Occurrence of Vitamin B₁₂ in Green, Blue, Red, and Black Tea Leaves

Hiromi Kittaka-Katsurai¹, Fumio Watanabe² and Yoshihisa Nakano³

¹Department of Food and Nutrition, Kyoto Women's University, Kyoto 605-8501, Japan
²Department of Health Science, Kochi Women's University, Kochi 780-8515, Japan
³Department of Applied Biological Chemistry, Osaka Prefecture University, Sakai 599-8531, Japan

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Summary Vitamin B₁₂ contents of green (0.046–0.263 and 0.125–0.535 µg/100 g dry weight), blue (0.068–0.81 and 0.525–0.528 µg/100 g dry weight), red (0.104–0.859 and 0.305–1.20 µg/100 g dry weight), and black (0.104–0.859 and 0.305–1.20 µg/100 g dry weight) tea leaves were obtained by intrinsic factor-chemiluminescence and microbiological methods, respectively. Although vitamin B₁₂ was found in all tea leaves tested by both assay methods, the higher values by the microbiological method were not due to occurrence of both deoxyribosides and deoxynucleotides (known as an alkali-resistant factor), but may have been due to that of inactive corrinoid compounds for mammals in the tea leaves.

Key Words cobalamin, tea, vegetarians, vitamin B₁₂

Vitamin B₁₂ (B₁₂) is synthesized only in certain bacteria (1). Usual dietary sources of B₁₂ are animal food products (meat, milk, egg, and shellfish), but not plant food products (2). Insufficient B₁₂ intake, as seen in strict vegetarians, can slowly lead to B₁₂ deficiency (3). Our previous study (4) has demonstrated that the tea leaves fermented by certain bacteria (black tea, Batabata-cha) contain considerable amounts of true B₁₂. Feeding of the black tea drink in B₁₂-deficient rats has indicated that the B₁₂ compound found in the black tea is bioavailable in mammals (4). However, it is unclear whether various tea leaves generally contain true B₁₂. If various kinds of tea leaves contain considerable amounts of true B₁₂, tea leaves and their drinks would contribute to human B₁₂ supply, especially for vegetarians. Here we describe the occurrence of B₁₂ in various tea leaves by use of both microbiological and chemiluminescence B₁₂ assay methods.

Materials and Methods

Materials B₁₂ was obtained from Wako Pure Chemical Industries (Osaka, Japan). A B₁₂ assay medium for Lactobacillus delbrueckii subsp lactic ATCC7830 (formerly L. leichmannii ATCC 7830) was obtained from Nissui (Tokyo, Japan). All other reagents were of the highest purity commercially available. All dried tea leaves were purchased from a local market in Japan. The fresh tea leaves was kindly provided from JA Kyoto Yamashiro (Kyoto, Japan). A Shimadzu (Kyoto, Japan) UV-visible spectrophotometer (UV-1600) was used for measuring the turbidity of L. delbrueckii test culture in the microbiological method. A fully-automated chemiluminescence B₁₂ analyzer ACS 180 (Chiron Diagnostics, East Walpole, MA) was also used for B₁₂ assay.

Extraction of B₁₂ from various tea leaves. Each sample (5 g) of dried tea leaves was powdered by a food mill (MK-K3, Panasonic, Osaka, Japan), and was suspended in 50 mL of 0.25 mol/L acetate buffer, pH 4.8, containing 0.2% (w/v) KCN. Total B₁₂ was extracted from the suspension by boiling for 60 min at 98 °C in the dark as described previously (4). The boiled suspension was centrifuged for 10 min at 5,000×g, and the supernatant was used for B₁₂ assay. Fresh tea leaves were harvested, washed with distilled water, and stored at −20 °C until use. Fifty grams of the stored tea leaves were homogenized by the food mill and suspended in 40 mL of the same buffer. B₁₂ was extracted under the same conditions. The B₁₂ extract was concentrated with a Sep-pak Vac C18 Cartridge (Waters Corp., Milford, MA) as described previously (4). The concentrated solution was evaporated to dryness under reduced pressure, dissolved in 1.0 mL of distilled water, and used for B₁₂ assay.

Assay of B₁₂. B₁₂ was assayed by the microbiological method with L. delbrueckii subsp. lactic ATCC 7830 and a B₁₂ assay medium and by the fully-automated chemiluminescence B₁₂ analyzer ACS 180 as described previously (4). The above B₁₂ extracts were directly applied to the chemiluminescence analyzer. They were diluted with distilled water up to a B₁₂ concentration range of 0.01–0.1 µg/L and used as samples for the microbiological method. Since L. delbrueckii ATCC 7830 can utilize both deoxyribosides and deoxynucleotides (known as an alkali-resistant factor) as well as B₁₂, the amount of true B₁₂ was calculated by subtracting the value of the alkali-resistant factor from that of total B₁₂ according to the reference cited (5).

Results and Discussion

In Asia, there are many types of teas, which are generally divided into five classes by their manufacturing processes (6). Table 1 shows the B₁₂ contents deter-
Table 1. Amounts of vitamin B12 in various tea leaves.

