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Consequences of ibotenic acid and muscimol during storage and processing of fly agaric"

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Change in Ibotenic Acid and Muscimol Contents in *Amanita muscaria* during Drying, Storing or Cooking*[1]

(*[1]Food Hygienic Studies of Toxigenic Basidiomycotina. III)

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It is believed by people in mountainous areas that toxins in *A. muscaria* are reduced by drying, storing or cooking. The main physiologically active substances, ibotenic acid (IBO) and muscimol (MUS), in *A. muscaria* were therefore investigated. IBO is readily transformed to into MUS through decarboxylation, and MUS is a hallucinogen. Drying *A. muscaria* in the sun or with a heater caused an increase of MUS in the mushroom, though a lot of precursor IBO was lost. It was suggested that the toxicity of the mushroom would be intensified by processing. IBO and MUS in the mushroom were stable on storage under dry or salt conditions. MUS increased in concentration while IBO decreased during heat-cooking, and the changes were more marked under acidic than alkaline conditions. However, general cooking (within 10 minutes) hardly reduced the toxic substances. Boiling or soaking *A. muscaria* in water caused most of the IBO and MUS in the mushroom to be released rapidly into the water.

Key words: イボテン酸 ibotenic acid; ムシモール muscimol; ベニテングタケ *Amanita muscaria*; 調理 cooking; キノコ中毒 mushroom poisoning; 幻覚 hallucination

Introduction

Fly agaric contains several toxic alkaloids, and the main bioactive components are ibotenic acid2)-5) (IBO) and muscimol51-9 (MUS).

Carbonated 5), 8-10), the decarboxylated product MUS is known to act on the central nervous system and cause mental confusion and hallucinations 5), 8, 9 On the other hand, IBO is also an umami component, (1) It is said that mushrooms are exceptionally delicious, and they are actually eaten in limited regions (2). There have been occasional reports of poisoning caused by consuming mushrooms mistakenly for edible mushrooms13).

* Food Hygiene Studies on Toxic Basidiomycetes (Part 3)

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In the previous report, I clarified the difference in toxicity of fly agaric from the concentration of IBO and MUS, but this time, from the viewpoint of accident prevention, IBO and changes in concentration due to cooking of MUS.

experimental method

1. Samples, reagents and equipment

1.1 Specimen

In September-October 1990, fly agaric mushrooms growing wild in the mountains of Yatsugatake Hara Village, Nagano Prefecture were collected, removed from dirt and soil, wrapped in newspaper one by one, and brought back to the laboratory on the same day or the next day. Stored refrigerated at 5°. As a refrigerated raw sample, it was used for each experiment within one week.

1.2 Reagents

Standard solutions of IBO and MUS Purified IBO' and Sigma MUS manufactured by Co., Ltd. was prepared with 70 v/v% methanol in five steps of 5, 10, 20, 30 and 50 ppm.

Methanol and Acetonitrile: for HPLC Wako Pure Chemical Industries

Manufactured by Kogyo Co., Ltd.

Special grade reagents such as sodium dodecyl sulfate and phosphoric acid

1.3 Apparatus and

Instruments High performance liquid chromatograph (HPLC): pump CCPE, detection Extractor UV-8010 Integrator SC-8010 Tosoh Corporation Shaking incubator: Model TN-303 Toyo Seisakusho Co., Ltd. Programmable incubator: TR-MC3 Trio Cy

Manufactured by Ensu Co., Ltd.

Blower constant temperature dryer FC-42D Toyo Seisakusho Co., Ltd. Homogenizer: Urtra-Turrax T-25 Janke & Kunkel

1.4 Measurement conditions 150

Analytical condition of HPLC

Column: IRICA RP-18T (ODS, 5 μm) 4.0 mm×25 cm,
Mobile phase: pH 2.2, 3 mM sodium dodesyl sulfate·20 mM phosphoric acid solution–methanol–acetonitrile (65 : 15 : 20, v/v),
Flow rate: 0.6 ml/min; Column temp.: 45°; Wave length: 210 nm;
Sensitivity: 0.02 AUFS; Injection vol.: 5 μl

1.5 Preparation of test solution15)

To prepare the test solution for the sample after each treatment, add 75 v/v% methanol to the dry sample, and add methanol to about 75 v/v% methanol to the raw sample, frozen sample, and liquid sample, and homogenize. After homogenization with , it was filtered with filter paper or centrifuged, the residue was washed with 75 v/v% methanol, and the volume was accurately adjusted according to the content of each experiment. After filtration through a membrane filter, the solution was diluted appropriately and used as the test solution for HPLC.

