microwave-treated milk sample did not differ from that in the 30 min boiled milk.

To determine whether the loss of B_{12} in the microwave-treated foods is derived from the conversion of B_{12} to some inactive B_{12} degradation products, OH-B_{12}, which predominates in foods, was analyzed with silica gel 60 TLC when treated by microwave heating for 6 min (Figure 2). The treated OH-B_{12} was separated into three red spots [major compound **I** with R_f of 0.03;

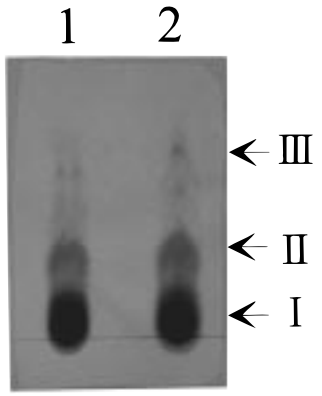

Figure 2. Formation of degradation products from purified hydroxo vitamin B_{12} by microwave heating. Two milliliters of 0.1 mmol/L purified OH-B_{12} was treated in the dark by boiling or microwave heating as described in the text. (Lane 1) OH-B_{12} solution with the 30 min boiling treatment. (Lane 2) OH-B_{12} solution with the 6 min microwave treatment. The data are representative of TLC patterns from five independent experiments.

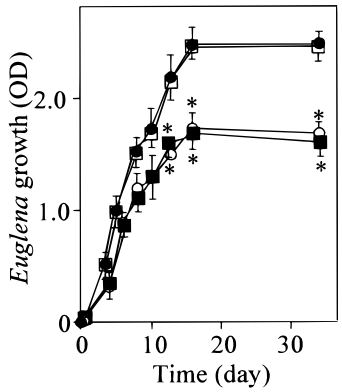

Figure 3. Effect of the B_{12} degradation product (compound **II**) formed by microwave heating on growth of *E. gracilis* Z. Authentic OH-B_{12} or the isolated compound **II** (10 μg/L) or both were added aseptically to a *Euglena* culture. *E. gracilis* Z was cultured as described in the text. The figure plots *Euglena* cell growth in the culture supplemented with (●) or without (○) OH-B_{12} and with compound **II** (■) and OH-B_{12} plus compound **II** (□). Data represent mean ± SD ($n = 3$). *Significantly different from control (OH-B_{12} supplemented *Euglena* cells), $P < 0.01$.

Table 2. Binding of Compound II to Hog Intrinsic Factor, a Mammalian Vitamin B_{12} Binding Protein

	B_{12} concn[a] (pg/mL)
OH-B_{12}	786 ± 31.8
compound **II**	56 ± 6.2*

[a] Each solution (800 pg/mL) of authentic OH-B_{12} and purified compound **II** was assayed by the chemiluminescent vitamin B_{12} assay system using hog intrinsic factor. All values represent means ± SD ($n = 3$). *Significantly different from authentic OH-B_{12}, $P < 0.01$.

identical R_f of intact OH-B_{12}, and minor compound **II** (18.2 ± 0.6%) with R_f of 0.16 and compound **III** (4.2 ± 0.3%) with R_f of 0.27]. These results strongly suggest that the loss of B_{12} caused by microwave heating in foods is due to the conversion of B_{12} to B_{12} degradation products.

To determine the biological activity of the purified compound **II** in a *E. gracilis* Z (a B_{12} requiring microorganism) culture, which is used for the bioassay for B_{12} in biological samples (Ross, 1952), authentic OH-B_{12} or the purified compound **II** or both were added aseptically

Table 3. Effects of Intravenous Administration of Compound II on Body Weight Gain, Urinary Methylmalonic Acid Excretion, and Plasma and Hepatic Vitamin B_{12} Concentrations in Rats[a]

	body wt gain (g/week)	liver wt (g)	urinary methylmalonic acid excretion (μmol/day)	vitamin B_{12} concn: plasma (pg/mL)	vitamin B_{12} concn: liver (ng/total liver)
control	28.33 ± 0.58	7.99 ± 0.68	ND	396.3 ± 74.5	700.8 ± 96.6
compound **II**	16.93 ± 5.96	7.12 ± 0.65	0.14 ± 0.21	406.2 ± 80.9	759.0 ± 140.8

