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Study of Quality in Fish and Shellfish as Food− I Concentration of Formaldehyde in Various Tissues of Cultured Eel by Formalin Bath

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The present study was undertaken to examine the concentration of formaldehyde in the various tissues of cultured eel by formalin bath. Formaldehyde is determined by the acetylacetone method. Formaldehyde in 4 l of water containing 50 and 200 ppm formalin, in which the test eels were, disappeared completely within 2 and 3 days, respectively. In control water, formaldehyde in water containing 50 and 200 ppm formalin decreased gradually and disappeared within 4 and 14 days, respectively. The biphasic curve of formaldehyde concentrated was obtained in the muscle, liver and blood of eel when it was exposed to the bath of formalin at 50 and 200 ppm. The levels in tissues decreased gradually and were almost equal to those of the control within 17−21 days post bath. The peak levels of formaldehyde in tissues post bath of formalin at 200 ppm showed similar heights to those of formalin at 50 ppm. It could be observed also that the tissues of Japanese eel naturally contain a small amount of formaldehyde.

Key words: formaldehyde, formalin, eel, drug bath

Formalin, 35−37.5% formaldehyde, is commonly used as an antiseptic agent because of its bactericide action. But the use of the drug as a food additive is prohibited since it has various kinds of toxicity. Formalin has been used also as a drug for exterminating or insecticiding various monogenetic trematoda, which parasite the skin surface and the gill of cultured fish. Recently, this drug has been taken under government control in Japan as well as in other countries and may not be used for edible fish. The presence of formaldehyde in foodstuffs might present serious problems not only in food hygiene but also in food quality.

The present work was undertaken to investigate the concentration and the disappearance of formaldehyde in the various tissues of cultured eel after formalin bath.

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Materials and Methods

Fish: Eels (150 g), *Anguilla japonica,* were purchased from the Eel Farm at Matsuzaka, Mie. Formalin and the other reagents were obtained from the Wako Pure Chemical Industries, Ltd.

Determination of formaldehyde: Aliquots of water were filtered with filter paper (Toyo No. 131), and the filtrate were subjected to analysis of formaldehyde described below.

Eels were placed in 40 l of aerated water containing 50 and 200 ppm formalin. Three eels were anesthetized at each sampling time and washed four times with distilled water to remove extra formaldehyde from the skin surface. Then, the blood, liver, and muscle were collected. To the blood and liver obtained, 4 volumes of 3% TCA were added (the same volumes of distilled water for the muscle.) and homogenized at fullspeed for 5 min by a Waring Blender (Sakuma Seisakusho Co. Ltd.). The homogenate was filtered with Filter-Cel (Nakarai Chemical Co. Ltd., 4 gals/sq. ft/hr). The filtrate was neutralized to pH 7.0 with 2 N NaOH and subjected to analysis of formaldehyde.

Formaldehyde was determined by the modified method of NASH (1953). To 3 ml of sample was added 3ml of acetylacetone reagent, pH 6.4, (Ammonium acetate 150 g, 3 ml of acetic acid, and 2 ml of acetylacetone were dissolved in 1,000 ml of distilled water.). The mixture was heated at 60°C for 15 min, cooled with running water and added to 5 ml of *n*-buthanol. After shaking vigorously, the suspension was allowed to stand for a few minutes. Then, the optical density of the buthanol layer was measured at 420 nm by a spectrophotometer (Hitachi Ltd., Type 101). The content of formaldehyde in formalin used here was 35.6%, which was measured by the method of iodine titration (OGATA 1968). The amount of formaldehyde was converted to that of formalin by the following equation.

$$\text{Formalin (ppm)} = \text{Formaldehyde (ppm)} \times \frac{100}{35.6}$$

The tested eels were placed in still water, and the water was not changed after the addition of formalin. The water temperature was 13−20°C.

Results

Recoveries of formaldehyde from various tissues of eel

As shown in Table 1, the recoveries from muscle, liver, and blood were 79, 65, and 58 %, respectively. Even three extractions from the tissues were not sufficient to recover 100% of the formaldehyde. Therefore, the first extraction

Table 1. Recoveries of formaldehyde from various tissues of eel

Tissues	Recovery (%)
Muscle	78.8 (94.7)
Liver	64.5 (67.0)
Blood	57.5 (66.3)

Formalin at 10 ppm was added to the tissues. The values are the average of three experiments. The parentheses represent total recovery of three extractions.

of formaldehyde was used in the following experiments.

Residual examination of formaldehyde in water

In order to examine the residue of formaldehyde in water with time after formalin bath, forty liters of water containing 50 and 200 ppm formalin were used. Twelve test eels were placed in the formalin water.