2. Experiment

2.1 Drying experiment 1

After weighing the refrigerated raw sample, it was dried in the sun for 3 days in the sun for 3 days. IBO and MUS in a dried sample (sunlight-I), a sample dried for 11 days due to unfavorable weather (sunlight-II), a sample dried by the radiant heat of a stove for 2 days (heater-III), and a control raw sample. was measured.

2.2 Drying experiment 2

Refrigerated raw samples (12 samples) were each divided vertically into 4 pieces, and 2 portions were used for the drying experiment, and one of the 2 portions was used for the control. 4) was used as one experimental sample. The experimental sample was weighed (g), and one of the dried experimental samples was dried in a dryer for each temperature and time (40°/40 hr, 50°/12 hr, 60%/ 10 hr, 80°/6 hr, 100°/6 hr). °/4 hr, 120°/3hr) enough to break by hand Air dried. IBO and MUS in the air-dried and control samples 2.3 was measured and compared.

Dry storage experiments

100 g of the refrigerated raw sample (three times) was freeze-dried, pulverized, stored in a desiccator containing silica gel as a desiccant, and stored at room temperature in the dark. In addition, the temperatures of IBO and MUS in the sample were measured.

100 g (three times) of the refrigerated raw sample was placed in a heat-resistant plastic bag, heated in a boiling bath for 10 minutes, cooled with tap water, added with 10% sodium chloride, and homogenized with a homogenizer. . The homogenous sample was stored at 5°C, and 5 g of the sample was taken every 0, 2, 5, 8, 14, 28, 74, and 136 days, and the concentrations of IBO and MUS in the preserved sample were measured.

2.5 Heating experiment

Add five volumes of ice water to each 100 g of chilled raw sample (7 times), homogenize with a homogenizer, and adjust to pH 4.0, 5.0, 5.98, 7.0, 8.0, 9.0 and 10.0 with phosphoric acid and sodium hydroxide. adjusted. The sample solution was heated in a boiling bath.5 ml of the heated solution was sampled at intervals of 0, 4, 10, 20, 35, 60, and 90 minutes, cooled in ice water, and used as the heating experimental sample. The concentrations of IBO and MUS in the samples were measured.

2.6 Elution experiment

Refrigerated raw samples (I: 37.7 g, II: 22.5 g, III: 22.7 g) were added to 5 times the volume of boiling water, boiled for 10 minutes, cooled with water, and weighed separately into broth and heated sample. About 3 times the amount of water for the heated sample

Table 1. Concentration (ppm*[1]) of IBO and MUS in *A. muscaria* after Drying

Sample	Raw S.*[2]		Sunlight I*[3]		Sunlight II*[4]		Heater III*[5]	
	IBO	MUS	IBO	MUS	IBO	MUS	IBO	MUS
No. 1	491	5	253	95	62	52	21	31
2	304	11	99	61	59	43	17	23
3	504	13	346	85	4	17	36	26
4	347	13	166	88	7	24	134	46
5	372	5	145	76	14	20	120	38
6	665	7	284	172	88	42	50	60
7	595	2			7	15	59	26
8	437	2			52	45	65	28
9	384	10			39	27	24	37
10	285	6			14	36	124	23
11	489	10			49	37	40	25
12	675	7					9	14
Mean	462	8	216	96	36	33	58	31
Remain (%)	100	100	47	1200	8	413	13	388

*[1] The value was reduced to the raw weight
*[2] Fresh raw sample
*[3] Sunning for three days
*[4] Sunning for eleven days
*[5] Drying near the oil heater for two days

After rinsing with water for 5 seconds and washing with water, the washing water and the washed sample were weighed. 1 ml of immersion water (three times) was collected every 660, 1300 and 2500 minutes. Concentrations of IBO and MUS in broth, washing water, and soaking water were measured.

