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Title	FUNDAMENTAL STUDIES ON FOOD POISONING CAUSED BY SEA FOODS : I. On the Fate of Histamine Contents during the Drying of Fish Meat.
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Citation	北海道大學水産學部研究彙報, 18(4), 385-391
Issue Date	1968-02
Doc URL	https://hdl.handle.net/2115/23337
Type	departmental bulletin paper
File Information	18(4)_P385-391.pdf



FUNDAMENTAL STUDIES ON FOOD POISONING CAUSED BY SEA FOODS

I. On the Fate of Histamine Contents during the Drying of Fish Meat.

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Introduction

Histamine is a kind of poisonous basic substance, namely ptomaine, in rotten meaty products. By the time poisoning caused by the ptomaine is formed, putrid and stimulative smell caused by such decomposing substances as ammonia-N, indole, mercaptane, etc. has grown so unbearable that the meat cannot usually be used for food. However, even before the decomposing process starts, when no decomposing can be recognized externally, poisoning caused by ptomaine sometimes takes place. Usually, this is because not only deamination but also decarboxylation produces amine when amino acid is decomposed by bacteria. When deamination and decarboxylation occur together, the amount of ammonia indicates the degree of putrefaction of the fish meat. But if they do not occur together, for instance if decarboxylation alone is working, the amount of ammonia does not increase and, therefore, putrefaction cannot be detected by smell.

According to Kimata and Kawai¹⁾, the quantity of histamine produced by the decomposition of fish meat does not agree with the degree of decomposition judged by the quantity of ammonia-N or by the sensory test. They report that it is dangerous to use histamine to decide the degree of freshness, since unlike ammonia-N a large quantity of histamine is often created when the meat is kept at lower temperature than 20°C or, even as low as 6~7°C. Although there is no established theory as to the formation of histamine it is said that histamine is produced from histidine by decarboxylation. It is also reported that the best conditions for the production of histamine are as follows: (1) a slightly acidic pH value, and (2) a temperature of 20°C. Also it is known that by the action of histaminase the histamine produced comes to possess the function of leaving the amino-radical and becoming non-poisonous.

Generally, fish meat is dried up after rigor mortis or autolysis, without going through the decomposing process, namely decomposing action by bacteria. When the meat is not fully dried, it sometimes tastes pungent and causes poisoning. However, the fully dried products do not possess the abovementioned properties and symptoms. These changes are related to the changes in the amounts of histamine during the drying process.

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This is also important in the inquiry into the difference between putrefactive poisoning, and bacterial poisoning. Therefore, the deterioration of fish meat, especially the formation of amines must be studied when the meat is being dried. This paper presents the results of an investigation on histamine which is supposed to have the biggest physiological effect of all the amines.

Experimental

Materials

Mackerel, cod and Atka mackerel were used in the present investigation. These fishes were obtained from the fish market in Hakodate. After removing the dark muscle, bone and skin, the muscle was minced with a masticator several times, ground well on a mortar, and then mixed thoroughly with a twentieth volume of toluene, and then the homogenate was separated into portions of approximately equal volume in weighing bottles, and stored at 20°C.

Analysis

i. Additional analysis of the water content

Analysis of the water content was carried out by the common method when the histamine was measured.

ii. Preparation of sample for the analysis of the histamine content

Ten grams of each fish muscle homogenate were mixed thoroughly with 80 ml of 80% ethyl alcohol, allowed to stand overnight, and the alcoholic extractives were filtered using Buchner funnel after heating for 10 min. with a reflux condenser.

