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Vitamin B_{12} Is the Active Corrinoid Produced in Cultivated White Button Mushrooms (*Agaricus bisporus*)

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Analysis of vitamin B_{12} in freshly harvested white button mushrooms (*Agaricus bisporus*) from five farms was performed by affinity chromatography and HPLC-ESI-MS techniques. The vitamin B_{12} concentrations obtained varied from farm to farm, with higher concentrations of vitamin B_{12} detected in outer peel than in cap, stalk, or flesh, suggesting that the vitamin B_{12} is probably bacteria-derived. High concentrations of vitamin B_{12} were also detected in the flush mushrooms including cups and flats. HPLC and mass spectrometry showed vitamin B_{12} retention time and mass spectra identical to those of the standard vitamin B_{12} and those of food products including beef, beef liver, salmon, egg, and milk but not of the pseudovitamin B_{12}, an inactive corrinoid in humans. The results suggest that the consumer may benefit from the consumption of mushroom to increase intake of this vitamin in the diet.

KEYWORDS: ***Agaricus bisporus*** **mushroom; vitamin B_{12}; pseudovitamin B_{12}**

INTRODUCTION

The term "vitamin B_{12}" is usually restricted to cyanocobalamin, which is a water-soluble vitamin and essential nutrient linked to human growth and cell development (*1*, *2*). Vitamin B_{12} is a biologically active corrinoid, a group of cobalt-containing compounds with a macrocyclic pyrrole ring (*3*). Vitamin B_{12} deficiency can occur in specific populations, especially the elderly (*4*, *5*) and vegans and their infants (*6*−*8*), who are at especially high risk. Cyanocobalamin, which is used in most supplements, is readily converted to the coenzyme forms of cobalamin (methylcobalamin and 5′-deoxyadenosylcobalamin) in the human body (*9*).

Vitamin B_{12} is synthesized by certain bacteria concentrated in the food chain, which provides the major dietary sources of vitamin B_{12} in meat and dairy products (*10*). Typical levels of vitamin B_{12} in foods range from the low nanograms per gram for cheese and fish to hundreds of nanogram per gram for liver and fortified cereals (*11*). Plants cannot synthesize vitamin B_{12} except for some edible algae,such as blue-green algae (cyanobacteria), in which large amounts of vitamin B_{12} have been found (*12*). However, vitamin B_{12} in algae has been reported to be biologically inactive, a form generally regarded as unavailable in the human diet (*13*). Indeed, it has been reported that 83% of vitamin B_{12} in edible cyanobacteria used as a vitamin B_{12} commercial supplement was pseudovitamin B_{12} (*14*), a poorly absorbed inactive corrinoid compound in the mammalian intestine due to its low binding affinity to intrinsic factor (IF) (*15*). In this regard, bacterial vitamin B_{12} supplementation may be ineffective in preventing vitamin B_{12} deficiency, especially among vegans and elderly subjects.

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Vitamin B_{12} production in mushrooms independent of bacteria is controversial. It has been suggested that the source of vitamin B_{12} in mushrooms is the microorganisms living on the surface of the mushrooms or the compost containing horse manure−wheat straw used for cultivating mushrooms, which then take up vitamin B_{12} from the compost bed (*16*). Low to undetectable amounts of vitamin B_{12} in white button mushrooms have been reported (*16*, *17*). However, treatment of compost beds with cobalt carbonate resulted in an enhanced production of vitamin B_{12} in a dose-dependent manner, with the highest concentration present in peel (*17*). The enhancing effect of cobalt on vitamin B_{12} synthesis was also observed in cyanobacteria (*18*), suggesting that vitamin B_{12} production in mushrooms is associated with the presence of surface bacteria and cobalt in the compost. However, widespread application of cobalt to bolster production of vitamin B_{12} in farmed mushrooms is unlikely to meet consumer acceptance or to be considered practical in terms of farm practices and environmental concern. Even with cobalt application, the levels of vitamin B_{12} produced in mushrooms from different farms are likely to be variable due to cultivation and harvesting practices, diverse vitamin B_{12}-producing bacteria species, weather, and storage conditions. Moreover, it is not clear whether the vitamin B_{12} produced is in fact the active bioavailable form as distinguished from the biologically inactive pseudovitamin B_{12} (*14*, *19*).

Here we report, using a combination of antibody affinity chromatography and HPLC-ESI-MS analysis, that high concentrations of vitamin B_{12} were obtained from farm-cultivated *Agaricus bisporus* mushrooms and that the vitamin B_{12} is the active corrinoid and not the pseudovitamin B_{12}, which is inactive in humans.

MATERIALS AND METHODS

Samples. The mushroom (*A. bisporus*) samples, casing, and compost were supplied by five different Australian mushrooms growers.

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The growers supplied button, cup, and flat mushrooms of three flushes. As soon as the samples reached the laboratory, they were separated into cap, stalk, peel, and flesh of button, cup, and flat mushrooms. The separated mushroom samples were then pooled and sliced into small pieces before freeze-drying. The freeze-dried mushrooms were ground into a powder, and the samples were frozen at −20 °C until analysis.

Chemicals. Vitamin B_{12} (cyanocobalamin) was purchased from Sigma-Aldrich Chemicals (Australia). Pseudovitamin B_{12} was kindly provided by Dr. F. Watanabe, Kochi Women's University, Japan. Methanol (HPLC grade), sodium acetate anhydrous (AR grade), and sodium hydroxide were purchased from Lomb Scientific Pty Ltd. (Australia). Sodium cyanide was purchased from May and Baker Ltd., London, U.K. Deionized water via an ultrawater system from Millipore (Bedford, MA) was used throughout the experiment.