Chilled raw samples (I: 40.9 g, II: 37.1 g, III: 27.7 g) were added to 5 times the volume of boiling water, boiled for 10 minutes, cooled with water, and weighed separately into broth and heated sample. Salt was added to the heated sample to make it 10%, and it was stored at 5°C for 3 months. Then, it was immersed in 10 times the amount of water as the washed sample, and 1 ml of immersion water (three times) was sampled every 0, 10, 30, 70, 150, 300, 660, 1300, 2500 minutes while stirring occasionally. . Concentrations of IBO and MUS in broth, washing water and each immersion water were measured.

Results and discussion

1. Drying Experiment 1: Concentrations of IBO and MUS in Sun and Stove Dried Samples

We experimentally examined the validity of the legend in Nagano Prefecture that drying in the sun or in a stove can be detoxified.The results are shown in Table 1. The mean concentrations of 12 were 462 ppm for IBO and 8 ppm for MUS. For Sunlight-I, 216 ppm for IBO and 96 ppm for MUS. For Sunlight-II, 36 ppm for IBO and 33 ppm for MUS. In Heater-III, IBO was 58 ppm and MUS was 31 ppm.

It was thought that drying increased the levels of hallucinogenic substances and MUS and strengthened their physiological effects. I thought I was

2. Drying experiment 2: Changes in concentration of IBO and MUS due to different drying temperatures

The concentrations of IBO and MUS in air-dried samples at 40, 50, 60, 100 and 120° were measured.

The results are shown in Table 2. Drying at 40° reduced IBO by 65% and increased MUS by 620% (approximately 6-fold) compared to the control. At 50°, IBO decreased to 64% and MUS increased to 575%. At 60°, IBO 54%, MUS 650%; at 80°, IBO 17%, MUS 640%; However, at 120°C, IBO decreased sharply to 0.5% and MUS halved to 50%. The average residual rate is shown in Fig. 1. Although the drying time became shorter as the temperature increased, the decrease in IBO was remarkable as the temperature increased, and it almost disappeared at 120°C. , sharply decreased after 80°.

Regarding the increase and decrease of IBO and MUS, there was no equivalence relationship in which MUS increased as much as IBO disappeared.

Table 2. Change in Concentration of Ibotenic Acid (IBO) and Muscimol (MUS) in *A. muscaria* by Drying

Sample*1	[I] at 40°				[II] at 50°				[III] at 60°				[IV] at 80°				[V] at 100°				[VI] at 120°			
	Raw S.*2		Dry S.*3		Raw S.		Dry S.		Raw S.		Dry S.		Raw S.		Dry S.		Raw S.		Dry S.		Raw S.		Dry S.	
	IBO	MUS	IBO	MUS	IBO	MUS	IBO	MUS	IBO	MUS	IBO	MUS	IBO	MUS	IBO	MUS	IBO	MUS	IBO	MUS	IBO	MUS	IBO	MUS
Conc. (ppm)*4																								
No. 1	295	2	238	23	419	2	293	27	665	7	354	56	625	6	142	57	504	13	62	43	384	11	1	4
2	285	2	224	21	437	3	274	30	686	6	407	41	675	8	127	48	487	12	60	50	342	11	1	3
3	505	10	299	53	595	5	366	23	456	6	257	32	304	5	30	29	347	13	31	21	313	10	2	8
4	489	6	266	49	605	10	393	35	491	6	235	35	372	6	38	26	379	13	52	34	304	12	2	7
Average	394	5	256	31	515	5	332	29	575	6	313	41	494	6	82	40	429	13	51	37	335	11	2	6
Remain (%)			65	620			64	580			54	683			17	666			12	280			0.6	54

*1 The samples were air-dried with a forced convection oven for 18.5 hours [I], 10.5 hours [II], 9.5 hours [III], for 5.5 hours [IV], 4 hours [V] and 4 hours [VI]; *2 Raw sample, control for dry sample; *3 Dry sample; *4 The value was reduced to the raw weight.