The procedures for extracting and filtrating were repeated once more. The muscle residue was washed off with a small amount of water, filtered, and the aqueous filtrates added to the above extractives. The total extractive was acidified with about 10 ml of 1N sulfuric acid, a portion of ethyl alcohol was thoroughly evaporated off, and the residual portion was concentrated to a proper quantity by heating on a water bath. Some drops of 20% silver nitrate were carefully added to this concentrated solution. The white precipitates, which formed after it was allowed to stand overnight, were filtered and washed well with water. The filtrate and washed solution were added together, and then increased to approximately 30 ml by adding an aliquot portion of hot baryta water. Then white precipitates appeared. These were removed by centrifugation and washed twice with about 5 ml of saturated baryta water. To the supernatant were added 10 ml of 1N sulfuric acid and 0.3 g of sodium chloride and the mixture was heated on a water bath. Thus, the orangish yellow precipitates which appeared were separated by filtration and washed with water. The filtrate and washed solution were mixed together and evaporated until dry on a water bath. The residue was subjected to an extracting process with mixed solvent which consisted of amyl-alcohol and ether (1:1). The residue was poured into extracting

bottle with 10 ml of water, and dissolved by addition of 2 g of sodium hydroxide and cooled to 0°C. After the addition of 20 ml of mixed solvent, a stopper was put on the bottle, and it was shaken vigorously for several minutes. The upper solvent layer was syphoned off. After these extracting procedures were applied twice more, each solvent layer was mixed together, extracted with water which had been acidified with 10% hydrochloric acid two times, and then the total acidic aqueous solution was evaporated to dryness. Thus, the residue was dissolved in 5 ml of water and subjected to an analysis of the histamine content.

iii. Analysis of the histamine content

Histamine was assayed by the diazo-test according to the Igarashi method³⁾ with minor modification; the optical density of the coloration resulting from the diazo-test was measured on the wave length of 420 $m\mu$ with a Hitachi photoelectric colorimeter model FPW 4.

One test tube containing one ml of each sample was added to 2 ml of diazo reagent, shaken immediately, and then mixed well with 5 ml of alkalic solution which consisted of 4 portions of 10% sodium hydroxide and 46 portions of water. To make a calibration curve the following procedure was carried out; histamine dihydrochloride was used as a standard compound. Each amount of 0.05, 0.1, 0.2, 0.3, 0.4, 0.5 mg of histamine dihydrochloride was weighed. These histamine samples were determined by the same procedure described above. The determined quantities were plotted as shown in Fig. 1.

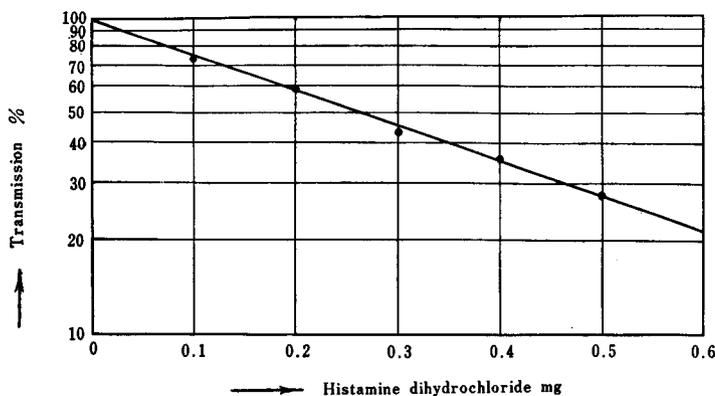


Fig. 1. Calibration curve obtained with histamine dihydrochloride

Results

1. Atka mackerel

The sample, which was not very fresh refrigerated Atka mackerel (caught near Hakodate in January), was kept at 20°C after being defrosted, and the quantities of histamine and water were determined every 24 hours as it was being dried. The

results are shown in Table 1 and Fig. 2. At first, the sample contained 24.1 mg/100 g of histamine and 83.2% of water. Twenty-four hours later, the sample contained 255 mg/100 g of histamine and 48 hours later, the sample contained 667 mg/100 g of histamine and 66.9% of water. Now the meat gave off a bad smell and decomposition was clearly going on. After this, however, the quantity of histamine began to decrease; 168 hours later, the sample contained 22.1 mg/100 g of histamine and 20.3% of water.

