# Comparison of pretreatent condition of cadmium in fish sample and diet by microwave digestion method for ICP-AES

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# Comparison of Pretreatment Condition of Cadmium in Fish Sample and Diet by Microwave Digestion Method for ICP-AES

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**Abstract:** As a part of a series of the study for creating healthy marine bio-resource, we investigated the most appropriate microwave digesting conditions for fish samples and the diet which contain complex biological components. Viscera of rainbow trout and the diet were treated by the combination of five oxidizers and three digestion programs, respectively, and then determined the concentration of cadmium by ICP-AES. The results indicated that 5 mL nitric acid and 1 mL hydrogen peroxide using microwave Program 1 gave the highest efficiency for fish samples. In the case of the diet, addition of double volume (2 mL) of hydrogen peroxide was required for the accurate ICP-AES measurement of the diet. No precise analytical data for the determination of toxic heavy metals such as cadmium ingested from the diet would be provided, if fish organs and the diet were digested by the same condition. However, the problem will be solved by the introduction of digestion method reported in this paper, and dependable analytical data will be guaranteed.

**Key words:** microwave digestion, oxidizers, microwave output, cadmium, fish, diet, ICP-AES

## Introduction

As a result of water pollution in coastal area, many problems on food safety like heavy metals accumulation have been pointed out in farmed fish which is one of the important fishery food resources. The heavy metals accumulated in fish not only have a bad influence on fish physiological and healthy conditions, but also in the case of the small fish which are consumed whole body including internal organs, affect the health of human being by the food chain. It is pointed out that remarkable heavy metals were contained in fish meals that are used as major raw materials for aquaculture feeds. Especially higher cadmium (Cd) is contained in Japanese soil compared to the foreign one, and we are also concerned an intake of such heavy metals through agricultural products. For this reason, a broad inspection of Cd content in food is started including the fishery product at the Center for Food Quality, Labeling and Consumer Services[1]. Although the Itai-itai disease easy breakable disease of Toyama Jintsu River area is well known human health damage being by high Cd accumulation, Cd induces people's kidney trouble as the symptoms of osteomalacia and osteoporosis. Therefore, grasp of the distribution of the toxic heavy metals in fish and fish meals for aquaculture feeds, and the establishment of simple and rapid determination method are important subjects from the viewpoint of the healthy crisis management incurred by Cd.

Although the atomic absorption spectrometry is also applicable to measure Cd concentration, in recent years, determination by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) is briskly used after the sample pretreatment by microwave digestion to assure the accuracy[2, 3]. ICP-AES is a sensitive measuring method[4], and the digestive pretreatment by the microwave oven, where an oxidizer coexist is important factor for accurate determination. An oxidizer has been commonly used to break down the combined heavy metals with biological materials, such as nitric acid, hydrochloric, sulfuric and perchloric acids, or hydrogen peroxide. It is necessary to determine the optimum digestive condition such as combination and proportion of oxidizer, and program of the microwave output power and heating time. In this case, we should also have to take into consideration the difference in digestion equipment, and the feature of the sample. For environmental sample, such as the atmosphere[5], soil[6], and environmental water[7], the pre-treating method was examined in detail. Many different digestive method for complicated biological materials containing organic matter such as protein and lipid, have been reported variously as follow. For example, it was reported that B. Perez Cid et al.[8] made an oxidizer nitric acid 4 mL + pure water 1 mL, F. J. Sanchez Lopez et al.[9] nitric acid 6 mL + hydrogen peroxide 1 mL, and H. Karadede et al.[10] used only nitric acid 7 mL as an oxidizer, moreover, as for Minagawa[5], about the output and time of microwave, 1) it was good to use the low output of 250 W and short-time for easily digestive ingredients, and to perform hardly digestive materials, such as a mineral, with the high output beyond 500 W for a long time. 2) the surface temperature of a digestion container was recommended to carry out at the time of a low

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output about 80 degrees C (internal temperature about 150 degrees C), and at the time of a high output with a maximum of 130 degrees C (internal temperature about 200 degrees C), suggesting the importance of the temperature and pressure on sample digestion process.

