



In Vitro Experiment to Evaluate ^{137}Cs Dissolution in the Digestion Process of Mushrooms

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ABSTRACT

Background: Several studies have reported that wild mushrooms contain high amounts of radioactive cesium (^{137}Cs). After the Fukushima Daiichi Nuclear Power Plant Accident, a significantly high concentration of ^{137}Cs has been detected in wild mushrooms, and their consumption may be the cause of the chronic internal exposure of local consumers to radioactivity. Therefore, an accurate evaluation of the internal radioactivity resulting from mushroom ingestion is needed.

Materials and Methods: The ^{137}Cs elution rate through the cooking and digestion stages was evaluated using *in vitro* experiments. The edible mushroom *Pleurotus djamor* was taken as a sample for the experiments. The mushrooms were cultivated onto solid media containing ^{137}Cs . We evaluated the internal dose based on the actual conditions using the elution rate data. For various cooking methods, the results were compared with those of other wild edible mushrooms.

Results and Discussion: From the elution experiment through cooking, we proved that 25%–55% of the ^{137}Cs in the mushrooms was released during soaking, boiling, or frying. The results of a simulated digestion experiment after cooking revealed that almost all the ^{137}Cs in the ingested mushrooms eluted in the digestive juice, regardless of the cooking method. The committed effective dose was reduced by 20%–75% when considering the dissolution through the cooking process.

Conclusion: We found that cooking lowers ^{137}Cs concentration in mushrooms, therefore reducing the amount of radioactivity intake. Besides, since there were differences between mushroom types, we demonstrated that the internal exposure dose should be evaluated in detail considering the release of ^{137}Cs during the cooking stages.

Keywords: Mushroom, Radioactive Cesium, Internal Dose, Existing Exposure, Ingestion, *In Vitro* Experiment

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Introduction

After the Fukushima Daiichi Nuclear Power Plant Accident, a study reported that a significantly high concentration of radioactive cesium (^{137}Cs) was detected in wild mushrooms [1]. Although the disaster occurred 8 years ago, the shipment of wild mushrooms is restricted in 55 out of 59 municipalities of the Fukushima Prefecture [2]. Presently, there is no concrete decontamination plan for forests areas, and a high ^{137}Cs concentration may continue to be detected in wild mushrooms in the long term.

Japan has established the standard limits for ^{137}Cs in general food as 100 Bq/g and all the food is inspected for radioactivity before shipment [3]; hence, mushrooms exceed-

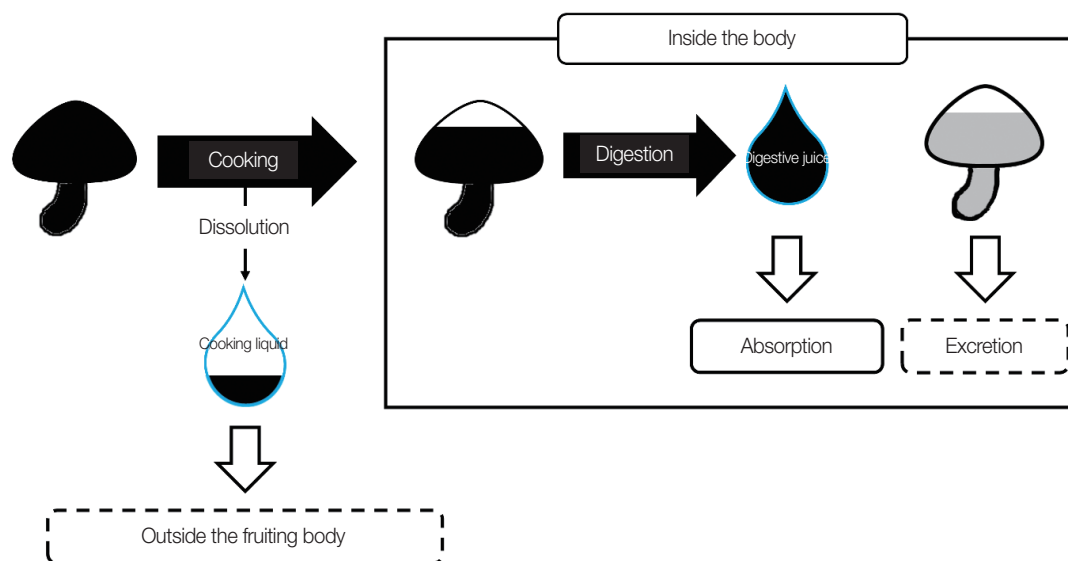


Fig. 1. A flow diagram depicting the transfer of ^{137}Cs in mushrooms from the cooking stage to body absorption.