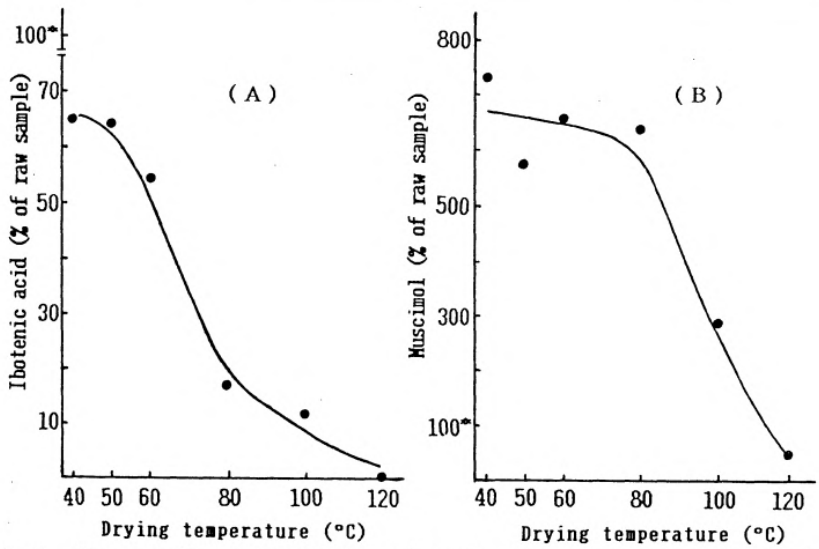

Fig. 1. Change of ibotenic acid (A) and muscimol (B) contents caused by drying
*Standard value, IBO or MUS contents in fresh raw sample

Table 3. Change in Concentration of IBO and MUS in Dry *A. muscaria* during Storage*[1]

Period (days)	0		14		30		60		90	
Conc. (ppm)*[2]	M*[3]	M. D*[4]	M	M. D	M	M. D	M	M. D	M	M. D
Ibotenic acid	418	2	414	3	409	7	406	8	415	20
Muscimol	22	1	21	1	22	2	23	1	20	1

*[1] At cool and dark place; *[2] The value was reduced to the raw weight; *[3] Mean value in triple tests; *[4] Mean deviation

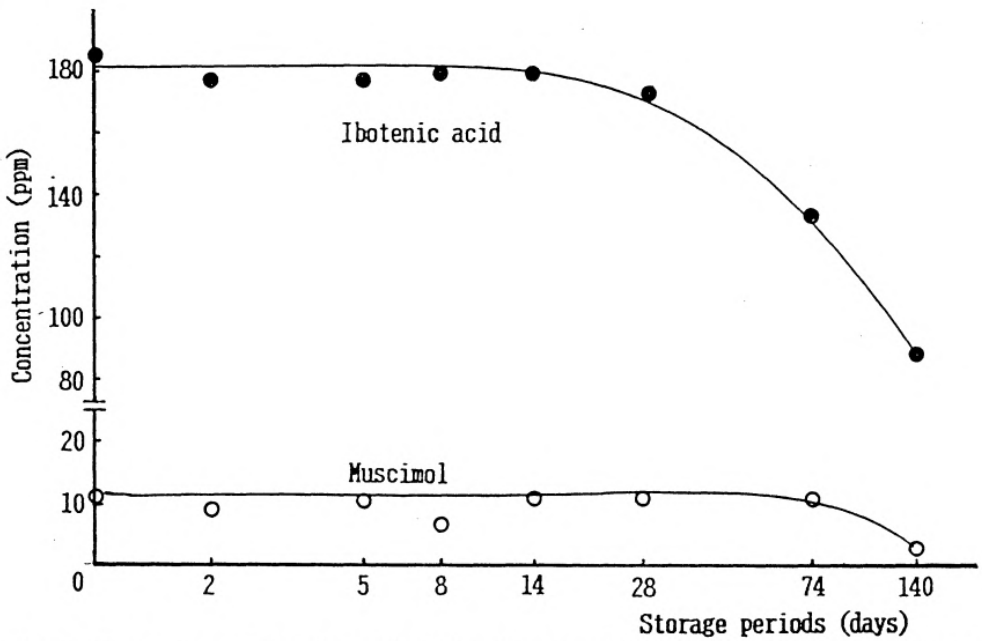

Fig. 2. Change in concentration of ibotenic acid and muscimol in *A. muscaria* during storage
The salted sample (10% NaCl-mushroom) was stored at 5℃ after boiling.