This paper deals with the optimum digestive conditions for rapid determination of Cd in fish sample and diet which contains many biological materials, as the first step of the research which is to develop and to secure a healthy fishery food material. The difference between the amount and combination of several oxidizers and a microwave outputs were examined to establish the exact and efficient simple digestive method.

## Materials and Methods

### 1. Reagents and samples

1) Reagents

Nitric acid (60.0–62.0%), sulfuric acid (96.0–98.0%), perchloric acid (60%) and hydrogen peroxide (30%) used as oxidizers were purchased from Wako Pure Chemical Industries, Ltd., heavy metals determination or atomic absorption spectrometry grade. Cd standard solutions were prepared from the 1000 ppm authentic standard for atomic absorption spectrometry (WPCI) by successive 10 times dilutions with deionized water. Cadmium nitric tetrahydrate (99.0%, WPCI) was used for Cd addition to assorted mixed diet.

2) Samples

In this study, 30 rainbow trouts (*Oncorhynchus mykiss*) were used with an average weight of 60 g kept in an indoor wooden water tank (96 L), given a diet once a day feeding rate of 1.2–1.0% of the body weight for nine weeks. The liver and the kidney of these fish were taken out after being sacrificed. All experimental diet used in this study were prepared at our lab according to the basic recipe shown in Table 1, and Cd was added to make their final concentration to 10 ppm (Diet A) and 30 ppm (Diet B).

### 2. Equipments and procedure of digestion

The microwave digestive equipment (MLS 1200 MEGA type, frequency of 2450 Hz) was used for digestion of a sample. Sample was weighted in the Teflon vessel, followed by an addition of one, two, or more oxidizers; it was sealed tightly and kept standing for 8 hours at the room temperature. Then after digestion and cooling down to the room temperature, it was diluted to 20 mL with deionized water.

**Table 1** Formula of the experimental diet for rainbow trout

| Ingredients | (%) | Ingredients | (%) |
|---|---|---|---|
| Jack mackerel meal | 50 | Pollock liver oil | 9.5 |
| Defatted soybean meal | 5 | P free-mineral mixture | 1 |
| Corn gluten meal | 5 | Vitamin premixture | 3 |
| Wheat flour | 15.9 | Choline chloride | 0.5 |
| Pregelatinized starch | 10 | Vitamin E (50%) | 0.1 |

**Table 2** Combination of oxidizers by microwave digestion

| Kinds of oxidizers | Proportion of oxidizers (mL) | |
|---|---|---|
| | Biological materials | Diet |
| $HNO_3$ | 5 | 5 |
| $HNO_3+H_2SO_4$ | 5+1 | 5+1 |
| $HNO_3+HClO_4$ | 5+1 | 5+1 |
| $HNO_3+H_2O_2$ | 5+1 | 5+1 |
| | 5+2 | 5+2 |
| | — | 5+3 |

Vessels and vitrics, and the polyethylene container for sample preservation were rigorously cleaned, soaked for 24h in 30% nitric acid, and thoroughly rinsed with deionized water before use.

### 3. Determination of Cd

Cd concentration was determined using ICP-AES (Seiko Instruments Inc., SPS7800ICP-AES) with power supply output 1.2 W, plasma gas flux 16 L/min, and auxiliary gas flux 1 L/min. The one point absolute calibration curve method was used for the determination.

### 4. Data calculation

After the liver and the kidney obtained from 30 fish were fully mixed and homogenized, four homogenate samples were taken out for Cd determination. Averaged value and the standard deviation were computed.

### 5. Oxidizer and microwave output

Nitric acid was selected as a basic oxidizer, and as shown in Table 2, one of sulfuric acid, perchloric acid, or hydrogen peroxide was added to nitric acid. Furthermore, the influence of microwave output was studied to find optimum condition as summarized in Table 3.

