Note

**Loss of Vitamin B\(_{12}\) in Fish (Round Herring) Meats during Various Cooking Treatments**

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**Summary** The loss of vitamin B\(_{12}\) in round herring meats during various cooking treatments was evaluated. Although amounts of vitamin B\(_{12}\) were three times greater in the viscera (37.5±10.6 \(\mu g/100\text{ g fresh weight}\)) than in the meats, about 73% of total vitamin B\(_{12}\) found in the whole fish body (except for head and bones) were recovered in the meats (5.1±1.0 \(\mu g\text{ of vitamin B}_{12}\)). The vitamin B\(_{12}\) contents of the round herring’s meats were significantly decreased up to ~62% during cooking by grilling, boiling, frying, steaming, and microwaving. There was, however, no loss of vitamin B\(_{12}\) during vacuum-packed pouch cooking. Model experiment using hydroxocobalamin suggest that loss of vitamin B\(_{12}\) is dependent on the degree of temperature and time used in conventional cooking, and is further affected by the concomitant ingredients of food. Retention of vitamin B\(_{12}\) was not dependent on vacuum or temperature (or both) used in the vacuum-packed pouch cooking.

**Key Words** cobalamin, cooking, *Etrumeus teres*, round herring, vitamin B\(_{12}\)

Vitamin B\(_{12}\) (B\(_{12}\)) is synthesized only in certain bacteria and then concentrated mainly in the bodies of higher predatory organisms in the natural food chain system (1). Animal foods (i.e., meat, milk, egg, fish, and shellfish), but not plant foods, are considered to be the major dietary sources of B\(_{12}\) (2). Japanese people obtain most (~84%) of daily B\(_{12}\) intake from both fish and shellfish (3). Fish is also a good source of minerals, vitamins, proteins (with high biological values), and unsaturated lipids (containing eicosapentaenoic acid and docosahexaenoic acid) (4). Many studies (5–7) have suggested that regular fish intake prevents atherosclerosis, thrombosis, and cardiac diseases. Therefore, the trend of fish intake is spreading throughout the world.

Bennink and Ono (8) have reported appreciable loss (~33%) of B\(_{12}\) during cooking of raw beef. Our previous studies have demonstrated that loss of B\(_{12}\) significantly occurs in beef, pork, and milk during microwave heating (9). Although the effects of various cooking methods on the proximate composition, mineral, and vitamin (A, E, B\(_{1}\), naiacin, and B\(_{6}\)) contents of certain fish fillets have been investigated (10), it is still unclear how much B\(_{12}\) is lost in raw fish meats during cooking; the lack of this information may be due to the difficulty of B\(_{12}\) assay in foods. If cooking processes lead to significant loss of B\(_{12}\) in the fish meats, it would be an important problem for assessment of the daily intake of B\(_{12}\) to prevent B\(_{12}\) deficiency. Here we describe the loss of B\(_{12}\) in a round herring’s (one of the most popular fishes) meat during various cooking treatments.

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**Materials and Methods**

**Materials.** B\(_{12}\) was purchased from Sigma (St. Louis, Missouri, USA). A B\(_{12}\) assay medium for *Lactobacillus delbrueckii* subspecies *lactis* (formerly *L. leichmannii*) ATCC7830 was obtained from Nissui (Tokyo, Japan). A Shimadzu (Kyoto, Japan) ultraviolet/visible spectrophotometer (UVmini-1240) was used for measuring the turbidity of *L. delbrueckii* test cultures in the microbiological B\(_{12}\) assay. All other reagents used were of the highest purity commercially available.

**Preparation for fish meats.** Raw big-eye round herrings (*Etrumeus teres*) (48–75 g body weight) were purchased from local markets in Kochi City, Japan (Fig. 1). The head, viscera, and bones of the round herring were removed to prepare the edible portions (meats), which were used for the experiments.