Extraction of Vitamin B_{12} from Mushroom Samples. Vitamin B_{12} was extracted as previously described by Watanabe et al. (*20*) with minor modification. Briefly, about 5.0 g of each homogenized mushroom sample was placed into a 250 mL conical flask, and 80 mL of freshly prepared 0.5 M acetate buffer (pH 4.8) and 2 mL of NaCN (1%) were added. Total vitamin B_{12} was extracted from the homogenate by boiling at 98 °C for 35 min under a nitrogen stream in the dark. The boiled homogenates were centrifuged at 9645*g* for 20 min at 4 °C. The recovery was determined using the addition of vitamin B_{12} (10, 50, 100, and 200 ng/mL) to mushroom samples. The same four concentrations were used to determine the recovery performance for all sample analyses. The supernatant was filtered through filter paper before a purification step using an immunoaffinity column.

Purification of Vitamin B_{12} from Mushroom Extract Using Immunoaffinity Column. The appropriate volume of sample was passed through the immunoaffinity column (Easi-Extract Vitamin B_{12}, 3 mL format, R-Biopharm Rhone Ltd., Glasgow, Scotland) at a flow rate of 2−3 mL/min. Finally, the immunoaffinity column was washed with 10 mL of distilled water. The column was then dried by the passage of 10 mL of air with a syringe. The vitamin B_{12} was then eluted with 3 mL of methanol repeated three times to ensure maximal recovery of vitamin B_{12}. The eluates were pooled, concentrated by rotary evaporator at 50 °C under reduced pressure, and then reconstituted in 250 μL of solvent (0.1% acetic acid in water) for LC-ESI-MS analysis.

Preparation of Standards for Calibration. A standard vitamin B_{12} stock solution of 1.0 μg/mL was prepared in 0.1% (v/v) acetic acid in water. For the HPLC-ESI-MS analysis, the stock solution was then diluted to 1 mL with 0.1% acetic acid in water to give calibration standard concentrations ranging from 10 to 250 ng/mL of vitamin B_{12}.

Table 1. Recovery of Vitamin B_{12} from the Mushroom Extracts

added amount (ng of vitamin B_{12}) per sample	mean recovery (%) ± SD[a]
10	93.90 ± 3.30
50	89.06 ± 1.75
100	77.05 ± 2.93
200	84.71 ± 4.05

[a] n = 3.

Table 2. Vitamin B_{12} Content in Button Mushrooms (*A. bisporus*)[a]

	ng/100 g on dry wt				
sample	farm 1	farm 2	farm 3	farm 4	farm 5
first flush					
cap	21.46 ± 1.19	9.67 ± 3.87	130.18 ± 0.20	117.45 ± 6.94	85.14 ± 12.06
flesh	7.24 ± 3.73	35.34 ± 2.42	115.49 ± 0.09	48.79 ± 2.15	17.91 ± 2.98
stalk	87.42 ± 2.00	22.85 ± 0.12	57.63 ± 1.51	147.48 ± 6.10	42.11 ± 4.00
peel	127.89 ± 1.01	263.12 ± 7.89	576.39 ± 32.63	699.86 ± 5.13	215.23 ± 9.45
second flush					
cap	10.61 ± 0.17	48.88 ± 0.94	65.02 ± 0.81	336.38 ± 3.81	217.83 ± 15.86
flesh	4.45 ± 0.03	48.46 ± 2.15	29.95 ± 0.18	69.60 ± 2.39	16.80 ± 8.34
stalk	11.31 ± 0.23	88.01 ± 2.07	54.58 ± 2.33	317.12 ± 0.98	233.44 ± 19.46
peel	35.91 ± 1.40	224.14 ± 2.79	199.63 ± 1.06	737.26 ± 3.92	1005.13 ± 14.65
third flush					
cap	48.12 ± 1.25	232.11 ± 2.47	88.87 ± 0.94	na	80.35 ± 0.97
flesh	5.77 ± 5.96	94.37 ± 7.18	27.46 ± 0.35	na	64.86 ± 3.99
stalk	44.22 ± 4.02	183.32 ± 0.10	86.93 ± 0.30	na	121.74 ± 4.55
peel	156.38 ± 26.51	377.30 ± 4.23	103.24 ± 1.84	na	309.91 ± 6.17

[a] Farm 1, Oakville mushroom (NSW), Australia; farm 2, ELF mushroom (NSW), Australia; farm 3, Adelaide mushroom (SA), Australia; farm 4, Mushroom exchange (VIC), Australia; farm 5, Mushroom exchange (QID), Australia. na, not analyzed (samples not supplied by the mushroom farm).