ing the maximum limits are excluded from being shipped and are not available in markets. However, local consumers eat self-picked wild mushrooms as a seasonal food, and self-consumption of wild mushrooms is not regulated. Therefore, the consumption of wild mushrooms might be the cause of the chronic internal exposure of local consumers to radioactivity. For this reason, an accurate evaluation of the internal radioactivity resulting from mushroom ingestion is needed.

Fig. 1 shows a flow diagram depicting the transfer of ^{137}Cs in mushrooms from the cooking stage to body absorption. Mushrooms are usually consumed cooked. With the method currently used for the estimation of the internal dose, the dilution coefficient after cooking is 1. However, Nabeshi et al. [4] and Tamaguchi et al. [5] reported that more than 50% of the ^{137}Cs in mushrooms is eluted into the cooking water by soaking dry mushrooms or after heat-treating, microwaving, or freezing treatments. ^{137}Cs may be eluted from mushrooms through other cooking methods. Thus, it was hypothesized that the uncertainty of the internal exposure evaluation might be reduced by considering the dissolution at the cooking stage. In contrast, the behavior of ^{137}Cs after ingestion is based on the ICRP Publication 100 (Human Alimentary Tract Model for Radiological Protection) [6], and it is believed that all the ingested ^{137}Cs is absorbed into the blood. However, since mushrooms contain a large amount of dietary fiber, most of them are relatively indigestible. Therefore, the rate of cesium eluting from mushrooms into digestive juices may be low, and it is possible that the ^{137}Cs in mushrooms is less absorbed than previously thought.

In this study, we aim to reduce the uncertainty of the internal dose estimation by the chronic intake of contaminated mushroom. The internal exposure dose was evaluated taking into account the dissolution rate of ^{137}Cs in the cooking and digestion processes based on an *in vitro* simulated digestion experiment. Wild edible mushrooms were used to compare the ratio of dissolution in the cooking and digestion stage for various cooking methods.

Materials and Methods

1. Samples

1) Cultivation of *Pleurotus djamor* containing ^{137}Cs

Due to the difficulty in collecting wild mushroom with similar ^{137}Cs concentrations, we used cultivated mushrooms to obtain data of the dissolution rate through cooking and the dissolution rate in the digestive juice, since the ingredients of the artificial growth medium were controlled. We used *Pleurotus djamor*, which grows relatively fast among cultivated mushrooms.

The culture medium composition was as follows: 10 kg (dry weight) of sawdust, 3.3 kg of wheat bran, and up to 70% of water. Benstrate wettable powder (0.2 g) was added as an insecticide. The ^{137}Cs -containing media were prepared by replacing a part of the substrate with fallen leaves collected at the Yamakiya district in Kawamata-machi, Fukushima Prefecture, 40 km northwest of the Fukushima Daiichi Nuclear Power Plant (FDNPP). The medium was mixed with the leaves in a dry weight ratio of 8 to 1. These leaves contained

adhered ^{137}Cs or accumulated cesium released from the FD-NPP. The ^{137}Cs concentration in the leaves and the medium (dry weight) were $2,321 \pm 9$ Bq/kg and 242 ± 27 Bq/kg, respectively. The medium was placed into 850 cm³ plastic wide-mouth bottles with 500 g in each to make 49 bottles. A hole was created with a boring machine in the center of the culture medium for seeding. The bottles containing the medium were capped and cooled down overnight. The bottles were then sterilized in a mushroom substrate sterilizer (FKS-U10W-C; Fuji High-Tech Co. Ltd., Nagoya, Japan). The sterilization conditions were 120°C for 120 minutes, followed by 118°C for 30 minutes. The young fruiting bodies of *P. djamor*, cultivated in advance, were scrapped off under aseptic conditions and used to inoculate the holes previously made in the culture medium.