**Extraction and assay of vitamin B\(_{12}\).** B\(_{12}\) was extracted and assayed by the microbiological method with *L. delbrueckii* ATCC 7830 as described in the Standard Tables of Food Composition in Japan (11). Raw meats and viscera of the round herring were homogenized with a food processor (MILLSER-II IFM-200D, Iwatani Co., Tokyo, Japan). Two grams of each homogenate were used for B\(_{12}\) assay. Total B\(_{12}\) was extracted by boiling with 0.005% (w/v) KCN at pH 4.5 to convert various B\(_{12}\) compounds with different α-ligands (e.g. coenzyme forms of B\(_{12}\)) to cyanocobalamin (CN-B\(_{12}\)). A portion of the above total B\(_{12}\) extract was adjusted pH to 11.0 and then treated with an autoclave (MC-23, ALP Co., Ltd., Tokyo, Japan) at 121˚C for 30 min in order to decompose B\(_{12}\) in the extract. The treated extract contains certain compounds (including deoxyribosides and deoxyribo-nucleotides) which are known as an alkali-resistant
factor. Since L. delbrueckii ATCC 7830 can utilize both deoxyribosides and deoxyribonucleotides (alkali-resistant factor) as well as B₁₂, the amount of true B₁₂ was calculated by subtracting the values of the alkali-resistant factor from the values of total B₁₂.

In the case of cooking experiments, B₁₂ was extracted and assayed from the cooked round herring meats under the same conditions as described above.

The amounts of the alkali-resistant factor in the raw (or cooked) fish meats and viscera were determined to be <0.07 and <6.59 μg/100 g fresh weight, respectively.

Cooking conditions. The whole meats (30–50 g of fresh weight) of the round herrings were treated with various cooking processes. The raw fish meats were cooked in a Hitachi (Tokyo, Japan) microwave oven MHI-530 (500 W) for 1.0 min, grilled in a conventional gas range (RTG-2080, Rinnai Co., Chiba, Japan). The sample (50 g) was put on a aluminum plate, and then directly treated for 7.5 min in a frying pan which was heated at 180˚C (as grilled samples).

The treated OH-B₁₂ solution was packed in pouches for vacuum-packed pouch cooking, treated with or without the vacuum sealing, and then heated for the indicated time and temperature under the same conditions. The OH-B₁₂ solution (100 g) was directly treated for 7.5 min in a frying pan which was maintained at 180˚C (as grilled samples).

The treated OH-B₁₂ solution was concentrated with a Sep-pak Plus C₁₈ cartridge (Waters Corp., Milford, USA) which had been washed with 2 mL of 75% (v/v) ethanol and then equilibrated with 3 mL of distilled water. The C₁₈ cartridge was eluted with 1.5 mL of 75% (v/v) ethanol. The eluate was evaporated at low temperature with a centrifugal concentrator (Integrated Speed Vac® System ISS110, Savant Instruments Inc., NY, USA). The residual fraction was dissolved with 0.5 mL of distilled water. The concentrated OH-B₁₂ solution was analyzed by HPLC using a JASCO HPLC apparatus (PU-2080 Plus Pump, UV-2070 Plus Spectrophotometer, DG-2080-53 Degasser, CO-2065 column oven) and CDS ver. 5 chromatography system (LASoft, Ltd., Chiba, Japan). The sample (50 μL) was put on a reversed-phase HPLC column (Wakosil-II 5C18RS, φ4.6×150 mm; particle size, 5 μm) equilibrated with 20% (v/v) methanol containing 1% (v/v) acetic acid at 40˚C. The flow rate was 1.0 mL/min. The treated OH-B₁₂ solution and authentic OH-B₁₂ were isocratically eluted with the same solution and monitored by measuring absorbance at 351 nm. Retention time of authentic OH-B₁₂ was 2.7 min.

Escherichia coli 215 bioautogram and reversed-phase HPLC analysis. After a cooked round herring extract was concentrated and partially purified with a Sep-pack Plus C₁₈ cartridge (Waters Corp.), 2 μL of the purified B₁₂ extract and authentic B₁₂ (cyancobalamian, 10 μg/L) were spotted on a silica gel 60 TLC sheet and developed with 2-propanol/NH₄OH (28%/water) (7:1:2 v/v) in the dark at 25˚C. After the TLC sheet was dried, agar containing basal medium and pre-cultured E. coli 215 was overlaid and then incubated at 30˚C for 24 h. After being sprayed with a methanol solution of 2,3,5-triphenyltetrazolium salt on the gel plate, B₁₂ compounds were visualized as red in color indicating E. coli growth.