As shown in Fig. 1, the formaldehyde in the water (containing 50 and 200 ppm formalin) in which the test eels were, disappeared completely within 2 and 3 days, respectively.

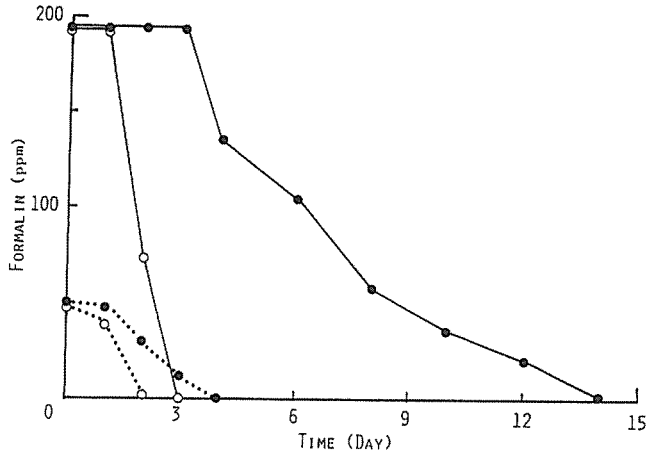

Figure 1. Changes of concentration of formaldehyde in 40 l of water containing 50 and 200 ppm formalin.
The dotted line and the solid line represent 50 and 200 ppm formalin, respectively. The water temperature was 20 ± 2.5°C. ○: with twelve eels, ●: without eels.

In the control water (containing 50 and 200 ppm formalin) the formaldehyde decreased gradually and disappeared within 4 and 14 days, respectively.

Concentration of formaldehyde in tissues of eel

The concentration of formaldehyde in the various tissues of eel after formalin bath is shown in Table 2.

These experiments were repeated three times. Figures 2 and 3 also show the mean values and the typical distribution pattern of the tissue levels of formaldehyde.

Formalin 50 ppm: The concentration of formaldehyde

Table. 2. Concentration of formaldehyde in tissues of eel after formalin bath

Formalin : 50 ppm

Tissues	Before bath	Time after bath (Day)			
		1−2	3−4	5−7	16−18
Muscle	3.3*3	5.8	2.1	4.1	2.7
Liver	4.7	7.3	3.8	7.8	3.0
Blood	4.6	7.6	4.0	6.8	3.2

Formalin : 200 ppm

Tissues	Before bath	Time after bath (Day)			
		2−3	4−7	8−9	17−21
Muscle	3.3	7.2	2.6	4.9	2.4
Liver	4.7	7.5	4.3	7.6	3.9
Blood	4.6	7.0	3.8	7.0	3.6

*3 ppm

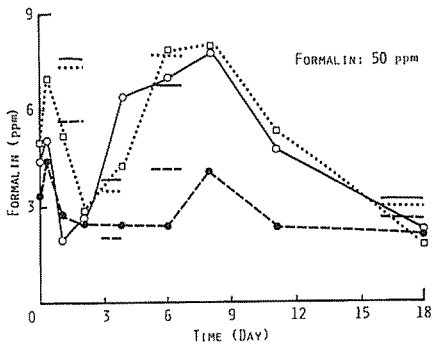

Figure 2. Concentration of formaldehyde in various tissues of eel after bath of formalin at 50 ppm.
The horizontal lines represent the average of three experiments. The water temperature was 16 ± 1°C.
● : Muscle, □ : Liver, ○ : Blood.

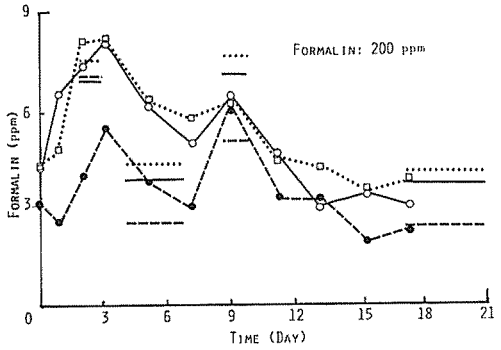

Figure 3. Concentration of formaldehyde in various tissues of eel after bath of formalin at 200 ppm.
The horizontal lines represent the average of three experiments. The water temperature was 13 ± 3°C.
● : Muscle, □ : Liver, ○ : Blood.

in muscle, liver, and blood reached the peak level of 5.8－7.6 ppm within 1－2 days post bath and decreased gradually to 2.1－4.0 ppm within 3－4 days post bath. Then, the second peak level of formaldehyde was attained within 5－7 days post bath and fell below the corresponding level of control tissues within 16－18 days post bath.