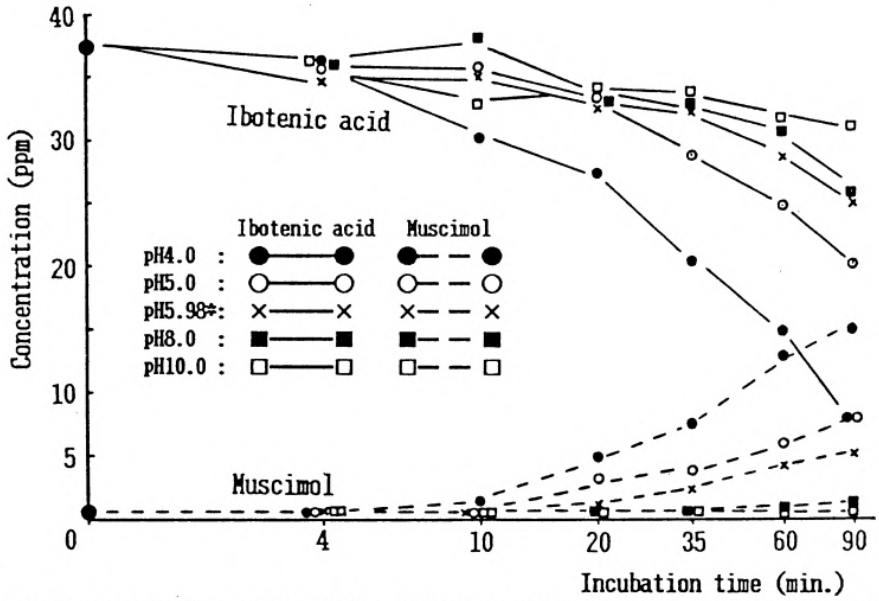

Fig. 3. Influence of pH on concentration of ibotenic acid and muscimol in boiling water
pH of the sample was adjusted with phosphoric acid or sodium hydroxide afte homogenizing *A. muscaria*.
Samples were incubated at 100℃ in water bath.
*Fresh sample

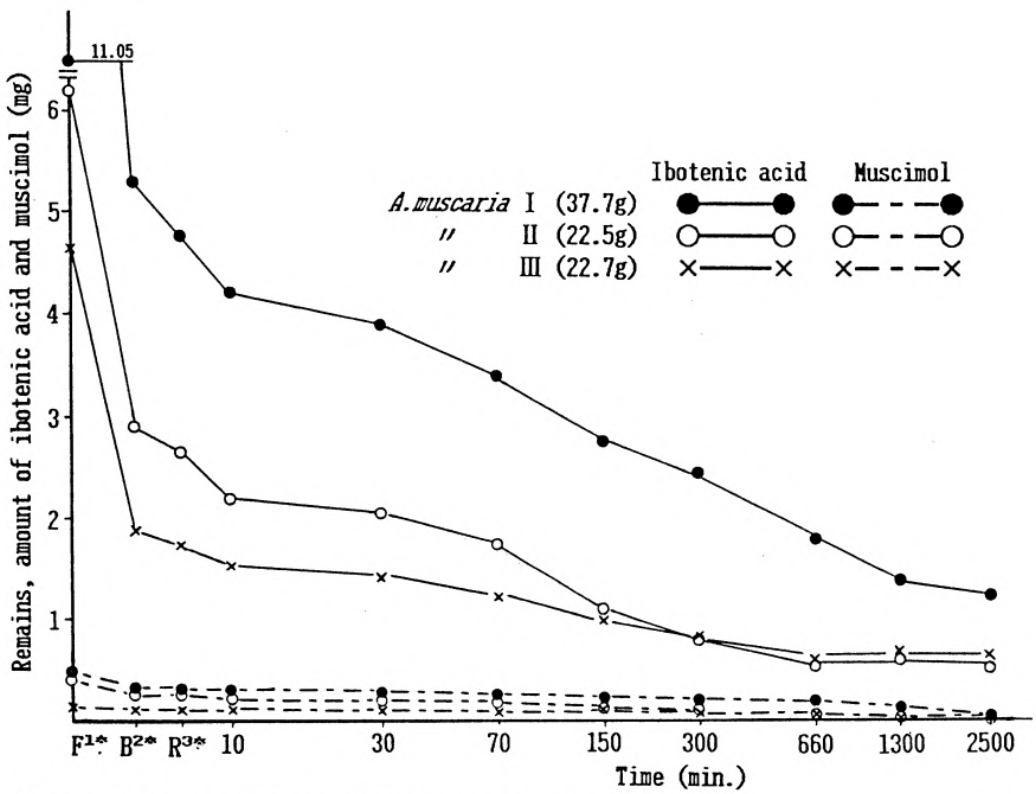

Fig. 4. Diffusion of IBO and MUS in *A. muscaria* into water
F*[1]: fresh raw *A. muscaria*; B*[2]: after boiling F*[1]-sample for 10 min.; R*[3]: after rinsing B*[2]-sample in a water bath for 3 sec