The mushroom was cultivated in an incubator at 26°C under lighting conditions in the whole day and at 80% of relative humidity. Approximately 2 weeks after inoculation, the mycelium spread throughout the culture medium. To stimulate growth of young fruiting bodies, approximately 2 cm-thick of culture medium was scraped off uniformly from the surface. Fruiting bodies were visible a few days later. The size and dryness of the fruit body and the shape of the grain support were assessed; mushrooms whose mosaics were 3 cm or larger and showed signs of aging were harvested. The culture conditions were maintained for the rest of the fruiting bodies to grow until ready to be harvested. The fruiting bodies were used immediately or stored in a zippered plastic bag in a refrigerator.

2) Wild mushroom collection

Edible wild mushrooms *Sarcodon aspratus* and *Lyophyllum fumosum* were collected at the Yamakiya district in 2016 and 2017, respectively. The mushroom ^{137}Cs concentrations were $72,948 \pm 80$ Bq/kg (dry weight) and 280 ± 5 Bq/kg (fresh weight) for *S. aspratus* and *L. fumosum*, respectively.

2. Cooking Procedures

The hard tips of the mushrooms were removed before cooking. The samples were cooked or processed as follows:

- (1) Chilled: placed in plastic zipper bags and refrigerated right after harvest;
- (2) Soaked: fresh mushrooms were dried in an incubator at 50°C and further soaked in water for 15.5 hours in a refrigerator;
- (3) Boiled (fresh sample): fresh mushrooms were boiled in

350 g of distilled water for 5 minutes and then the water was drained;

- (4) Boiled (dry sample): mushrooms were air-dried until completely dried (for 2–7 days) and boiled as indicated in process 3;
- (5) Deep-fried: mushrooms were fried in 350 g canola oil at 160°C for 5 minutes right after harvest. Process 1 corresponds to the control. Processes 2, 3, and 5 were set to investigate the effect of the drying and heat treatments using water or cooking oil, respectively, on ^{137}Cs elution. In addition, for processes 2 and 3, we compared the boiling after sampling with the one with the dry product.

3. In vitro Digestion

An *in vitro* digestion experiment was simulated on fruiting bodies after each cooking procedure. The experiment was based on the method by Minekus et al. [7]. Approximately 10 g of mushrooms was treated in a simulated digestion experiment. The detailed method is described below.

1) Oral phase

The oral bolus was mixed with simulated gastric fluid to obtain a 50:50 food-to-simulated digestive juice final ratio (v/v). HCl (1 M) was added to reduce the pH to 3.0 and the mixture was incubated for 2 hours at 37°C in a tube rotator mixer to mix the food and the digestive juice. The pH was checked after 1 hour and HCl was added as necessary.

2) Stomach phase

The oral bolus was mixed with simulated gastric fluid to obtain a 50:50 food-to-simulated digestive juice final ratio (v/v). HCl (1 M) was added to reduce the pH to 3.0 and the mixture was incubated for 2 hours at 37°C in a tube rotator mixer to mix the food and the digestive juice. The pH was checked after 1 hour and HCl was added as necessary.

3) Intestinal phase

The gastric chyme was mixed with simulated intestinal fluid at a 50:50 ratio (v/v). Next, 1 M NaOH was added to adjust the pH to 7.0 and the mixture was incubated at 37°C for 2 hours. The pH of the samples was checked after 1 hour and NaOH was added as necessary.

The digests were centrifuged at 3,500 rpm (2,910 g) for 5 minutes to pellet the undigested mushroom, and the supernatants were used as samples. The centrifugation procedure was repeated until there was no more supernatant.

To understand the pharmacokinetics of ¹³⁷Cs in digestion, the elution rates for each digestion stage were compared. The treatment of the sample was performed under three conditions: (1) oral phase only, (2) oral and gastric phases, and (3) oral, gastric, and intestinal phases. The solid (food mass) and the liquid portions (simulated digestive juice) were separated using filter paper and each was used as a sample.

The elution rates were compared for each digestion method to evaluate the factors affecting the elution of ¹³⁷Cs in each digestion stage. We added three types of reagents after the mincer treatment: (i) distilled water, (ii) simulated digestion fluid without enzyme, and (iii) simulated digestion fluid with enzyme; we then conducted the simulated digestion experiments and centrifuged the samples to separate the solid and liquid portions. Determinations were conducted with the liquid portion.