[a] All values represent means ± SD (n = 4). ND represents not detectable.

to a 0 time B_{12} deficient culture (Figure 3). The growth of *E. gracilis* Z was increased significantly by the addition of OH-B_{12} (control) or OH-B_{12} plus compound **II**, while in the 32-day cultures supplemented only with compound **II**, *Euglena* cell growth was inhibited up to ~70% of the control culture; an identical growth curve was also obtained in the B_{12} deficient culture. Addition of compound **II** did not cause further growth inhibition of *Euglena* cells. These results indicate that compound **II** is inactive in *E. gracilis* Z but does not act as a B_{12} antagonist. *Euglena* cells have been unable to utilize B_{12} analogues lacking the α-ligand (the cobalt-coordinated nucleotide) for cell growth (Watanabe et al., 1992), and *Euglena* B_{12} binding protein has shown an absolute requirement for the α-ligand and intact (*b*)-propionamide side chain of B_{12} molecule in B_{12} binding (Watanabe et al., 1993).

Intrinsic factor, which is involved in the intestinal absorption of B_{12} (Seetharam and Alpers, 1982), recognizes the structure of the B_{12} molecule more strictly than the *Euglena* B_{12} binding proteins (Watanabe et al., 1993). When authentic OH-B_{12} and compound **II** (an identical amount, 800 pg/mL) were assayed with the chemiluminescent B_{12} assay system using hog intrinsic factor (Table 2), the amount of compound **II** was estimated to be ~7.1% of that of OH-B_{12}. The results indicate that the intrinsic factor cannot bind compound **II**, which would not be absorbed in mammalian intestine by the intrinsic factor-mediated system.

To determine whether compound **II** has an acute toxicity in mammals, effects of intravenous administration of the compound on body weight gain, plasma and hepatic B_{12} concentrations, and urinary methylmalonic acid excretion as an index of B_{12} deficiency were studied in rats (Table 3). The 6-week-old rats were injected with 1 μg of compound **II** every 24 h for 7 days. The administration of compound **II** did not affect liver weights or plasma and hepatic B_{12} concentrations in the rats. The urinary methylmalonic acid excretion (0.14 ± 0.21 μmol/day) became detectable in the compound **II** treated rats, but it was not significantly different from the control. Severely B_{12} deficient rats have been reported to excrete 214.3 ± 115.2 μmol of methylmalonic acid/day in urine (Watanabe et al., 1991). The body weight gain of the 7-day compound **II** treated rats was reduced relative to the control, but the differences between them are not significant. These results indicate that compound **II** neither has toxicity nor acts as a B_{12} antagonist in mammals within the experiment time course examined.

In our preliminary experiments, the ^{1}H NMR spectrum of compound **II** was measured on a JEOL GX-270 at 270 MHz in D_2O (δ = 4.71 ppm; internal reference). The ^{1}H NMR spectrum of authentic CN-B_{12} showed the presence of three aromatic protons assigned to the dimethylbenzimidazolyl moiety: δ_H 6.36 (1H, s), 6.94 (1H, s), and 7.13 (1H, s) ppm. The spectra of compound **II** showed the lack of these signals in the low-field region. The signals due to ribose anomeric proton at δ_H 6.22 (1H, d, J = 3.1 Hz) and H-10 proton δ_H 5.95 (1H, s) disappeared, and the signals of the sugar moiety and methyl groups were also changed. These findings show that compound **II** may be due to the elimination of the base portion and the skeletal alterations by microwave heating. The NMR studies support the observation that compound **II** is inactive in *Euglena* cells and mammals. Structural information on compound **III** with R_f of 0.27 is not available because a purified sample was not obtained for NMR study.

These results indicate that biologically inactive B_{12} degradation products are formed in foods by microwave heating. Bennink and Ono (1982) have reported that there is a 27−33% loss of B_{12} during cooking of foods. They have assayed the B_{12} content of raw and cooked beefs by a radioisotope dilution method with hog intrinsic factor. Our result that the intrinsic factor could not bind compound **II** indicates that the compound is unable to be assayed according to this method. The loss of B_{12} during cooking as reported by Bennink and Ono would be due to the conversion of B_{12} to biologically inactive B_{12} degradation products.