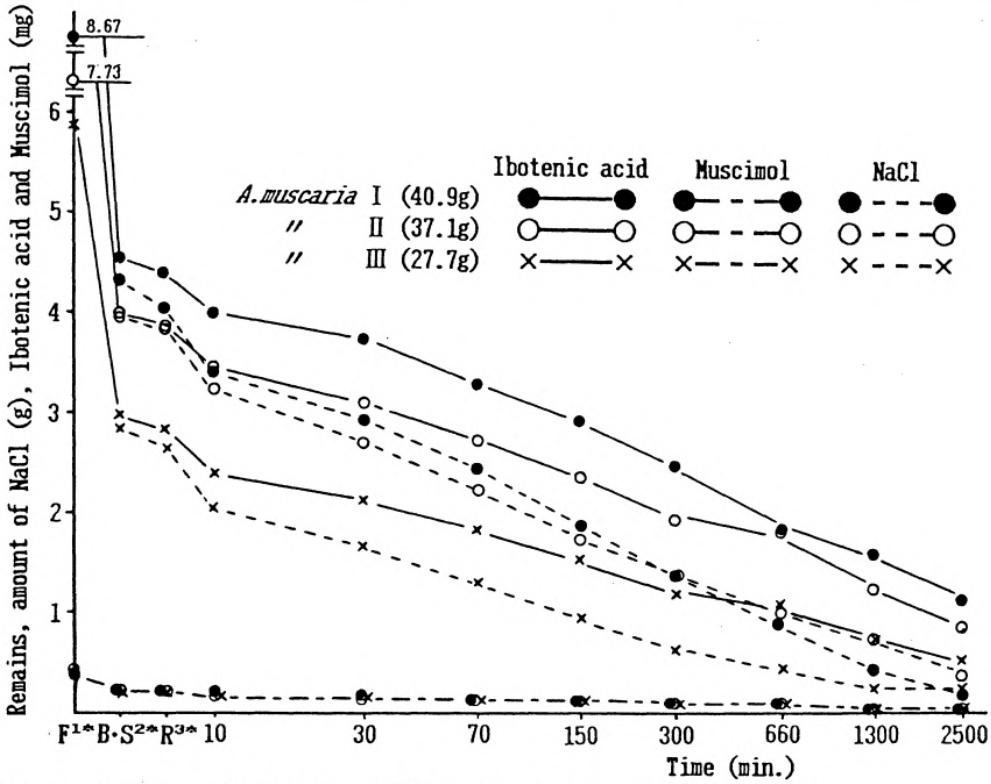

Fig. 5. Diffusion of NaCl, IBO and MUS in the salted *A. muscaria* into water
F*[1]: fresh raw *A. muscaria*
B·S*[2]: The salted sample (10% NaCl), it was salted with sodium chloride after boiling F*[1]-sample with water for 10 min.
R*[3]: after rinsing *[2]B·S-sample in a water bath for 3 sec

From the above, it is thought that IBO decreases at reported drying temperatures such as the sun and stoves, but MUS increases, albeit less than the equivalent amount, and the effect of MUS becomes stronger.

3. Dry storage experiment: Concentration change of IBO and MUS in dried sample

Traditionally, the method of preserving fly agaric is drying and pickling. there is A 90-day storage test was performed on the dried samples, and the results are shown in Table 3. During the storage period, no change in the concentrations of both IBO and MUS was observed in the well-dried samples. No decrease in MUS and no decrease in precursor IBO was observed during storage, and detoxification by storage was not expected at all.

4. Salting Preservation Experiment: Changes in Concentration of IBO and MUS in 10% Salted Preserved Samples

The concentrations of both substances tended to decrease gradually throughout the 136 days of refrigerated storage of the salted sample. Very little can be expected in short-term storage.

5. Heating experiment: Concentration change of IBO and MUS due to pH difference in boiling

Assuming a general cooking method, the changes in IBO and MUS with respect to pH and boiling were tested. The results are shown in Fig. 3. In the boiling bath, IBO decreased and MUS increased with the passage of time. It was found that the lower the pH, the greater the decrease in IBO and the increase in MUS. rice field.