The liquid part was used as a measurement sample. The effects of mechanical crushing were examined from (i), the effects of digestive juices were compared from (i) and (ii), and the effects of digestive enzymes were examined from (ii) and (iii).

4. Determination of ¹³⁷Cs Concentration and Calculation of the Elution Rate

Each sample was placed in a U-8 container and dried in an infrared oven. The ¹³⁷Cs concentrations in the medium, fresh cooked mushrooms, soup, stock liquid, cooking oil, digestive juice, and digested mushroom were determined using the calibrated High Purity Germanium radiation detector (GEM40-76; SEIKO EG&G, Tokyo, Japan) for 80,000 seconds. The natural background radiation was reduced with a 10 cm-thick lead shield around the detector. The gamma-ray analysis software Gamma Studio (SEIKO EG&G) was used to calculate the ¹³⁷Cs concentration. The ¹³⁷Cs dissolution rate in the cooking and digestion stages were determined as a ratio of the decrease in radioactivity before and after treatment and the activity before treatment.

Results and Discussion

1. Dissolution of ¹³⁷Cs by Cooking in Water or Oil

Table 1 shows the changes in the weight of *P. djamor* and the ¹³⁷Cs dissolution rate due to cooking. Except for frying, mushrooms absorbed water and their weight increased 2.6 to 6.5 times compared to the initial amount. In contrast, the

Table 1. ¹³⁷Cs Concentrations of *Pleurotus djamor* and its Dissolution Rate in Digestive Juice

	Fresh weight (g)	Cooked weight (g)	Weight change (%)	¹³⁷ Cs activity (Bq)	Dissolution rate (%)
Chilled	-	-	-		
Sample 1				1.040 ± 0.026	74.8 ± 1.9
Sample 2				0.931 ± 0.023	67.4 ± 1.7
Sample 3				1.023 ± 0.024	73.9 ± 1.8
Average				0.998 ± 0.059	72.1 ± 4.1
Soaked	33.31	204.78	615		
Sample 1				0.173 ± 0.015	87.6 ± 7.6
Sample 2				0.208 ± 0.016	102.2 ± 7.7
Sample 3				0.216 ± 0.016	107.3 ± 7.9
Average				0.199 ± 0.023	98.9 ± 10.5
Boiled (fresh sample)	37.40	97.08	260		
Sample 1				0.253 ± 0.015	100.8 ± 5.9
Sample 2				0.250 ± 0.014	98.2 ± 5.7
Sample 3				0.232 ± 0.013	88.8 ± 5.0
Average				0.245 ± 0.012	95.9 ± 6.3
Boiled (dry sample)	20.38	132.45	650		
Sample 1				0.152 ± 0.013	92.9 ± 8.0
Sample 2				0.192 ± 0.013	102.0 ± 6.9
Sample 3				0.195 ± 0.013	101.3 ± 6.6
Average				0.180 ± 0.024	98.7 ± 5.1
Deep-fried	67.32	55.04	82		
Sample 1				0.890 ± 0.024	99.4 ± 2.7
Sample 2				0.824 ± 0.024	92.5 ± 2.7
Sample 3				0.907 ± 0.024	101.2 ± 2.7
Average				0.874 ± 0.044	99.7 ± 4.6

Values are presented as mean ± standard deviation.

The treatment of the sample was performed under three conditions: (Sample 1) oral phase only, (Sample 2) oral and gastric phases, and (Sample 3) oral, gastric, and intestinal phases.

mushroom weight decreased by 20% after frying. The dissolution ratio for *P. djamor* during cooking ranged from 25% to 55%.

Nabeshi et al. [4] and Yamaguchi et al. [5] reported that more than 50% of the ¹³⁷Cs in the fruiting body was released by soaking the dry mushroom, but according to our results the percentage was as low as 26%. This difference might be due to the surface area of the sample. Nabeshi et al. and Yamaguchi et al. cut the fruiting bodies into 5 mm-thick sections and four divisions before drying. However, in this study, the number of cuttings of the fruiting bodies was low and the surface area was small. From these facts, we concluded that ¹³⁷Cs leached out when re-watering the dried mushrooms and consequently, the intake of ¹³⁷Cs was reduced. Moreover, when the fruiting body was cut finely before reconstitution, the rate of elution into the immersion liquid increased, and

the ^{137}Cs intake might be further reduced.