The tissue levels of formaldehyde in liver and blood were much higher than those in muscle.

Formalin 200 ppm: The biphasic curve of formaldehyde concentrated was obtained in muscle, liver, and blood. The first and the second peak levels of formaldehyde in these tissues were attained within 2－3 and 8－9 days post bath, respectively. Then, the levels in tissues decreased gradually and were almost equal to those of the control tissues within 17－21 days post bath. The time of disappearance of formaldehyde concentrated in tissues post bath of formalin at 200 ppm took a litte longer than that of formalin at 50 ppm. It was also observed that the peak levels of formaldehyde in tissues post bath of formalin at 200 ppm showed similar heights to those of formalin at 50 ppm.

Discussion

The water distillation and the acetylacetone methods are commonly used for the determination of formaldehyde in foodstuffs. The former is established by the official hygiene method.[*2] In a preliminary experiment, the recovery of formaldehyde from tissues of eel was about 10－13% when measured by this method. Our experimental results agree with those reported by HARADA *et al.* (1974). The latter is known as the method

*[2] The Ministry of Public Welfare, ed., 1963. X Formalin. A guide to examination of food hygiene (II), Japan Public Hygiene Association, Tokyo, 44－42.

of NASH (1953). AMANO *et al.* (1963) reported that this method which had the specificity for formaldehyde under a mild condition was reliable for its determination. Therefore, the acetylacetone method was used in the present experiment.

Formaldehyde is oxidized and decomposed by sunlight and oxygen in the atmosphere. Besides these, it might be suggested that the water temperature and the presence of aquatic organisms such as planktons and fish have a significant effect of the decomposition of formaldehyde. In a preliminary experiment, formaldehyde in a pond with various planktons disappeared quickly when compared to that in the water without these.

It has been reported that the tissue levels of formaldehyde in eel post bath of formalin at 40 ppm disappeared quickly within 1 day when measured by the distillation method (NOZAWA *et al.* 1982). Our data show that the concentrations of formaldehyde in tissues post formalin bath were equal or lower within 21 days than in the control tissues. This fact might be due to a difference in the methods used for the formaldehyde determination. Further work is needed to evaluate the disappearance of formaldehyde post formalin bath. It should include all possible factors such as the water temperature, the presence of aquatic organisms, the intensity of solar radiation, the density of planting fish, and so on.

It is known that the methabolic patterns of fish drugs in tissues are conjugation, oxidation, reduction, enzymatic hydrolysis, and so on. Our data also show that two peak levels of formaldehyde in tissues were attained after formalin bath. From this result, it is suggested that formaldehyde is concentrated in fish tissues after formalin bath and excreted outside the body. But some of the formaldehyde concentrated in the tissues might be absorbed with protein or be present as a conjugation form, and then might be released later by some changes in the physiological condition of the fish tissues. This is under investigation at present.

In the present experiments, formaldehyde (3－5 ppm). was detected in the control tissues of eel. The presence of formaldehyde in the tissues of several fish was reported by AMONO *et al.* (1963) and HARADA *et al.* (1970). Consequently, the tissues of Japanese eel might naturally contain a small quantity of formaldehyde.

References

AMANO, K., K. YAMADA and M. BITO, 1963. Detection and identification of formaldehyde in codoid fish. *Bull. Jap. Soc. Sci. Fish.*, **29**: 695－701 (In Japanese).

HARADA, K., S. MIURA, S. SHIDA and K. YAMADA, 1970. Thinlayer chromatographic identification of formaldehyde in aquatic animals. *Ibib.*, **36**: 188－191 (In Japanese).

IWAMI, K., K. YASUMOTO and H. MITSUDA, 1974. Determination of formaldehyde in vegetables and edible mushrooms by modified acetylacetone method. *J. Jap. Soc. of Food and Nutrition*, **27**: 387－391 (In Japanese).

NASH, T., 1953. The colorimertic estimation of formaldehyde by means of the Hantzsch reaction. *Biochem. J.*, **55**: 416－421.

NOZAWA, M., M. YAMAGATA and K. TOKUOKA, 1982. Examination of residue of formalin. Report of Study

of Technical Development for Fish Disease. Nippon Reito Shokuhin Kensa Kyokai, Tokyo, 28–31 (In Japanese).

OGATA, A. ed., 1968. Formalin. The 7th Japanese Pharmacopoeia Dispensatory. The First Commentary, Hirokawa-Shoten, Tokyo, C–1313–1315 (In Japanese).