**Table 3** Microwave digestion programs

| Program 1 | | | Program 2 | | | Program 3 | | |
|---|---|---|---|---|---|---|---|---|
| Step | Power (W*) | Time (min) | Step | Power (W) | Time (min) | Step | Power (W) | Time (min) |
| 1 | 250 | 5 | 1 | 250 | 1 | 1 | 250 | 5 |
| 2 | 0 | 2 | 2 | 0 | 1 | 2 | 0 | 2 |
| 3 | 250 | 10 | 3 | 250 | 5 | 3 | 400 | 5 |
| 4 | 400 | 5 | 4 | 400 | 5 | 4 | 0 | 3 |
| 5 | 500 | 3 | 5 | 600 | 5 | 5 | 600 | 5 |
| 6 | 0 | 2 | 6 | Ventilation | 5 | 6 | Ventilation | 5 |
| 7 | Ventilation | 3 | | | | | | |

* W: watt

**6. Digestion times**

Upon establishment of the best oxidizer and microwave output, digestion times were examined. The most ideal digestion conditions about these three factors were defined. For safety reason, the digestive vessel was kept leaving for 30 minutes after the next microwave treatment.

**7. Recovery ratio test**

Authentic Cd standard solution was added to the fish sample to check the recovery rate, and more detailed exploration is required of the determination result of ICP-AES.

## Results and Discussion

**1. Influence of oxidizer and microwave output on determination result of Cd**

The determination results of the liver and the kidney are shown in Fig. 1 and Fig. 2, respectively. Selection of an oxidizer gave big difference in the concentration of Cd. The highest value was obtained by nitric acid + hydrogen peroxide, followed by nitric acid, nitric acid + perchloric acid and nitric acid + sulfuric acid in this order. The same tendency was seen in both samples of the liver and the kidney. In addition digestibility difference clearly depended on the usage of an oxidizer. Although the organic matter was able to digest well when sulfuric acid was used[11], addition of 1ml sulfuric acid to 5mL nitric acid showed apparent Cd decrease than by 5 mL nitric acid alone in any diet or biological sample. The reason was supposed to the loss of gas from the vessel due to their intense chemical reaction; moreover the viscosity of sulfuric acid interrupted physically the determination by ICP-AES. Furthermore, in the case of nitric acid alone, nitric acid + sulfuric acid and nitric acid + perchloric acid, a lot of yellow digestion gas escaped when vessel was opened. Since the inner pressure of a digestion vessel kept high for a long time when perchloric acid was used, it was diluted to a final volume of 20 mL after one hour cooling. On the other hand there was no gas loss when hydrogen peroxide was added