The elution rate in boiled mushrooms was approximately 10% higher compared to the air-dried ones. Comparing the change in weight before and after cooking, the fresh mushrooms boiled absorbed approximately 1.5 times their weight, while the dry mushrooms boiled absorbed approximately 5.5 times their weight. Therefore, ^{137}Cs eluted into water upon heating, but dried mushrooms contained less water in the fruiting body before cooking, and the water used for boiling containing ^{137}Cs was retained in the fruiting body. As a result, the amount of ^{137}Cs emitted from the mushroom decreased and the dissolution rate decreased.

The change rate of ^{137}Cs concentration in mushrooms before and after cooking was 28.8% for mushrooms boiled immediately after harvesting, while that of air-dried and boiled samples was 8.4%. Air-dried mushrooms showed a 20% higher reduction in the ^{137}Cs concentration compared to freshly cooked samples. These results suggest that boiling may reduce the amount of ^{137}Cs in mushrooms by approximately 55%, and that it is possible to further reduce this percentage by cooking dried mushrooms after their reconstitution in water.

The elution rate was similar for the deep-fried and dried mushrooms, but the change rates of ^{137}Cs concentration were 94.4% and 14.4%, respectively. The mushroom weight increased about six times due to the absorption of water, and the ^{137}Cs concentration was reduced in the reconstitution of dried mushrooms compared to the chilled ones.

The elution rate of ^{137}Cs was approximately half that of boiled cooking compared to fried cooking. This may be due to the fact that ^{137}Cs in the mushroom is water-soluble. In addition, Watanabe et al. [8] studied ^{137}Cs removal in wild edible plants and showed that the ^{137}Cs elution rate in the case of fried edible plants is lower than that of boiled ones.

2. Dissolution in the Digestive Juice of ^{137}Cs in Cooked Mushrooms

The cooked mushrooms were *in vitro* digested. The sample consisted of 10 g of fruiting bodies harvested from the culture medium and used for three experiments under the same condition.

Table 1 shows ^{137}Cs concentration and the dissolution rate in the digestive juice. The elution rate was almost 100% except for the samples stored under refrigeration. This result implies that almost all the ^{137}Cs contained in the cooked mushrooms was eluted in the digestive juice. Unlike the oth-

er samples, the refrigerated ones were not damaged by heating or drying; thus, the fiber remained and the size of the crushed pieces after the mincer treatment was not uniform since some samples were slightly larger. As described in the previous section, mechanical crushing has a large effect on the ^{137}Cs elution rate. For that reason, the elution rate might be lower because of the difference in the sample size. In fact, the elution rate was $93 \pm 2\%$ after removing the large pieces from the refrigerated stored mushrooms crushed with the mincer and standardizing the sample size.

Fig. 2 depicts the comparison of the dissolution rate during cooking and the residual rate based on the amount of ^{137}Cs in mushrooms after harvesting. ^{137}Cs eluted through cooking did not contribute to the internal exposure because the body does not uptake it. In addition, ^{137}Cs remaining in mushrooms was excreted immediately after digestion, and therefore had a minor contribution to the internal exposure. Therefore, the ratio that mainly contributed to the internal exposure was only the blood absorption ratio. The remaining rate of ^{137}Cs was 1%–2% for all the cooking procedures compared to that in mushrooms stored in the refrigerated. We found that almost all the ^{137}Cs contained in the mushroom