Table 3. Vitamin B_{12} Content in Cup Mushrooms (*A. bisporus*)

	ng/100 g on dry wt				
sample	farm 1	farm 2	farm 3	farm 4	farm 5
first flush					
cap	18.75 ± 0.32	45.54 ± 0.58	23.54 ± 0.02	77.39 ± 1.01	31.24 ± 0.22
flesh	14.91 ± 0.41	22.41 ± 0.63	6.30 ± 0.38	19.12 ± 0.01	5.26 ± 0.65
stalk	66.82 ± 9.14	43.89 ± 0.25	36.65 ± 0.73	72.61 ± 0.39	21.09 ± 0.84
peel	1155.91 ± 0.88	88.06 ± 2.68	50.06 ± 0.04	334.63 ± 14.45	227.82 ± 5.57
second flush					
cap	75.18 ± 1.2	8.45 ± 4.11	24.92 ± 0.37	294.19 ± 5.15	567.99 ± 1.45
flesh	12.65 ± 0.59	7.03 ± 0.11	18.71 ± 1.26	66.46 ± 2.76	82.96 ± 6.75
stalk	64.44 ± 0.99	6.77 ± 0.16	20.21 ± 2.28	131.71 ± 0.10	254.92 ± 8.34
peel	75.31 ± 0.64	20.13 ± 1.85	209.70 ± 3.72	361.70 ± 0.40	1014.65 ± 5.63
third flush					
cap	60.97 ± 1.30	15.84 ± 3.22	52.88 ± 2.01	34.80 ± 0.82	54.02 ± 2.25
flesh	9.72 ± 2.24	3.20 ± 0.09	61.23 ± 4.18	15.40 ± 0.54	24.74 ± 3.29
stalk	20.23 ± 0.05	9.42 ± 0.07	63.08 ± 1.46	95.61 ± 2.70	61.84 ± 2.26
peel	104.46 ± 5.19	27.30 ± 0.78	80.49 ± 1.37	455.38 ± 0.83	278.29 ± 0.55

High-Performance Liquid Chromatography with Electrospray Ionization Mass Spectrometry (HPLC-ESI-MS) Analysis. The identification and quantification of vitamin B_{12} were carried out by HPLC-ESI-MS. The HPLC-ESI-MS analysis was performed on a Varian ProStar model 210 gradient solvent delivery module and a Varian ProStar model 430 autosampler with a Varian 1200 L tandem MS/MS detector. Positive electrospray ionization (ESI) was employed. The autosampler was fitted with a 100 μL loop. Separation was achieved on a Polaris 5 μm C18 amide column (100 × 2.00 mm), with a flow rate of 0.18 mL/min. A linear gradient eluent of methanol (A) and 0.1% acetic acid in water (B) was used. The gradient elution was programmed as follows: 0−3 min, 100% B; 3−11 min, 100−75% B; 11−19 min, 75 −65% B; 19−20 min, 65 −90% B; 20−26 min, 90−100% B; 26−30 min, 100% B. Different HPLC-ESI-MS conditions were determined by the injection of the pure standard vitamin B_{12} into the MS detector in order to determine the settings necessary to obtain the optimum amount of the parent and daughter ion(s). The ESI-MS was operated in positive ion mode. The API housing and drying gas temperatures were maintained at 49.5 and 350 °C. The electrospray capillary and detector were set at 100 and 1850 V, respectively. Argon was used at 2.0 mTorr as the collision gas. For the HPLC-ESI-MS analysis, identification was achieved by comparison of retention times of sample and standard. The identities of vitamin B_{12} (m/z 1356.1 representing $[M+H]^+$) and pseudovitamin B_{12} (m/z 1345 $[M+H]^+$) were confirmed by comparison of the observed molecular ions. Quantifier ions used for vitamin B_{12} were m/z 930.8 and 1356.1, respectively.

RESULTS AND DISCUSSION

HPLC-ESI-MS Analysis of Vitamin B_{12}. The water-soluble vitamin B_{12} was analyzed in ESI positive mode. Two ions at m/z 930.8 and 1356.1 were selected from the ESI-MS spectrum of standard vitamin B_{12} and used for qualitative and quantification of vitamin B_{12} in samples. Vitamin B_{12} was identified in samples by comparing the HPLC retention time and mass spectra with those of standard vitamin B_{12}. The m/z 1356.1 corresponds to $[M+H]^+$, and 930.8 represents $[M+H - base - sugar - PO_3 - CN - Co]^+$ (*21*). The quantification of vitamin B_{12} was achieved by extrapolation from a standard curve. The 10 point calibration curves using a linear fit had an r^2 value of 0.99. The average recovery of B_{12} was 86.18% for both standard and mushroom samples spiked with known concentrations of vitamin B_{12} with an average RSD of 3.01 (**Table** 1).

Vitamin B_{12} Levels in Farm-Cultivated Mushrooms. The vitamin B_{12} levels in mushroom samples from individual farms were variable (**Tables** 2−4). Higher concentrations of vitamin B_{12} in button mushrooms were found in peel than in cap, stalk, or flesh, irrespective of whether the mushrooms were harvested from first, second, or third flush (**Table** 2). The high concentration of vitamin B_{12} in peel suggests that it was not synthesized within the mushrooms but was either absorbed directly from the compost or synthesized by bacteria on the mushroom surface. The latter is more likely because mushrooms have no root system to take up the vitamin in the compost as is the case with the uptake of vitamins by root plants from the soil containing fertilizers (*22*). In the present study, higher levels of vitamin B_{12} continued to be produced in the second and third flush mushrooms from some farms (2, 4, and 5), whereas in other farms (1 and 3) the amounts of vitamin B_{12} produced were variable or decreased relative to the first-flush yield (**Table** 2). A similar trend was observed in the analysis of vitamin B_{12} in cup and flat mushrooms, in which higher yields of vitamin B_{12} were also found in peel (**Tables** 3 and 4). The lower levels observed in the flats may be related to a dilution effect on vitamin B_{12} through the expansion of the cap. Taken together, the concentrations of vitamin B_{12} using freshly harvested freeze-dried mushrooms are at least 10-fold higher than those previously reported in the analysis of first- and second-flush *A. bisporus* mushrooms, for which higher concentrations of vitamin B_{12} were obtained only from fresh mushrooms cultivated in compost beds fortified with cobalt (*17*). To our knowledge, the compost used in the cultivation of mushrooms in the farms studied had no exposure to cobalt at any stage of production and handling. The compost consisted of cereal crops and straw, which have very little cobalt, although the chicken manure added to the compost could provide a source of cobalt, but this is unlikely because the manure was collected from caged birds. On the other hand, the higher levels of B_{12} detected in our mushrooms may be due to the greater sensitivity of the HPLC-ESI-MS analytical system, which is considered to be more accurate, reliable, and reproducible than the microbiological assay (*17*, *23*).