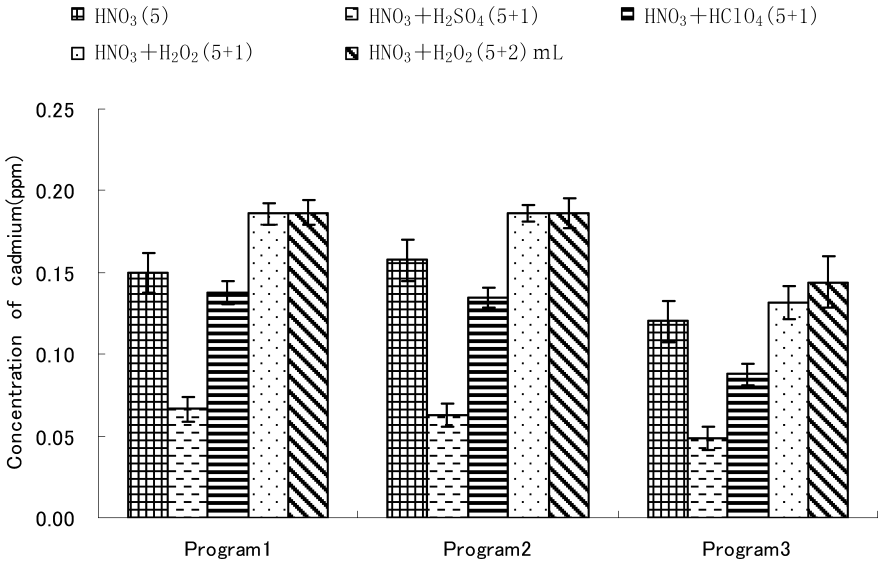


**Fig. 1.** Cd concentration in liver by different oxidizers and programs.

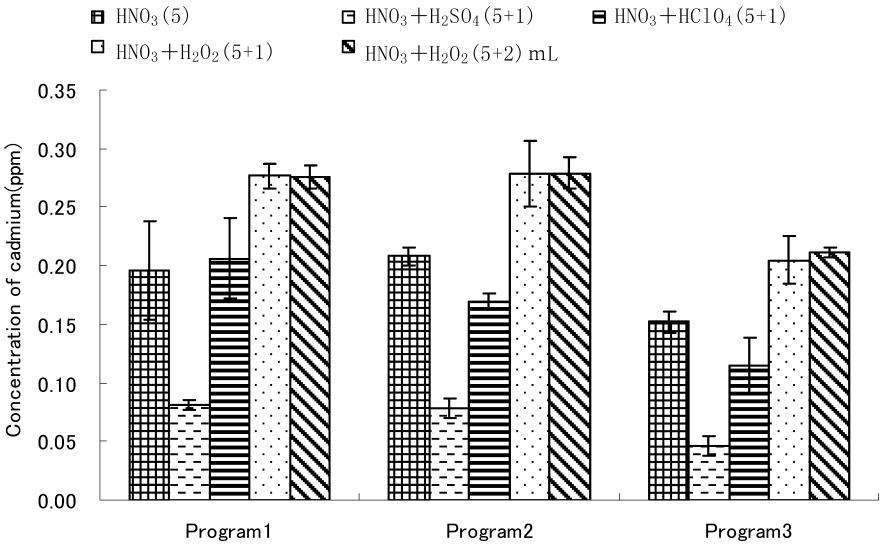


**Fig. 2.** Cd concentration in kidney by different oxidizers and programs.

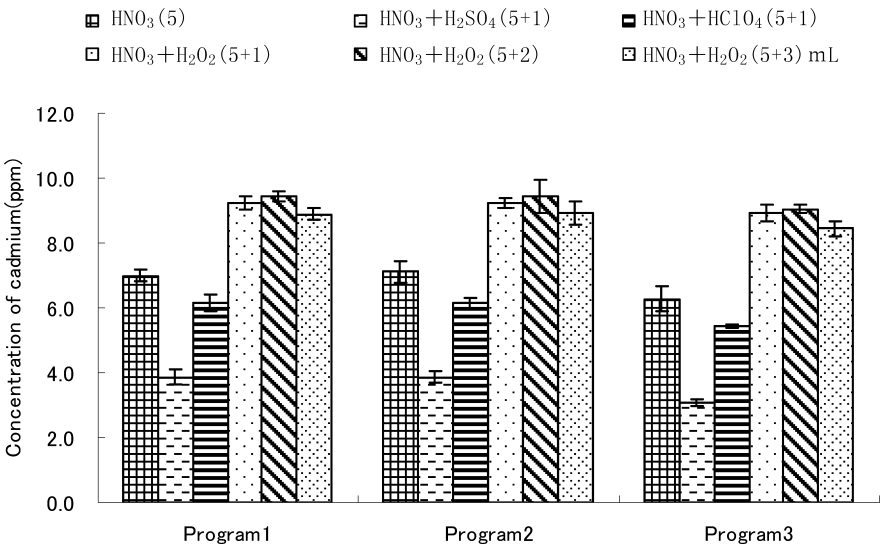


**Fig. 3.** Cd concentration in Diet A by different oxidizers and programs.

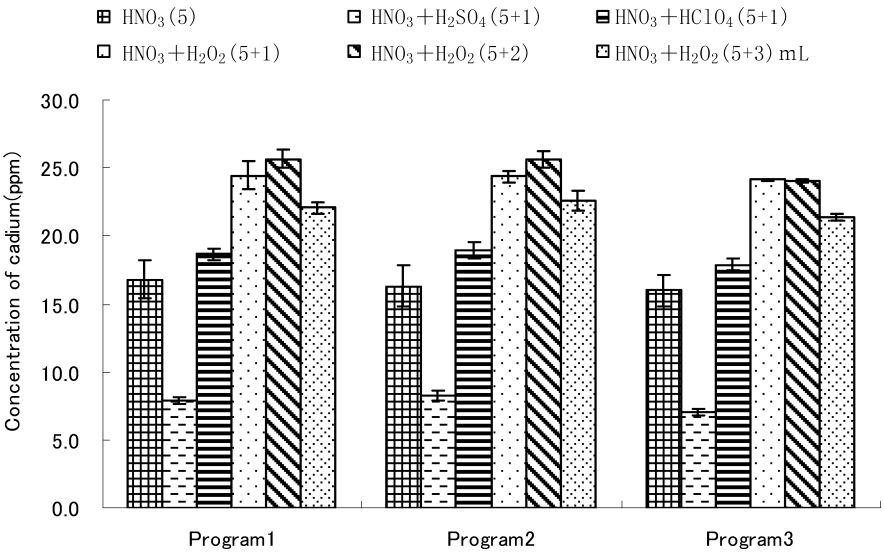


**Fig. 4.** Cd concentration in Diet B by different oxidizers and programs.

and further almost transparent and colorless digested liquid was obtained. It was thought that the sample was digested completely without any loss. When hydrogen peroxide was added and Program 2 was used, Cd concentration determined by ICP-AES in the liver and the kidney increased about 33% and 34%, respectively. From these results, nitric acid + hydrogen peroxide were chosen for the oxidizer of digestion. In addition the determination result was compared after 1–2 mL hydrogen peroxide was added to biological materials. The levels of cadmium were 0.1865 ppm and 0.1859 ppm in liver, and were 0.2782 ppm and 0.2789 ppm in kidney, respectively. Since these was almost no difference between both condition, it was thought that 1mL hydrogen peroxide was sufficiency.

Among the output of microwave we tested, Cd concentration of kidney and liver, always showed the highest value (0.1865 ppm and 0.2782 ppm) by Program 2. Therefore, digestion of biological materials was decided to carry out by Program 2, and was able to process it promptly (22 min). Though we focused on the digestibility of internal organs in this study, the digestive condition set up could also be applied to edible portions, such as muscle and the skin, because they consisted of a little more easily digestible compounds than internal organs we tested.