An aliquot (10 mL) of each extract was loaded onto an immunoaffinity column (EASI-EXTRACT® Vitamin B₁₂ Immunoaffinity Column (P80), R-Biopharm AG, Darmstadt, Germany) and then B₁₂ was purified according to the manufacturer's recommended protocol. The purified B₁₂ solution was analyzed by HPLC using a
JASCO HPLC apparatus (PU-2080 Plus Pump, UV-2070 Plus Spectrophotometer, DG-2080-53 Degasser, CO-2065 column oven) and CDS ver. 5 chromatogram processing system (LaSoft, Ltd.). The sample (100 μL) was put on a reversed-phase HPLC column (Wakosil-II 5C18RS, φ4.6×150 mm; particle size, 5 μm) equilibrated with 20% (v/v) methanol containing 1% (v/v) acetic acid at 40°C. The flow rate was 1.0 mL/min. The B12 compound and authentic B12 were isocratically eluted with the same solution and monitored by measuring absorbance at 361 nm. Retention time of authentic B12 was 10.4 min.

**Results and Discussion**

**B12 contents of the raw round herring meats**

B12 contents were determined in the meats and viscera of the round herrings using the microbiological B12 assay method (Table 1). Amounts of B12 (per 100 g of fresh meat weight) were three-times greater in the viscera (37.5 ± 10.6 μg) than in the meats (12.2 ± 2.1 μg), which value was similar to the raw fish meat B12 content (14.2 μg) described in Standard Tables of Food Composition in Japan, 5th revised and enlarged edition (12). About 73% of total B12 found in the whole fish

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Weight (g)</th>
<th>Vitamin B12 contents (μg/100 g fresh weight)</th>
<th>Distribution (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meats</td>
<td>41.4±1.4</td>
<td>12.2±0.7</td>
<td>73.1±2.5</td>
</tr>
<tr>
<td>Viscera</td>
<td>5.0±0.4</td>
<td>37.5±3.4</td>
<td>26.9±2.5</td>
</tr>
</tbody>
</table>

The values represent mean±SE (n=10).
**Distribution represents ratio of B12 contents* found in the meat or viscera of each fish to the sum of them.

**Table 2. Effect of various cooking methods on vitamin B12 contents of the meats of raw round herring.**

<table>
<thead>
<tr>
<th>Cooking methods</th>
<th>Changes in weight (g)</th>
<th>Vitamin B12 contents (μg/100 g meats)</th>
<th>Cooking conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw (without cooking, n=10)</td>
<td>41.4±1.4</td>
<td>5.1±0.3</td>
<td>100</td>
</tr>
<tr>
<td>Grilling (n=5)</td>
<td>46.8±1.7</td>
<td>3.4±0.2</td>
<td>heated at 180°C for 7.5 min</td>
</tr>
<tr>
<td>Boiling-1 (n=3)</td>
<td>33.0±0.9</td>
<td>1.9±0.3</td>
<td>heated for 5 min in boiling water</td>
</tr>
<tr>
<td>Boiling-2 (n=5)</td>
<td>40.1±1.2</td>
<td>3.4±0.3</td>
<td>heated for 8 min in water (boiled for 6.0 min)</td>
</tr>
<tr>
<td>Steaming-1 (n=5)</td>
<td>37.1±1.1</td>
<td>4.2±0.1</td>
<td>steamed for 4.5 min</td>
</tr>
<tr>
<td>Steaming-2 (n=5)</td>
<td>44.8±1.3</td>
<td>2.3±0.1</td>
<td>steamed for 9.0 min</td>
</tr>
<tr>
<td>Frying-1 (n=4)</td>
<td>42.8±2.4</td>
<td>3.3±0.4</td>
<td>heated at 180°C for 2.0 min</td>
</tr>
<tr>
<td>Frying-2 (n=5)</td>
<td>48.3±2.0</td>
<td>2.5±0.2</td>
<td>heated at 180°C for 4.0 min</td>
</tr>
<tr>
<td>Microwaving (n=5)</td>
<td>38.3±0.8</td>
<td>2.7±0.1</td>
<td>heated at 500 W for 1.0 min</td>
</tr>
<tr>
<td>Vacuum-packed pouch cooking (n=5)</td>
<td>29.4±2.8</td>
<td>3.6±0.5</td>
<td>heated at 70°C for 30 min</td>
</tr>
</tbody>
</table>

The values represent mean±SE.
*These values were adjusted by using the ratio of mean weights of raw fish meats (control meats/meats used for cooking).
body (except for head and bones) was recovered in the meats. The raw meat of half a fish (about 21 g) can supply the recommended dietary allowance (2.4 μg of B12 per day) for adults (13).