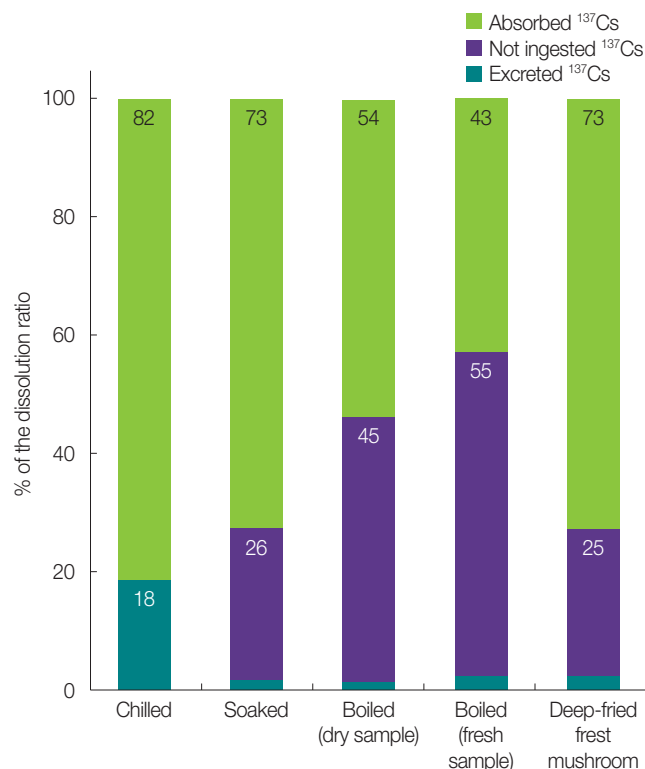


Fig. 2. Comparison of the dissolution rate during cooking and the residual rate based on the amount of ^{137}Cs in mushrooms after harvesting.

Table 2. Measured Value at Each Digestion Step and Dissolution Rate into Digestive Fluid

	Oral phase		Stomach phase		Intestine phase	
	Indigestion	Digestive juice	Indigestion	Digestive juice	Indigestion	Digestive juice
Mushroom weight before experiment	10.328	-	10.358	-	10.352	-
Sample weight (g)	3.696	0.055	3.311	5.194	3.620	17.071
^{137}Cs concentration (Bq/g)	0.429 ± 0.007	2.813 ± 0.216	0.188 ± 0.006	0.191 ± 0.004	0.217 ± 0.004	0.094 ± 0.002
Supernatant weight (g)	-	2.466	-	20.856	-	48.203
^{137}Cs concentration before dry (Bq/g)	-	0.063 ± 0.005	-	0.048 ± 0.001	-	0.033 ± 0.001
Dissolution ratio into digestive juice (%)	22.3 ± 1.7		51.5 ± 1.1		83.2 ± 1.4	
Dissolution ratio by digestion stage (%)	-		29.2 ± 2.0		31.7 ± 1.8	

Values are presented as mean \pm standard deviation.

after cooking was absorbed into the blood. Subsequently, the dissolution rate at the cooking stage was 25%–55%, indicating that cooking might reduce the ^{137}Cs intake.

Mushrooms are difficult to digest, but the amount of ^{137}Cs remaining after digestion is 1%–2% of the initial, and almost all the ^{137}Cs contained in the mushrooms after cooking is digested. After ^{137}Cs is transferred to the liquid, it is absorbed by the body. However, the amount of ^{137}Cs intake might be reduced by 25%–55% through the cooking procedure, indicating that cooking is an effective way to reduce the internal exposure dose.

3. Evaluation of the Dissolution Rate and the Digestion Effect at Different Digestion Stages

A simulated digestion experiment was performed using *L. fumosum* to examine whether ^{137}Cs was eluted in the mouth, stomach, or intestine through digestion. Table 2 shows the results and the calculation of the dissolution rates for each stage. In the mouth stage, the treatment time was set to 2 minutes, while in the stomach and intestinal stages, it was 2 hours for each one. Although these times were different, each dissolution rate (20%–30%) was similar.

The digestion includes mechanical crushing by chewing, chemical digestion by digestive juices, and biological digestion by enzymes. In order to investigate the effects of each process, a simulated digestion experiment was performed using three kinds of reagents on *P. djamor* crushed with a mincer: water, digestive juice, and digestive juice with enzyme. The dissolution rates were as follows: $67 \pm 3\%$ in water, $72 \pm 1\%$ in digestive juice, and $83 \pm 10\%$ in digestive juice with enzyme. Therefore, the mechanical crushing through the mincer treatment affected the elution of ^{137}Cs .

Next, in order to investigate the effects of the mechanical crushing in more detail, three different size of dry *P. djamor* crushed to different sizes were used in the simulated oral

phase, and the dissolution rates in digestive juices were compared. Comparing the elution rate of the mincer-crushed sample of 25 mm^2 to 1 cm^2 , which is the standard crush size in the simulated digestion experiment, the elution rates of a 2 cm^2 sample and a sample cut to 2 cm wide and 5 cm long were 0.98 in each case. The values of elution rates were 0.98 ± 0.09 and 1.06 ± 0.09 , respectively, and were not significantly different from the dissolution rate of the mincer-crushed sample. In the dried mushrooms, the crushed size did not contribute to the elution of ^{137}Cs . These results suggest that the cell wall damage caused by drying and further reconstitution is greater than the physical disruption by a mincer.