The results of the diet are shown in Fig. 3 and Fig. 4. Lyophilized diet for rainbow trout contained organic ingredients, such as proteins, starch and oils, in higher concentration than biological test samples. Large volume of reaction gas was generated during acid digestion and it was more difficult to decompose the diet than raw biological materials. Although the efficiency of ①nitric acid + hydrogen peroxide, ② nitric acid alone, ③nitric acid + perchloric acid,④nitric acid + sulfuric acid looked almost the same as observed in the biological materials by aforesaid order, addition of double volume of hydrogen peroxide gave us a little higher measured values. While in the case of 3ml hydrogen peroxide addition showed downward tendency conversely, because the absorption efficiency of microwave energy was supposed to

**Table 4** Discussion of times with Cd concentration by microwave digestion ($n=4$)

| Times | Liver*1 (ppm) | Kidney*1 (ppm) | Diet A*2 (ppm) |
|---|---|---|---|
| Once | 0.18±0.02 | 0.28±0.02 | 9.33±0.01 |
| Twice | 0.17±0.01 | 0.28±0.02 | 8.25±0.18 |
| Three times | 0.18±0.01 | 0.27±0.01 | 8.55±0.27 |

*1 Biological materials were digested with 5 mL $HNO_3$ and 1 mL $H_2O_2$ by Program 2.
*2 Diet was digested with 5 mL $HNO_3$ and 2 mL $H_2O_2$ by Program 1.

**Table 5** Recoveries of cadmium by different oxidizers ($n=4$)

| Sample | Digestive oxidizers | | Not added (ppm) | Added (ppm) | Found (ppm) | Recoveries (%) | CV (%) |
|---|---|---|---|---|---|---|---|
| | $HNO_3$ (mL) | $H_2O_2$ (mL) | | | | | |
| Liver 0.82 g | 5 | — | 0.16 | 0.25 | 0.38 | 89±8.17 | 9.17 |
| Liver 0.82 g | 5 | 1 | 0.18 | 0.25 | 0.45 | 107±2.12 | 2.54 |
| Kidney 0.82 g | 5 | — | 0.21 | 0.96 | 1.10 | 92±5.09 | 5.52 |
| Kidney 0.82 g | 5 | 1 | 0.21 | 0.96 | 1.33 | 107±4.06 | 3.77 |
| Diet A 0.20 g | 5 | 1 | 9.27 | 6.84 | 15.90 | 97±5.72 | 5.91 |
| Diet A 0.20 g | 5 | 2 | 9.42 | 6.84 | 16.27 | 100±2.76 | 2.75 |
| Diet B 0.20 g | 5 | 1 | 24.39 | 10.32 | 34.29 | 96±7.47 | 7.79 |
| Diet B 0.20 g | 5 | 2 | 25.37 | 10.32 | 35.27 | 97±3.44 | 3.55 |