<table>
<thead>
<tr>
<th>Types of tea (Chinese classification)</th>
<th>Manufacturing process</th>
<th>Name of product (Producing district)</th>
<th>B12 content (µg/100 g dry weight) Mean±SD</th>
<th>C/M*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Chemiluminescence method (C) Microbiological method (M)</td>
<td></td>
</tr>
<tr>
<td>Steamed-green tea (Green tea)</td>
<td>dried after steamed and rubber tea dried after roasted and rubbed</td>
<td>Sen-cha (Shizuoka, Japan) Kamarih-cha (Miyazaki, Japan) Lung ching (China)</td>
<td>0.263±0.037 0.535±0.025</td>
<td>Medium</td>
</tr>
<tr>
<td>Roasted-green (Green tea)</td>
<td></td>
<td></td>
<td>0.173±0.037 0.187±0.017</td>
<td>High</td>
</tr>
<tr>
<td>Half-fermented tea (Blue tea)</td>
<td>dried after withered and half-oxidized</td>
<td>Tung-ting oolong (Taiwan) Wang-jiin-gui oolong (China)</td>
<td>0.068±0.002 0.528±0.032</td>
<td>Low</td>
</tr>
<tr>
<td>Fully-fermented black tea (Red tea)</td>
<td>dried after withered and fully-oxidized dried after heated and fermented by bacteria</td>
<td>Keemun (China) Pu’er (China) Ruy bao (China) Lapet-chin chau (Myanmar) Babatata cha (Toyama, Japan) Awa-bun cha (Tokushima, Japan) Goishi cha (Kochi, Japan)</td>
<td>0.061±0.008 0.663±0.118</td>
<td>Low</td>
</tr>
<tr>
<td>Fermented-black tea by bacteria (Black tea)</td>
<td></td>
<td></td>
<td>0.229±0.006 0.524±0.004</td>
<td>Medium</td>
</tr>
</tbody>
</table>

B12 was assayed in the tea leaves (n=3) by the two methods.
*Teas were divided into three groups by C/M ratios: High (C/M≥0.7), Medium (0.3≤C/M<0.7), and Low (C/M<0.3).

mined by both microbiological and chemiluminescence methods in various tea leaves. B12 contents of green (0.046–0.263 and 0.125–0.535 µg/100 g dry weight), blue (0.068–0.081 and 0.525–0.528 µg/100 g dry weight), red (0.061 and 0.663 µg/100 g dry weight), and black (0.104–0.859 and 0.305–1.20 µg/100 g dry weight) tea leaves were obtained by the chemiluminescence and microbiological methods, respectively. Remarkably a certain amount of B12 was found in all tea leaves without bacterial fermentation in the manufacturing processes. To clarify whether tea leaves themselves contain B12 or not, B12 content of fresh tea leaves was determined by the chemiluminescence method. The fresh tea leaves contained 0.044±0.004 µg of B12 per 100 g wet weight (equivalent to about 0.22 µg of B12 per 100 g dry weight). As plants cannot synthesize B12, the tea tree may have the ability to take up B12 from soil containing some organic fertilizers such as the fish meal that is usually used in Japan. Our preliminary experiments suggest that B12 contents of green tea tend to be increased with increase of the organic fertilizer used in soil. Some forms of B12 found in the black tea fermented by bacteria would be derived from the B12 synthesized by the bacteria.

When the ratio (C/M) of the values determined by the chemiluminescence method to those determined by the microbiological method was calculated, teas were divided into three groups: High (C/M≥0.7), Medium (0.3≤C/M<0.7), and Low (C/M<0.3). Although half- and fully-fermented teas were classified under the Low group, green teas and black teas fermented by bacteria were distributed into the High or the Medium group (Table 1).

The values determined by the microbiological method were several folds greater than the values determined by the chemiluminescence method. Since *L. delbrueckii* ATCC 7830 can utilize both deoxyribosides and deoxynucleotides (known as an alkali-resistant factor) as well as B12, the contents of alkali-resistant factor were determined in these tea leaves by the microbiological method. The values of the half- and fully-fermented teas were from 0 to 8% of the total B12, but those of green teas and black teas fermented by bacteria were 54 and 31%, respectively. The amount of true B12 was calculated by subtracting the value of the alkali-resistant factor from total B12. The higher values by the microbiological method were not due to occurrence of the alkali-resistant factor, but may have been due to that of inactive corrinoid compounds for mammals in the tea leaves.
Our previous study (7) has indicated that except for foods containing substantial amounts of inactive B\textsubscript{12} and/or B\textsubscript{12}-substitutive compounds, the observed correlation coefficient between values obtained by the microbiological and chemiluminescence methods in foods is excellent. These results may suggest that true B\textsubscript{12} predominates in the tea leaves with C/M of >0.7. The high prevalence of protein-bound B\textsubscript{12} (or food-bound B\textsubscript{12}) malabsorption is found in peoples older than 50 y and/or in peoples with certain gastric dysfunctions (3). Preliminary experiments indicated that most of the B\textsubscript{12} found in the commercial black tea (Batabata-cha) drink were recovered in the free B\textsubscript{12} fraction, but not in the macromolecular fraction on Bio-Gel 10DG gel filtration column chromatography. Our previous study (4) has demonstrated that although B\textsubscript{12} content (about 2.0 ng/100 mL) of the black tea drink is slightly low, the feeding of the tea drink can improve considerably B\textsubscript{12} status in B\textsubscript{12}-deficient rats. Judging from the results presented here, two (Ryu bao and Batabata-cha) of black tea leaves may be available as plant sources of B\textsubscript{12} for vegetarians and/or elderly peoples.

REFERENCES