Table 4. Vitamin B_{12} Content in Flat Mushrooms (*A. bisporus*)

	ng/100 g on dry wt				
sample	farm 1	farm 2	farm 3	farm 4	farm 5
first flush					
cap	12.47 ± 0.44	50.50 ± 1.48	77.41 ± 0.44	na	na
flesh	7.13 ± 0.98	nd[a]	9.27 ± 0.13	na	na
stalk	28.36 ± 0.18	26.24 ± 5.78	14.54 ± 0.74	na	na
peel	175.95 ± 1.04	96.75 ± 0.35	110.89 ± 9.62	na	na
second flush					
cap	138.36 ± 5.33	na	20.94 ± 2.77	na	161.24 ± 0.21
flesh	37.43 ± 1.39	na	11.27 ± 0.42	na	83.89 ± 5.30
stalk	118.28 ± 4.40	na	15.07 ± 0.06	na	465.36 ± 20.23
peel	181.03 ± 14.42	na	100.41 ± 0.58	na	354.01 ± 55.54
third flush					
cap	99.82 ± 8.08	na	17.13 ± 0.02	na	45.25 ± 1.72
flesh	45.79 ± 0.15	na	3.80 ± 0.23	na	25.95 ± 1.39
stalk	62.92 ± 1.53	na	12.46 ± 0.15	na	109.33 ± 9.02
peel	174.18 ± 3.67	na	67.86 ± 2.19	na	247.62 ± 0.51

[a] nd, not detected.

Table 5. Vitamin B_{12} Content in Compost and Casing

	ng/g on dry wt				
sample	farm 1	farm 2	farm 3	farm 4	farm 5
compost					
phase 1	260.88 ± 4.99	604.34 ± 27.58	367.57 ± 0.94	460.45 ± 0.90	201.51 ± 0.06
phase 2	154.26 ± 5.17	612.63 ± 8.55	663.51 ± 6.60	294.78 ± 0.24	156.12 ± 0.37
phase 3	na	370.88 ± 0.14	471.73 ± 5.17	236.92 ± 10.59	182.01 ± 1.25
first flush	181.36 ± 4.55	233.97 ± 9.50	136.14 ± 1.79	265.96 ± 14.94	106.86 ± 0.13
second flush	104.52 ± 3.34	168.71 ± 4.94	119.61 ± 2.96	94.44 ± 0.43	94.49 ± 0.13
third flush	52.96 ± 13.52	143.77 ± 3.83	105.48 ± 0.32	106.58 ± 3.42	83.07 ± 0.35
casing					
first flush	na	14.88 ± 0.10	2.89 ± 0.05	7.64 ± 0.08	6.92 ± 0.31
second flush	na	14.48 ± 0.52	4.80 ± 0.12	5.38 ± 0.09	12.05 ± 0.25
third flush	na	11.39 ± 0.11	6.82 ± 0.09	11.17 ± 0.07	4.98 ± 0.09

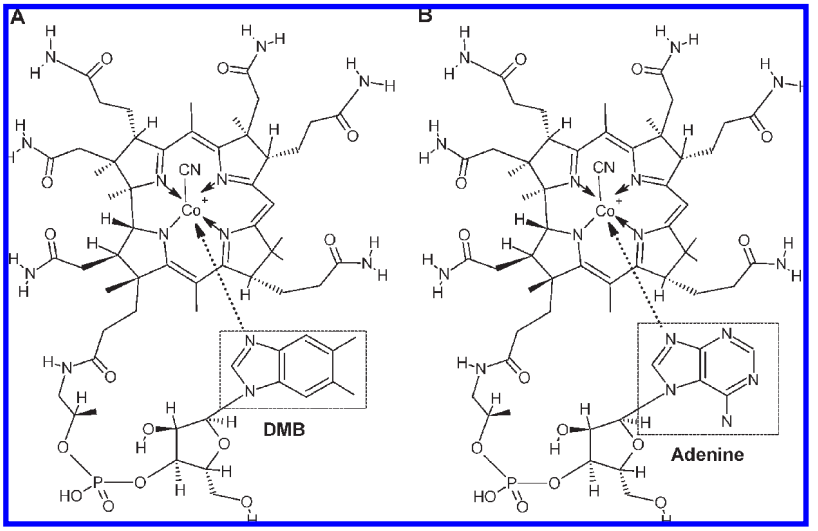

Figure 1. Chemical structures of (**A**) vitamin B_{12} and (**B**) pseudovitamin B_{12} with the lower ligands containing 5,6-dimethylbenzidazole (DMB) and the adenine moieties, respectively.