4. Comparison of the Dissolution Rate for Each Type of Mushroom

Wild *L. fumosum* and *S. aspratus* collected in the Fukushima Prefecture were cooked in the same manner as that for *P. djamor*, and a simulated digestion experiment was performed with those mushrooms. *L. fumosum* was refrigerated after collection because this mushroom senesces rapidly and is easily self-digested and damaged. In contrast, *S. aspratus* was dried after harvest and further rehydrated.

Table 3 shows the dissolution rate results. The elution rate after cooking was three times higher for *S. aspratus* than for *P. djamor* mushrooms. *P. djamor* was mostly composed of a cap, and its weight increased before and after rehydration, but no morphological change was observed. In contrast, *S. aspratus* mushrooms consisted of a cap and a stem, and the inside of the dried stem was hollow, and disappeared after rehydration. The mycelium cells might be more susceptible to damage during the drying and reconstitution treatments, and the elution rate in the reconstituted juice varied depending on the fruiting body structure.

The elution rate in the digested juice was almost the same

Table 3. Elution Rate Comparison by Mushroom Type

	Chilled		Soaked (dry sample)	
	<i>Pleurotus djamor</i>	<i>Lyophyllum fumosum</i>	<i>Pleurotus djamor</i>	<i>Sarcodon aspratus</i>
Dissolution rate by cooking (%)	-	-	26±9	74±0
Dissolution rate by digestion (%)	72±4	83±1	99±11	97±1
Absorbed ratio (%)	72	83	73	26
Not ingestion ratio (%)	-	-	26	74
Excreted ratio (%)	28	17	1	1

Values are presented as mean ± standard deviation.

Absorbed ratio = (100 – Dissolution rate by cooking) × Dissolution rate by digestion. Not ingestion ratio = Dissolution rate by cooking. Excreted ratio = 100 – Dissolution rate by cooking – Absorbed ratio.

for cooked *L. fumosum* and *P. djamor*, but the elution rate for *L. fumosum* was approximately 10% higher than that for *P. djamor*. *S. aspratus* and *P. djamor* mushrooms were both dried and then rehydrated, and the cell damage due to mechanical disruption that had the greatest effect on cesium release into digestive juices was comparable. However, ¹³⁷Cs diffused and leaked out of *L. fumosum*, which showed an autolysis effect because its mycelial cells were more susceptible to damage than those of *P. djamor*.

These results suggest that mushrooms differ in the ¹³⁷Cs elution rate at the cooking and digestive juice stages depending on the type of mushroom.

5. Estimation of the Committed Effective Dose after Mushroom Ingestion

The current conventional calculation method for the estimation of the committed effective dose after mushroom ingestion is shown in Equation (1) and the calculation method used in this study is shown in Equation (2).

$$D (\mu\text{Sv}) = h \times A \times m \times F_m \times F_c \times 103 \quad (1)$$

$$D (\mu\text{Sv}) = h \times A \times m \times F_m \times (1 - R_c) \times R_d \times 103 \quad (2)$$

where

h = Dose coefficient (mSv/Bq);

A = ¹³⁷Cs concentration in the fresh mushroom (Bq/g);

m = Annual intake per person (g);

F_m = Market dilution factor;

F_c = Reduction factor due to cooking;

R_c = Dissolution rate by cooking;

R_d = Dissolution rate by digestion.

In this study, the dose was evaluated considering the dis-

solution rate into the digestive juice and comparing with the conventional method (Equation (2)). In the conventional method, the dilution factor for cooking was set to 1, but in this study, different values were used for different cooking methods using the dissolution rate obtained in the *in vitro* experiments. The dissolution rate of the refrigerated sample into the digestive juice was used as the dissolution rate of the sample without standardization of the crushed size in order to approximate to the actual eating conditions.