ABBREVIATIONS USED

AdoB$_{12}$, 5′-deoxyadenosyl vitamin B_{12}; B_{12}, vitamin B_{12}; CN-B_{12}, cyano vitamin B_{12}; HPLC, high-pressure liquid chromatography; MeB$_{12}$, methyl vitamin B_{12}; OH-B_{12}, hydroxo vitamin B_{12}; TLC, thin layer chromatography.

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LITERATURE CITED

Bennink, M. R.; Ono, K. Vitamin B12, E and D content of raw and cooked beef. *J. Food Sci.* **1982**, *47*, 1786−1792.

Cross, G. A.; Fung, D. Y. C. The effect of microwaves on nutrient value of food. *Crit. Rev. Food Sci. Nutr.* **1982**, *16*, 355−381.

Frenkel, E. P.; Prough, R.; Kitchens, R. L. Measurement of tissue vitamin B_{12} by radioisotopic competitive inhibition assay and quantitation of tissue cobalamin fractions. *Methods Enzymol.* **1980**, *67*, 31−40.

Hoffman, C. J.; Zabik, M. E. Effects of microwave cooking/reheating on nutrients and food systems: A review of recent studies. *J. Am. Diet Assoc.* **1985**, *85*, 929−933.

Resources Council, Science and Technology Agency. In *Standard Tables of Food Composition in Japan—Vitamin K, B_6 and B_{12}*; Resources Council, Science and Technology Agency: Tokyo, 1995; pp 41−114.

Ross, G. I. M. Vitamin B_{12} assay in body fluids using *Euglena gracilis*. *J. Clin. Pathol*. **1952**, *5*, 250−256.

Schneider, Z. Cobalamin in food and feeding stuff. In *Comprehensive B_{12}*; Schneider, Z., Stroiñski, A., Eds.; de Gruyter: Berlin, 1987; pp 194−198.

Schneider, Z.; Stroiñski, A. Historical outline. In *Comprehensive B_{12}*; Schneider, Z., Stroiñski, A., Eds.; de Gruyter: Berlin, 1987; pp 1−6.

Seetharam, B.; Alpers, D. H. Absorption and transport of cobalamin (vitamin B_{12}). *Annu. Rev. Nutr*. **1982**, *2*, 343−369.

Smith, E. L.; Martin, J. L.; Gregory, R. L.; Shaw, W. H. L. Standardisation of hydroxocobalamin. *J. Soc. Anal. Chem*. **1962**, *87*, 183−186.

Toyoshima, S.; Saido, H.; Watanabe, F.; Miyatake, K.; Nakano, Y. Assay for urinary methylmalonic acid by high-pressure liquid chromatography. *Biosci., Biotechnol., Biochem*. **1994**, *58*, 1882−1883.

Watanabe, F.; Nakano, Y.; Tachikake, N.; Saido, H.; Tamura, Y.; Yamanaka, H. Vitamin B12 deficiency increases the specific activities of rat liver NADH- and NADPH-linked aquacobalamin reductase isozymes involved in coenzyme synthesis. *J. Nutr*. **1991**, *121*, 1948−1954.

Watanabe, F.; Nakano, Y.; Stupperich, E. Different corrinoid specificities for cell growth and the cobalamin uptake system in *Euglena gracilis* z. *J. Gen. Microbiol*. **1992**, *138*, 1807−1813.

Watanabe, F.; Tamura, Y.; Stupperich, E.; Nakano, Y. Uptake of cobalamin by *Euglena* mitochondria. *J. Biochem*. **1993**, *114*, 793−799.

Watanabe, F.; Abe, K.; Tamura, Y.; Nakano, Y. Adenosylcobalamin-dependent methylmalonyl-CoA mutase isozymes in the photosynthetic protozoon *Euglena gracilis* Z. *Microbiology* **1996**, *142*, 2631−2634.

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