6. Leaching Experiment: Outflow Diffusion of IBO and MUS during Cooking Process

A model experiment was conducted on the outflow and diffusion of IBO and MUS during the cooking process. At time point R, the outflow and diffusion were investigated over time after the sample B was washed with water for 3 seconds and then exposed to water for 2500 minutes. It is thought that most of the elution is from the many-folded and thin fungal cap. In the water washing at time B

About 5% flowed out, and about 25% gradually flowed out after a day and night (1440 minutes) exposure to water, and the total decreased to about 1/8 of the initial value. MUS is also considered to exhibit the same behavior as IBO, albeit at a low level.

Spilling hot water after blanching and soaking in water, which are part of the local cooking method, are due to the fact that the known toxic alkaloids (muscarine, muscaridine, bufotenine, etc.) in fly agaric are readily soluble in water. Therefore, it was speculated that the detoxification effect was great.

7. Elution experiment of salt-preserved samples: outflow diffusion of IBO and MUS by exposing samples preserved in 10% salt to water

Since salt-preserved products have a high salt concentration, it was expected that the outflow effect of IBO and MUS would be promoted during desalting. The outflow diffusion of NaCl by exposure to water was rapid, and was steeper than the outflow gradient of IBO. Since the outflow tendency of IBO and MUS in the unsalted sample immediately after boiling in ``Dissolution Experiment 7'' showed almost the same decrease slope, it was considered that there was no synergistic effect of outflow and diffusion due to salt.

In addition to the method of detoxification described above, the methods of preservation and cooking that have been handed down are believed to be greatly influenced by individual differences, the amount consumed, and the effects of other ingredients. should not be recommended.

summary

From the results of this experiment, 1) Although drying in the sun or stoves drastically reduces IBO, it seems to increase hallucinogenic substances and MUS and strengthen their effects. In addition, long-term storage in salt gradually decreased both substances, but short-term storage for 1 to 2 months showed no change and hardly led to detoxification.) In the boiling bath, the decrease in IBO and the increase in MUS were more pronounced at lower pH. However, adding vinegar or cooking with boiling water for about 10 minutes increased or decreased the level of both slightly, and did not lead to detoxification. 4) Boiling and soaking in water during cooking drastically reduce the contents of both substances, and are presumed to contribute greatly to detoxification together with the removal of other toxic alkaloids that are water-soluble.

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Literature

1) Theodor, W.: Science **159**, 946～952 (1968).

2) Johnston, G. A. R., Curtis, D. R., De Grout, W. C., Duggan, A. W.: Biochem. Pharmacol. **17**, 2,488～2,489 (1968). 3)

Tsunematsu Takemoto, Tetsuro Yokobe, Tadashi Nakajima: Yakushi, 84, 1, 186-1, 188 (1964).

4) Bowden, K., Drysdale, A. C., Mogey, G. A.: Nature (London) **206**, 1,359～1,360 (1965).

5) Gore, M. G., Jordan, P. M.: J. Chromatogr, **243**, 323～328 (1982).

6) Onda, M., Fukushima, H., Akagawa, M.: Chem. Pharm. Bull. **12**, 751 (1964).

7) Bowden, K., Drysdale, A. C.: Tetrahedron Lett. **12**, 727 ～728 (1965).

8) Muller, G. F. R., Eugster, C. H.: Helv. Chim. Acta **48**, 910 ~926 (1965).

9) Eugster, C. H., Muller, G. F. R., Good, R.: Tetrahedron Lett. 23, 1,813-1,815 (1965).

10) Takemoto T., Nakashima T.: Yakushi 84, 1, 232-1, 233 (1964).

Yokobe, T.: Nutrition and Food 18, 172-175 (1965) 12) Kamiko Food Sanitation Association ed.: "Mushrooms of Nagano Prefecture" 1st edition (1986).

13) Ministry of Health and Welfare, Food Health Division, Environmental Hygiene Bureau: "National Food Poisoning Incident Records" (1977-1986). ~24 (1993). 15)

Tsunoda, M., Inoue, N., Aoyagi, Y., Sugawara, T.: Ibid. 34, 12~ 17 (1993).