Note

Characterization of Vitamin B\textsubscript{12} Compounds in the Wild Edible Mushrooms Black Trumpet (Craterellus cornucopioides) and Golden Chanterelle (Cantharellus cibarius)

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Summary This study determined the vitamin B\textsubscript{12} content of six wild edible mushrooms which are consumed by European vegetarians. Zero or trace levels (0.01–0.09 µg/100 g dry weight) of vitamin B\textsubscript{12} were determined in porcini mushrooms (Boletus spp.), parasol mushrooms (Macrolepiota procera), oyster mushrooms (Pleurotus ostreatus), and black morels (Morchella conica). By contrast, black trumpet (Craterellus cornucopioides) and golden chanterelle (Cantharellus cibarius) mushrooms contained considerable levels (1.09–2.65 µg/100 g dry weight) of vitamin B\textsubscript{12}. To determine whether C. cornucopioides or C. cibarius contained vitamin B\textsubscript{12} or other corrinoid compounds that are inactive in humans, we purified a corrinoid compound using an immunoaffinity column and identified it as vitamin B\textsubscript{12} based on LC/ESI-MS/MS chromatograms.

Key Words Cantharellus cibarius, cobalamin, Craterellus cornucopioides, edible mushrooms, vitamin B\textsubscript{12}

Vitamin B\textsubscript{12} (B\textsubscript{12}) is synthesized only by certain bacteria (1). The B\textsubscript{12} synthesized by bacteria is concentrated mainly in the bodies of higher predatory organisms in the natural food chain system. Animal foods (i.e., meat, milk, egg, fish, and shellfish), but not plant foods, are considered to be the major dietary sources of B\textsubscript{12} (2). Thus, strict vegetarians have a greater risk of developing B\textsubscript{12} deficiency compared with nonvegetarians (3). The major symptoms of B\textsubscript{12} deficiency are neuropathy and megaloblastic anemia (4). Thus, we need to identify plant foods that contain high levels of B\textsubscript{12} to prevent vegetarians from developing B\textsubscript{12} deficiency.

Wild mushrooms are becoming increasingly important in our diet because of their nutritional and medicinal characteristics (5, 6). Many species of wild mushrooms are consumed widely. Six wild edible mushroom species are popular with vegetarians in European countries, i.e., porcini mushrooms (Boletus spp.), parasol mushrooms (Macrolepiota procera), oyster mushrooms (Pleurotus ostreatus), black morels (Morchella conica), black trumpets (Craterellus cornucopioides), and golden chanterelles (Cantharellus cibarius). However, little information is available on the B\textsubscript{12} content of these mushrooms, particularly whether these mushrooms contain “true” (authentic) B\textsubscript{12} or an inactive corrinoid such as pseudo B\textsubscript{12} (2). If certain edible mushrooms contain considerable or high levels of B\textsubscript{12}, they would be good sources of B\textsubscript{12} for vegetarians.

In this study, we analyzed the B\textsubscript{12} content of the six edible mushrooms consumed by European vegetarians, and characterized the B\textsubscript{12} compounds found in black trumpet and golden chanterelle mushrooms.

Materials and Methods

Materials. B\textsubscript{12} was obtained from Sigma (St. Louis, Missouri, USA). A B\textsubscript{12} assay medium based on Lactobacillus delbrueckii subspecies lactis (formerly L. leichmannii) ATCC7830 was obtained from Nissui (Tokyo, Japan). Silica gel 60 thin layer chromatography (TLC) aluminum sheets were obtained from Merck (Darmstadt, Germany). The dried mushroom samples were purchased in Germany and Japan.

Extraction and assay of B\textsubscript{12} in edible mushrooms. Each mushroom sample (approximately 10 g) was homogenized using a mixer (TML160; Tescom & Co., Ltd., Tokyo, Japan). A portion (5.0 g) of the homogenate was used as the test sample. Total B\textsubscript{12} compounds were extracted by boiling at pH 4.8 in the presence of 4.0×10\textsuperscript{−4} % KCN and assayed using a microbiological technique based on L. delbrueckii ATCC 7830, according to the method described in the Standard Tables of Food Composition in Japan (7). L. delbrueckii ATCC 7830 can utilize deoxyribosides, deoxyribonucleotides (known as an alkali-resistant factor), and B\textsubscript{12}. Hence, the correct B\textsubscript{12} values were calculated by subtracting the results for the alkali-resistant factor from those for total B\textsubscript{12}.

Bioautogram of vitamin B\textsubscript{12} compounds using vitamin B\textsubscript{12}-dependent Escherichia coli 215. A bioautogram of B\textsubscript{12} compounds was prepared according to a published method (8). The B\textsubscript{12} extract (10 mL) prepared above was
partially purified and concentrated using a Sep-pak® Plus C18 cartridge (Waters Corp., Milford, USA) that had been washed with 5 mL of 75% (v/v) ethanol and equilibrated with 5 mL of distilled water. The C18 cartridge was washed with 5 mL of distilled water and B12 compounds were eluted using 2 mL of 75% (v/v) ethanol. The eluate was evaporated in a centrifugal concentrator (Integrated SpeedVac® System ISS110; Savant Instruments Inc., NY, USA). The residual fraction was dissolved in 1.0 mL of distilled water. Next, 2 mL of the concentrated B12 extracts as well as authentic and pseudo B12 (each 10 μg/L) were spotted onto the silica gel 60 TLC sheet and developed in the dark using 2-propanol/NH4 OH (28%)/water (7 : 1 : 2 v/v) at room temperature (25˚C). After the TLC sheet was dried, it was overlaid with agar containing basal medium and pre-cultured E. coli 215, and incubated at 37˚C for 20 h. The gel plate was then sprayed with a methanol solution containing 2,3,5-triphenyltetrazolium salt and B12 compounds were visualized as red, indicating E. coli growth.