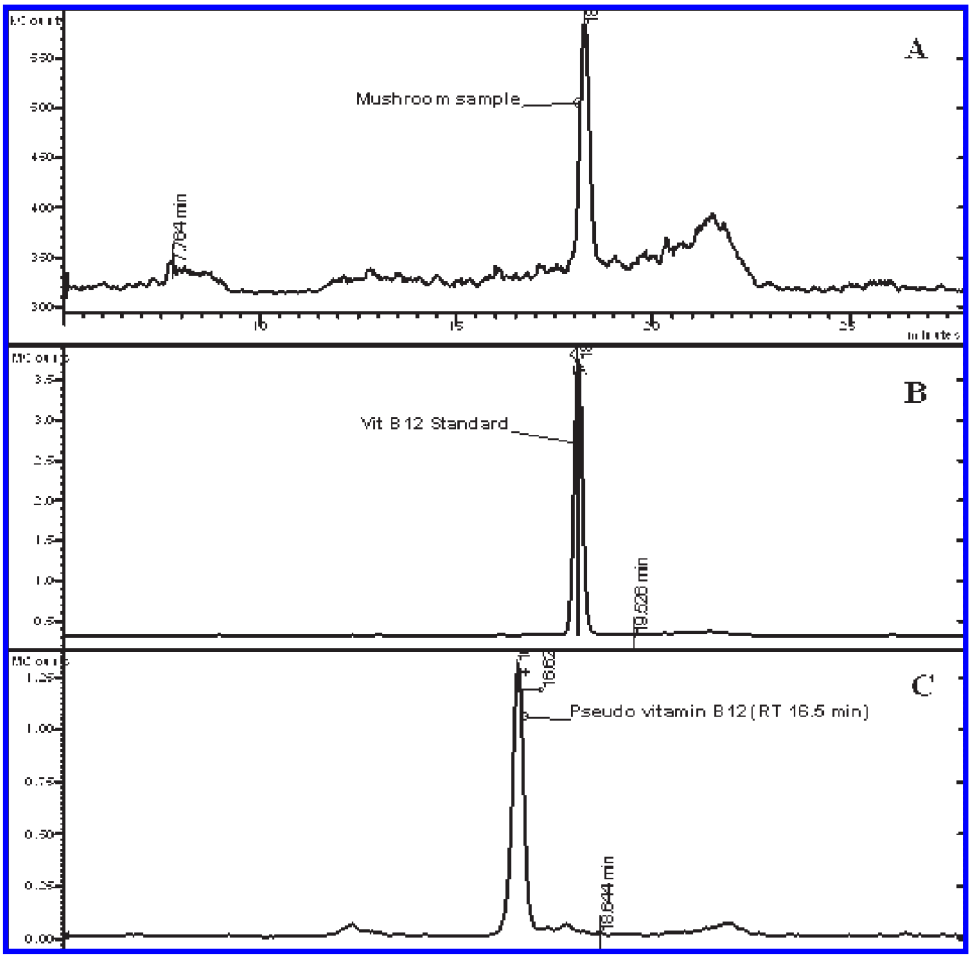

Figure 2. HPLC-ESI/MS chromatograms showing the separation of (**A**) corrinoid purified from mushroom extract, (**B**) cyanocobalamin (vitamin B_{12}) standard, and (**C**) pseudovitamin B_{12} standard. HPLC chromatograms of the two standards display peaks corresponding with retention times of 17.5 and 16.5 min, respectively.

The source of vitamin B_{12} is most likely to be derived from bacteria present in the composts because synthesis of vitamin B_{12} by bacteria has been widely reported (*24*, *25*). However, the variable yields of vitamin B_{12} in mushrooms from individual farms may be attributable to differences in the bacterial flora in composts in terms of species and bacterial growth rates, which may contribute to the variability of vitamin B_{12} content observed. In this study, detection of vitamin B_{12} in compost and casing appeared to provide a clue as to the source of the vitamin B_{12}. As shown in **Table** 5, high levels of vitamin B_{12} were found in compost compared with the levels detected in casing from either first, second, or third flush, thus suggesting that the compost