The committed effective dose was calculated assuming that an adult consumes 3,715 g of *P. djamor* per year with a ¹³⁷Cs concentration of 93 ± 25 Bq/kg. The annual intake of mushrooms per person is based on the “changes in annual household purchases of mushrooms” published by the Forestry Agency of Japan (2013) [9] and the “average household staff” published by the Ministry of Health, Labour and Welfare of the Japanese Government [3]. The consumption was calculated and the intake pattern was a chronic daily ingestion. It was assumed that for the dry cooking and after the mushrooms were reconstituted in water, the reconstituted juice was discarded and only the mushrooms were consumed. F_c , the market dilution factor, indicates the ratio of the consumption of contaminated food and the annual food intake by the consumer. In this study, the coefficient value was set to 1 under the assumption that all the mushrooms ingested by the subjects contained ¹³⁷Cs. Table 4 shows the calculated effective doses for each cooking method for *P. djamor* and *S. aspratus* intake.

The results of the present method changed with the cooking procedure. Our method, which considers the dilution due to cooking and the rate of elution into digestive juices, showed 27%–57% lower values for *P. djamor* and 75% lower for *S. aspratus* than the conventional method. The dissolution rate of the cooked sample into the digestive juice was almost 1 for both, suggesting that there is little uncertainty in evaluating the internal exposure dose during the digestion stage. In contrast, the dilution by cooking showed lower values in this method, 25%–55% for *P. djamor* and 74% for *S. aspratus*. Reduction of the intake by cooking directly affects the reduction of internal exposure dose, and has 25%–74% of uncertainty, which depends on the type of mushroom and the cooking method.

In order to evaluate the internal exposure dose according to the actual situation, it is necessary to consider the ¹³⁷Cs elution rate at the cooking and digestion stages, and this rate differs at each stage depending on the type of mushroom. It

Table 4. Committed Effective Doses by Storage and Cooking Method for Ingesting *Pleurotus djamar* or *Sarcodon aspratus*, Calculated by the Current and the New Methods

	<i>Pleurotus djamar</i> (93 Bq/kg)						<i>Sarcodon aspratus</i> (72,948±80 Bq/kg)					
	Chilled		Soaked		Boiled (fresh sample)		Boiled (dry sample)		Deep-fried		Soaked (dry sample)	
	Current	Present	Current	Present	Current	Present	Current	Present	Current	Present	Current	Present
Dilution factor by cooking (F_c , $1-R_c$)	1	1	1	0.74±0.09	1	0.45±0.01	1	0.55±0.01	1	0.75±0.06	1	0.26±0.00
Dissolution ratio in digestion stage (R_d)	1	0.72±0.04	1	0.99±0.11	1	0.96±0.06	1	0.99±0.05	1	0.87±0.04	1	0.97±0.01
Committed effective dose (μSv)	4.49±1.22	3.24±0.18	4.49±1.22	3.29±0.53	4.49±1.22	1.94±0.55	4.49±1.22	2.46±0.68	4.49±1.22	2.95±0.85	4.49±1.22	(3.52±0.00)×10 ³ (0.89±0.01)×10 ³

F_c , reduction factor due to cooking; R_c , dissolution rate by cooking; R_d , dissolution rate by digestion.

is necessary to obtain and analyze data on the dissolution rate for various kinds of mushroom and cooking methods.

Conclusion

The purpose of this research was to reduce the uncertainty in the estimation of the ¹³⁷Cs dose due to the intake of contaminated mushrooms. We estimated the dose considering the dissolution rate in cooking and digestion stages based on *in vitro* experiments.

In the process of cooking, 25%–55% of ¹³⁷Cs in the mushrooms was released into the liquid. In wild mushrooms, the elution rate at the cooking stage was three times higher than that in the cultivated mushrooms. The ratio of the unabsorbed form of ¹³⁷Cs in the ingested cooked mushrooms was only 1%. Regardless of the cooking method and the mushroom types, almost all the ¹³⁷Cs contained in the mushrooms was eluted in the digestive juice at the time of ingestion. Thus, we found that cooking could leach ¹³⁷Cs in mushrooms and reduce its intake.

From the results obtained on effective committed dose estimation, we found that the dilution effect due to cooking had an uncertainty of 25% or more. This result suggests that we may reduce the uncertainty of the internal exposure dose evaluation by calculating the dissolution rate at the cooking stage in detail.

Applying these methods to other mushrooms and obtaining more data would result in a more realistic evaluation of the internal dose through mushroom intake.

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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