Effects of various cooking methods on B12 contents of the raw round herring meats

To clarify how much B12 was lost in the raw fish meats during various cooking methods, B12 was extracted and analyzed from the cooked fish meats (Table 2). When the fish meats were treated under the appropriate cooking conditions, the B12 contents of the fish meats were significantly decreased up to about 59, 47, 41, 43, and 59% during the cooking by grilling (for 7.5 min), boiling (for 5.0 min), steaming (for 9.0 min), frying (for 4.0 min), and microwaving (1.0 min), respectively, but not at all during vacuum-packed pouch cooking. The stability of B12 was dependent on the treatment temperatures and times judging from the data of the steam and fry cooking. B12 content of broth in boiling with boiling water was about half of that in boiling with water; decrease in the leakage of B12 from the treated meats may be due to the meat surface proteins being denatured quickly by the boiling water.

Table 3 shows the loss of a naturally occurring B12 compound, OH-B12, solution (at the same B12 concentration as the raw round herring meats shown in Table 1) during various heat treatments as a model system. OH-B12 levels were the similar to those of the fish meat B12 for grilling, boiling, steaming (4.5 min), and vacuum-packed pouch cooking (70°C for 30 min), and greater for steaming (9.0 min) and microwaving; the larger loss of B12 in the fish meats may be due to the B12 destruction stimulated by the interaction with the ingredients of the meats. As shown in Tables 2 and 3, the loss of B12 appears to be dependent on the temperature and time used in the conventional cooking.

As there is a lack of detailed data on nutritional aspects of vacuum-packed pouch cooking, it is difficult to evaluate whether the little (or no) loss of vitamins during vacuum-packed pouch cooking is only due to the lower temperature used or due to vacuum packaging (14). Petersen (15) has described the effects of the vacuum-packed pouch cooking, steaming, and boiling of broccoli florets on stability of vitamin C, vitamin B6,
and folate. The stability of all vitamins was lowest for boiling, while the stability was greatest for vacuum-packed pouch cooking and a little lower for steaming. Although stability of vitamin C is highly dependent on the degree of vacuum in the package, that of vitamin B₆ is independent on the degree of vacuum. Vitamins sensitive to oxidation have better stability in vacuum-packed pouch cooking than in conventional or traditional cooking. In the case of B₁₂, retention of B₁₂ in meat and fish dishes by vacuum-packed pouch cooking has been reported to be 87% (beef), 100% (veal), 100% (lamb), 100% (pork), 92% (salmon), and 72% (cod). The results in Table 3 indicate that the loss of B₁₂ is not dependent on the vacuum or temperature (or both) used in vacuum-packed pouch cooking.

These results indicate that the usual cooking methods for fish meats (by grilling, frying, steaming, and microwaving) results in significant B₁₂ decrease (≈50%), while vacuum-packed pouch cooking is an excellent method to prevent loss of B₁₂ during the cooking of fish meats.

Our previous studies have demonstrated that appreciable loss (≈40%) of B₁₂ occurs in certain foods during microwave heating (9). Prolonged heat-treatments (boiling for 30 min or microwaving for 6 min) of authentic OH-B₁₂ solution have demonstrated the accelerated formation of B₁₂ degradation products, some of which are identified as the compounds with various changes to lower-ligand moiety (cobalt-coordinated nucleotide) (9, 17).

Since some of these B₁₂ degradation products are detectable by a B₁₂-dependent E. coli 215 bioautogram and a reversed-phased HPLC (18), B₁₂ extracts of the fish meats treated by frying and microwaving were qualitatively analyzed (Fig. 2). The B₁₂ compound found in each extract was given as a single spot and peak, whose respective Rᵣ values and retention times were identical to that of authentic B₁₂ according to the bioautogram and HPLC. These results suggest that no unidentified B₁₂ compounds were formed in the fish meats under these cooking conditions. The decreased B₁₂ contents of the various cooked fish meats appear to be due to destruction of B₁₂ by heating.

The results presented here indicate that the B₁₂ content of fish meats is decreased ≈50% during normal cooking (grilling, boiling, frying, steaming, and microwaving); conveniently, 50% loss of B₁₂ should be estimated in assessment of the daily intake of B₁₂. We also demonstrate for the first time that vacuum-packed pouch cooking is an excellent method to prevent loss of B₁₂ during the cooking of round herring.

REFERENCES