[Recoveries=〔concentration of added sample−concentration of not added sample〕/concentration of added]

become small when oxidizer concentration was too high. However, Cd concentration obtained by Program 1 and 2 were almost the same as 9.4322 ppm and 9.4291 ppm, the appearance of two resultants looked quite different ca., transparent and a little muddy. The latter sample was not supposed to be digested completely. Therefore, it was decided to use Program 1 for digestion of diet.

As mentioned above, it turned out that nitric acid 5 mL+hydrogen peroxide 1 mL was applicable to digestion of biological materials under Program 2, and nitric acid 5 mL+hydrogen peroxide 2 mL to digest diet under Program 1.

### 2. Influence of repeated digestion on Cd concentration

It was found that from above test when digestion was performed once, combination of the mixed acids of 5 mL nitric acid and 1 mL hydrogen peroxide and the microwave processing by Program 2 was the best digestion condition. Further digestion was repeated on the same conditions to see if improvement would occur in the color tone of muddiness of digestion liquid, the existence of a residual substance, and the determination result of ICP-AES as shown in Table 4. However, no obvious refinement was obtained in concentration or accuracy of Cd determination.

### 3. Addition and recovery test

The addition and recovery rate was discussed based on the determination result shown above. The result compared with the negative control sample is shown in Table 5. In the case of the biological materials, Cd recovery rates was 89–92% when adding nitric acid only. When 1 mL hydrogen peroxide was added and processed, the recovery rates improved to 100%. Moreover, Diet A (low Cd concentration) was digested by hydrogen peroxide 2 mL, the recovery rate improved from 97% to 100%, too. The result of this addition and recovery test was in agreement the result of the abovementioned oxidizer selection test. Although the same recovery rate was obtained with Diet B (high Cd concentration), irrespective the volume of hydrogen peroxide added, coefficients of variation (CV) of 4 times fell down 54% when hydrogen peroxide 2 mL was added, as shown in Table 5. From coefficient of variation (2.54–3.77%) and standard deviation (2.12–4.06%), the error span of digestion by the selected method seemed negligible. Due to the little variation in the determined value and the good recovery rate, the proposed procedure could be the optimum for the pretreatment for both of fish meals for aquaculture feeds and fish body sample.

## Conclusion

1) The microwave digestion with 5 mL nitric acid and 1 mL hydrogen peroxide was able to digest biological materials, such as the internal organs of fish quickly.
2) The fish meals for aquaculture feeds was digested in 30 minutes by microwave treatment with 5 mL nitric acid and 2 mL hydrogen peroxide.
3) Our proposed quick and high sensitive procedure gave the good recovery rates of 97–107% with 2.54–3.77% CV on the biological materials.

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## ICP-AES による魚体と飼料中カドミウム定量に及ぼすマイクロ波分解条件の影響

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健全な水産食素材を創出する研究の一環として，複雑な生体成分を含む魚体試料中および配合飼料中の有害重金属 Cd を正確，かつ迅速簡便に測定できる分解条件を設定することを目的とした。数種類の酸化剤を用いて，その使用量，配合割合，ならびにマイクロ波出力の違いが Cd 測定結果に及ぼす影響を検討した。魚の内臓では硝酸 5 mL と過酸化水素 1 mL を用い，出力プログラム 1 により迅速に分解することができた。しかし養魚用固形配合飼料は魚体とは構成成分がかなり違うので，硝酸は同量で良かったが過酸化水素は魚体試料の 2 倍量添加しないと，正しい値が得られないことが判明した。つまり飼料から魚体へ移行する重金属を測定する際に，同一条件で両者の分解を行うと正しい結果が得られないが，本報で述べた条件を用いることにより解決できることが分かった。

**キーワード**：マイクロ波分解，酸化剤，マイクロ波出力，カドミウム，魚，飼料，ICP-AES