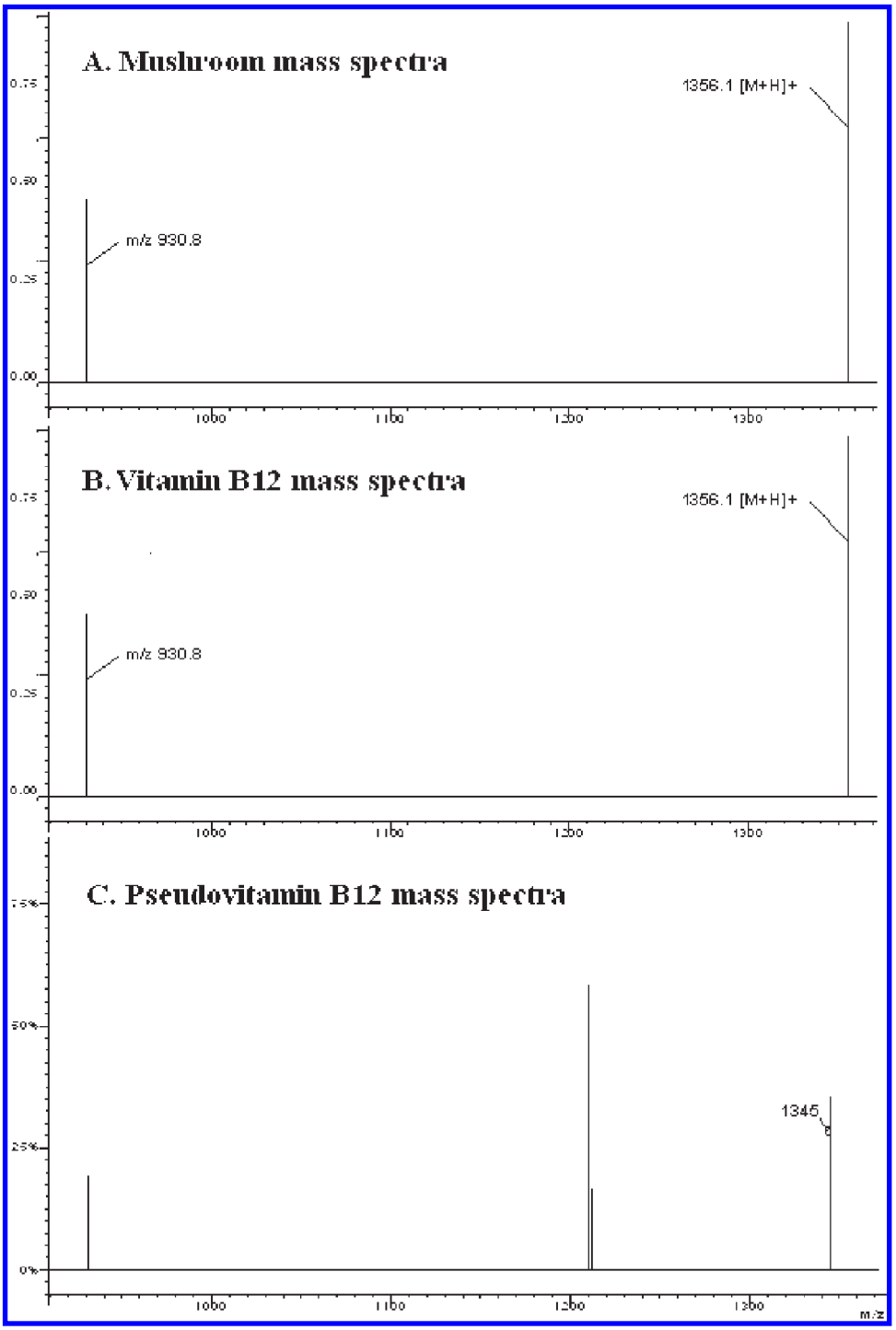

Figure 3. Mass spectra of (**A**) mushroom corrinoid and (**B**) cyanocobalamin (vitamin B_{12}) standard display similar peaks with *m*/*z* values of 930.8 and 1356.1, respectively. Mass spectrum of (**C**) pseudovitamin B12 has a different *m*/*z* value (1345). MS spectral information is acquired in the positive-ion mode.

provides the main source of vitamin B_{12} in mushrooms, most likely in the form produced by bacteria localized on the mushroom surface. However, the vitamin B_{12} was not permanently retained on mushrooms because around 26 and 45% of vitamin B_{12} was removed from peel and cap, respectively, after washing (data not shown). Nevertheless, the amounts retained by the mushrooms after washing may still be beneficial to the consumer in terms of contributing to the vitamin B_{12} dietary requirement.

Characterization of Vitamin B_{12} in *A. bisporus*. Vitamin B_{12} exists in a variety of analogues of which some are generally not available in the diet. Although they share a similar structural architecture that consists of a corrin ring with a cobalt ion chelated at the core (**Figure** 1), differences in the range and nature of functional groups among the analogues have been reported (*10*). For example, the chemical structure of the corrinoid, pseudovitamin B_{12}, is similar to that of the vitamin B_{12} (cyanocobalamin) except that the 5,6-dimethylbenzimidazole (DMB) moiety is substituted by the adenine base (**Figure** 1). Furthermore, unlike the cyanocobalamin analogue, the pseudovitamin B_{12} is unavailable in the active form of B_{12} in the diet (*14*), implying that it serves no role as a vitamin in human metabolism. In the present study, these analogues were used as reference standards with the HPLC-ESI-MS technique to identify the corrinoid purified from mushroom extract by antibody affinity chromatography.