Identification of mushroom B12 compounds by LC/ESI-MS/MS. The mushrooms samples that contained high levels of B12 (Craterellus cornucopioides and Cantharellus cibarius) (each 50 g wet weight, moisture content of 90%) were suspended in 500 mL of distilled water and homogenized in a mixer (TML160). Each homogenate was added to 50 mL of 0.57 mol/L acetic buffer, pH 4.5, with 0.05 g KCN, and boiled for 30 min to extract B12 compounds. The extraction procedures were performed in a draught chamber (Dulton Co., Japan). The boiled suspension was centrifuged at 5,000 ×g for 10 min. An aliquot (approximately 200 mL) of the supernatant was placed in Sep-pak®Vac 20 cc (5 g) C18 cartridges (Waters Corp.) that had been washed with 75% (v/v) ethanol and equilibrated with distilled water. The C18 cartridges were washed with 30 mL of distilled water and B12 compounds were eluted using 30 mL of 75% (v/v) ethanol. The remaining supernatant was treated in the same way. The combined eluates were evaporated to dryness under reduced pressure. The residual fraction was dissolved in 5 mL of distilled water and centrifuged at 10,000 ×g for 10 min to remove any insoluble material. The supernatant fraction was loaded onto an immunoaffinity column [EASI-EXTRACT® B12 Immunoaffinity Column (P80); R-Biopharm AG, Darmstadt, Germany] and B12 compounds were purified according to the manufacturer’s
Fig. 2. LC/ESI-MS/MS chromatograms of authentic B₁₂, and the B₁₂ compounds purified from black trumpet (C. cornucopioide) and golden chanterelle (C. cibarius). Vitamin B₁₂ was analyzed using an LCMS-IT-TOF system (Shimadzu) as described in the text. The total ion chromatogram (TIC) of authentic B₁₂ is shown in panel A. Panels B and C show the TICs and reconstructed chromatograms of the purified B₁₂ compounds (m/z 678.29) from black trumpet (C. cornucopioide) and golden chanterelle (C. cibarius), respectively. The mass spectra of authentic B₁₂ and the B₁₂ compounds purified from black trumpet (C. cornucopioide) and golden chanterelle (C. cibarius) at 7.35 min are shown in panels D, E, and F, respectively (the magnified spectrum ranging from m/z 678 to m/z 680 is shown as an insert in each panel). The MS/MS spectra for the m/z 678.292 peak of authentic B₁₂ and the B₁₂ compounds purified from black trumpet (C. cornucopioide) and golden chanterelle (C. cibarius) are shown in panels G, H, and I, respectively.
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7.35 min. The mass spectrum of authentic B12 was eluted as a peak with a retention time of 7.35 min in these purified compounds, containing the B12 divalent ions with m/z values of 678.2892 and 678.2884 (Fig. 2B, C, E, and F). The MS/MS spectrum of each compound was identical to that of authentic B12 (Fig. 2G, H, and I). These results indicated that black trumpet (C. cornucopioides) and golden chanterelle (C. cibarius) mushrooms contained considerable levels of authentic B12, but not pseudo B12 that is inactive in humans. Thus, black trumpet and golden chanterelle mushrooms could be useful plant B12 sources for vegetarians.

Consumption of approximately 100 g of dried black trumpet [or approximately 1 kg of fresh mushroom (moisture content of 90%)] could provide the recommended daily dietary allowance for adults (2.4 µg/d) (9), although they would not be able to ingest such large amounts of this mushroom daily. However, a moderate mushroom intake may contribute slightly to the prevention of severe B12 deficiency in vegetarians.

There is little information available on why C. cornucopioides and C. cibarius contain higher levels of B12 than the other mushrooms tested. Thus, further phylogenetic, biochemical, and genetic studies are needed to determine whether C. cornucopioides and C. cibarius have the ability to synthesize B12 de novo or if it is derived from B12 synthesized by bacteria living on the surfaces of these mushrooms.

**References**


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**Results and Discussion**

The levels of B12 were assayed in six edible mushrooms that are commonly consumed by European vegetarians using a microbiological method based on L. delbrueckii ATCC 7830 (Table 1). Zero or trace levels (0.01–0.09 µg/100 g dry weight) of corrected B12 were found in Boletus spp., Macrolepiota procera, Pleurotus ostreatus, and Morchella conica, whereas Craterellus cornucopioides and Cantarellus cibarius contained considerable levels (1.09–2.65 µg/100 g dry weight) of corrected B12. High levels (0.12–1.49 µg as B12 equivalent/100 g dry weight) of the alkali-resistant factor were found in all mushrooms.

The B12 compounds found in the edible mushrooms were analyzed using an E. coli 215 bioautogram after being separated by silica gel 60 TLC (Fig. 1). Each extract of C. cornucopioides and C. cibarius produced a single clear spot, the Rf value of which was identical to that of the authentic B12 form but not to that of the pseudo B12 form that is inactive in humans. Thus, black trumpet and golden chanterelle mushrooms contained considerable levels of authentic B12, but not pseudo B12 that is inactive in humans. Thus, black trumpet and golden chanterelle mushrooms could be useful plant B12 sources for vegetarians.

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**References**