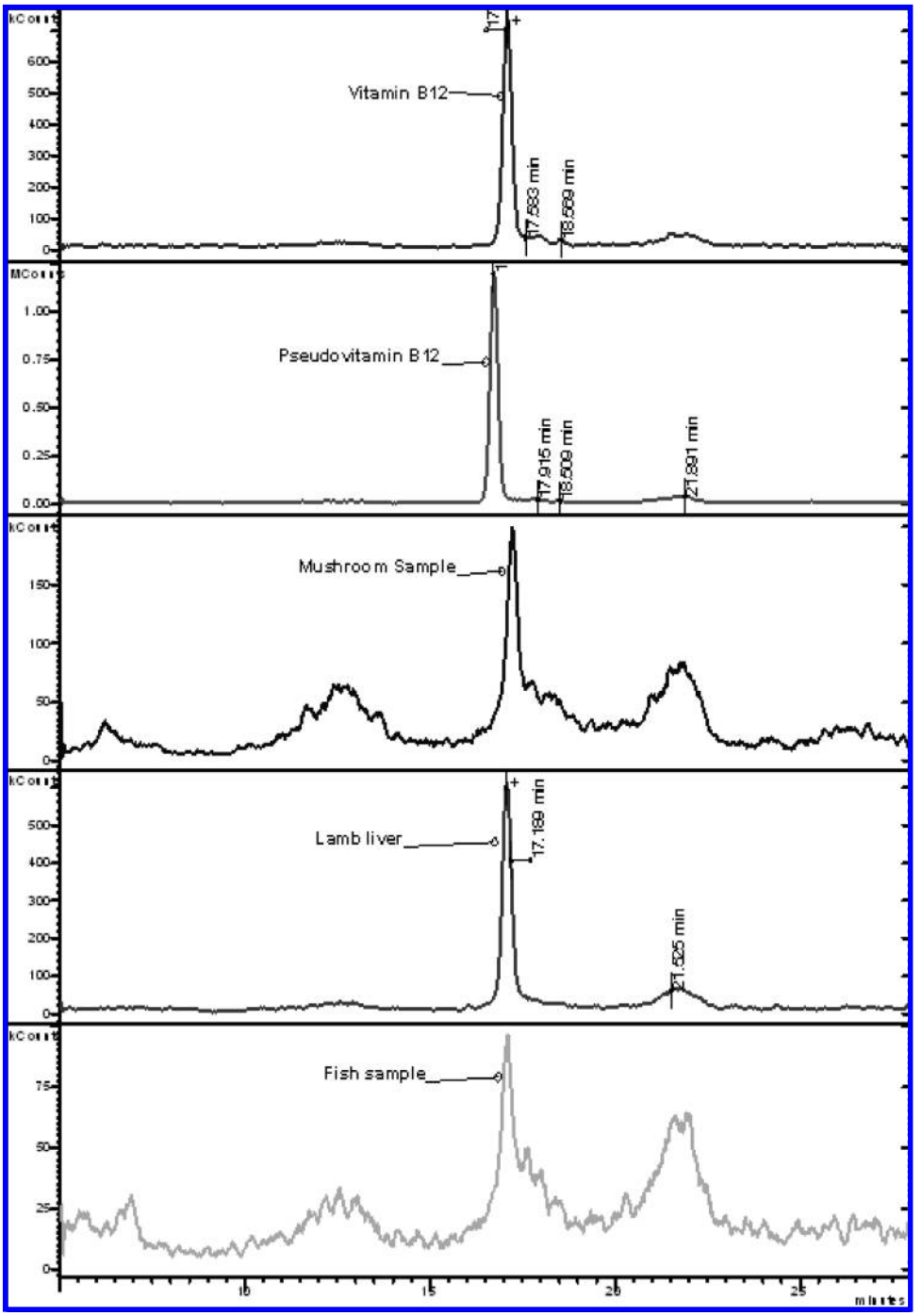

Figure 4. HPLC chromatograms comparing the corrinoid purified from mushroom extract with those extracted from other food sources and the vitamin B_{12} standard in the spiked sample. All of the spectra of the various corrinoids except pseudovitamin B_{12} display similar retention times compared with the cyanocobalamin (vitamin B_{12}) standard.

The separation of the cyanocobalamin (vitamin B_{12}) and the pseudovitamin B_{12} standard is shown in **Figure** 2. Cyanocobalamin standard had a retention time of 17.5 min compared to the retention time of 16.5 min displayed by the pseudovitamin B_{12}. The mass spectral *m*/*z* values of the two standards were 1356.1 and 1345, corresponding to $[M+H]^+$, respectively. This observation is consistent with that reported in the literature (*10*, *14*). The HPLC chromatogram (**Figure** 2) of the corrinoid purified from mushroom extract identified a peak with a retention time of 17.5 min, which is similar to that of the cyanocobalamin standard (17.5 min). The mass spectrometry data (**Figure** 3) recorded for the corrinoid purified from mushroom extract showed that the molecule has *m*/*z* values (930.8 and 1356.1) similar to those of the cyanocobalamin (*m*/*z* 930.8 and 1356.1, respectively) standard (**Figure** 3). Taken together, the HPLC chromatogram and the mass spectrum data confirmed that the corrinoid extracted from white button mushroom is not a pseudovitamin B_{12}, the inactive corrinoid, commonly produced by various microorganisms (*10*, *14*, *24*). Furthermore, the corrinoid purified from mushroom showed an HPLC chromatogram peak similar to those of vitamin B_{12} extracted from the spiked sample and the food sources including lamb liver and salmon fish (**Figure** 4), none of which contained the inactive pseudovitamin B_{12}. On the basis of all the spectral information acquired, the corrinoid present in farm-cultivated

white button mushrooms is the active vitamin B_{12}, although its source as discussed earlier remains to be determined. Although the vitamin B_{12} levels are low by comparison with that required in the human diet at the recommended daily allowance of 2.4 μg per day, the consumer may still benefit from consumption of the mushroom to increase the intake of this important vitamin.

LITERATURE CITED

(1) B-12. In *Biochemistry and Medicine*; Dolphin, D., Ed.; Wiley: New York, 1982; Vol. *2*, pp 1−30.

(2) Gimsing, P.; Nexo, E. The Forms of Cobalamin in Biological Materials. In *The Cobalamins*; Hall, C. A., Ed.; Churchille Livingstone: New York, 1983, pp 1−30.

(3) Herbert, V. Vitamin B-12. In *Present Knowledge in Nutrition*; Ziegler, E. E., Filler L. J., Jr., Eds.; Int. Life Sci. Inst., Nutr. Found: Washington, DC, 1996; Vol. *20*, pp 191−205.

(4) Baik, H. W.; Russell, R. M. Vitamin B_{12} deficiency in the elderly. *Annu. Rev. Nutr*. **1999**, *19*, 357–377.

(5) Houston, D. K.; Johnson, M. A.; Nozza, R. J. Age-related hearing loss, vitamin B_{12}, and folate in the elderly women. *Am. J. Clin. Nutr*. **1999**, *69*, 564–571.

(6) Kunhe, T.; Bubi, R.; Baumgartner, R. Maternal vegan diet causing a serious infantile neurological disorder due to vitamin B_{12} deficiency. *Eur. J. Pediatr*. **1991**, *150*, 205–208.

(7) Shinwell, E. D.; Gorodischer, R. Totally vegetarian digest and infant nutrition. *Pediatrics* **1982**, *70*, 582–586.

(8) Crane, M. G.; Sample, C.; Pathcett, S. Vitamin B_{12} studies in total vegetarians (vegans). *J. Nutr. Med*. **1994**, *4*, 419−430.

(9) Ball, G. F. M. Vitamin B_{12}. In *Bioavailability and Analysis of Vitamins in Foods*; Chapman and Hall: London, U.K., 1998; pp 497−515.

(10) Watanabe, F. Vitamin B_{12} sources and bioavailability. *Exp. Biol. Med*. **2007**, *232*, 1266–1274.

(11) Souci, S. W.; Fachmann, N.; Kraut, H. *Food Composition and Nutrition Tables*, 6th ed.; CRC Press: Boca Raton, FL, 1994.

(12) van den Berg, H.; Dagnelie, P. C.; van Staveren, W. A. Vitamin B_{12} and seaweed. *Lancet* **1988**, *1*, 242−243.

(13) Herbert, V.; Drivas, G. Spirulina and vitamin B_{12}. *JAMA−J. Am. Med. Assoc*. **1982**, *248*, 3096–3097.

(14) Watanabe, F.; Katsura, H.; Takenaka, S.; Fujita, T.; Abe, K.; Tamura, Y.; Nakatsuka, T.; Nakano, Y. Pseudovitamin B_{12} is the predominant cobamide of an algal health food, *Spirulina* tablets. *J. Agric. Food Chem*. **1999**, *47*, 4736–4741.

(15) Stupperich, E.; Nexo, E. Effect of the cobalt-N coordination on the cobamide recognition by the human vitamin B_{12} binding proteins intrinsic factor, transcobalamin, and haptocorrin. *Eur. J. Biochem*. **1991**, *199*, 299–303.

(16) Mattila, P.; Konko, K.; Eurola, M.; Pihlava, J.; Astola, J.; Vahteristo, L.; Hietaniemi, V.; Kumpulainen, J.; Valtonen, M.; Piironen, V. Contents of vitamins, mineral elements and some phenolic compounds in cultivated mushrooms. *J. Agric. Food Chem*. **2001**, *49*, 2343–2348.

(17) Walker, C. D. Vitamin B_{12} fortification of *Agaricus bisporus* mushrooms. *Cultiv. Mushroom Res. (CMR) Newsl*. **1996**, *3*, 31–38.

(18) Vladeanu, G.; Mitrea, N. The effect of cobalt and nickel microelements upon the content increase of vitamin B_{12} in *Spirulina platensis* biomass. *Stud. Cercet. Biol., Ser. Biol. Veg*. **1992**, *44*, 607–610.

(19) Miyamoto, E.; Tanioka, Y.; Nakao, T.; Barla, F.; Inui, H.; Fujita., T.; Watanabe, F.; Nakano, Y. Purification and characterization of a corrinoid-compound in an edible cyanobacterium *Aphanizomenon flos-aquae* as a nutritional supplementary food. *J. Agric. Food Chem*. **2006**, *54*, 9604–9607.

(20) Watanabe, F.; Katsura, H.; Takenaka, S.; Enomoto, T.; Miyamoto, E.; Nakatsuka, T.; Nakano, Y. Chracterization of vitamin B_{12} compounds from edible shellfish, clam, oyster, and mussel. *Int. J. Food Sci. Nutr*. **2001**, *52*, 263–268.

(21) Luo, X.; Chen, B.; Ding, L.; Tang, F.; Yao, S. HPLC-ESI-MS analysis of vitamin B_{12} in food products and in multivitamin−multimineral tablets. *Anal. Chim. Acta* **2006**, *562*, 185–189.

(22) Mozafar, A. Enrichment of some B-vitamins in plants with application of organic fertilizers. *Plant Soil* **1994**, *167*, 305–311.

(23) AOAC. *Official Methods of Analysis*; Cunniff, K., Ed.; Association of Official Analytical Chemists: Gaithersburg, MD, 2000; p 47.

(24) Martens, J. H.; Barg, H.; Warren, M. J.; Hahn, D. Microbial production of vitamin B_{12}. *Appl. Microbiol. Biotechnol*. **2002**, *58*, 275–285.

(25) Taranto, M. P.; Vera, J. L.; Hugenholtz, J.; F. de Valdez, G.; Sesma, E. *Lactobacillus reuteri* CRL 1098 produces cobalamin. *J. Bacteriol*. **2003**, *185*, 5643–5647.

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