Table 1. Variation of moisture and histamine content in the meat during the drying process of Atka mackerel

Sample number	Drying time (hrs)	Moisture (%)	Dilution (fold)	Transmission (%)	Histamine (mg/100g)
1	0	83.2	5	28	24.1
2	24	75.5	80	42	255
3	48	66.9	160	34	667
4	72	56.0	160	43	510
5	96	51.5	80	36	310
6	120	42.5	40	42	131
7	144	30.5	40	45	114
8	168	20.3	5	32	22.1

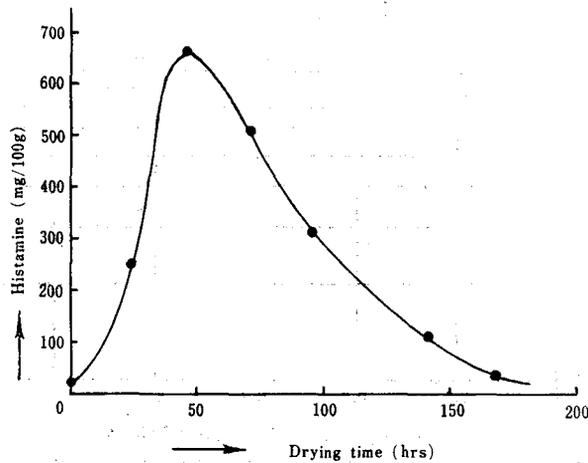


Fig. 2. Relation between the drying time and histamine content in the meat of Atka mackerel

2. Codfish

The sample, which was very fresh, was kept at 20°C and the quantities of histamine and water were measured every 30 hours as it was being dried. The results are shown in Table 2 and Fig. 3. At first, the sample contained a trace of

histamine and 73.4% of water. Thirty hours later the sample contained 0.5 mg/100 g of histamine; and 90 hours later, it had 4.3 mg/100 g of histamine and 41.2% of water. After this, both histamine and water lessened; 150 hours later, the sample contained 0.4 mg/100 g of histamine.

Table 2. Variation of moisture and histamine content in the meat during the drying process of codfish

Sample number	Drying time (hrs)	Moisture (%)	Dilution (fold)	Transmission (%)	Histamine (mg/100g)
1	0	73.4	2	99	trace
2	30	62.6	2	96	0.5
3	60	50.1	2	64	3.3
4	90	41.2	2	56	4.3
5	120	30.9	2	70	2.5
6	150	20.1	2	95	0.4

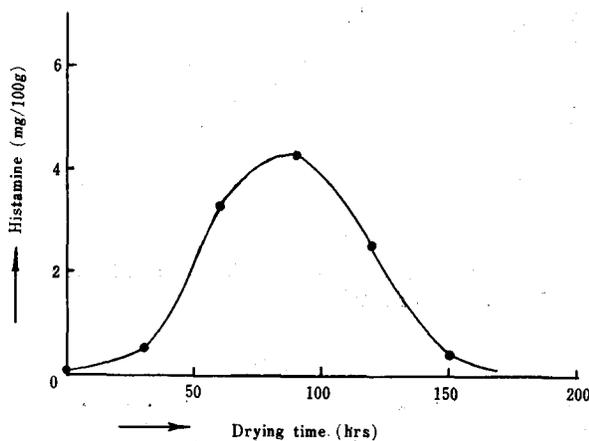


Fig. 3. Relation between the drying time and histamine content in the meat of codfish

3. Mackerel

Very fresh mackerel, to which 1/20 of toluene was added kept at 20°C, and the quantities of histamine and water were measured as the meat was being dried. The results are shown in Table 3 and Fig. 4. At first, the sample contained 10.7 mg/100 g of histamine and 76.5% of water. Thirty hours later, it contained 16.2 mg/100 g of histamine and 63.1% of water. After this, both histamine and water lessened; 150 hours later, the sample contained 2.3 mg/100 g of histamine and 18.6% of water.

Table 3. Variation of moisture and histamine content in the meat during the drying process of mackerel

Sample number	Drying time (hrs)	Moisture (%)	Dilution (fold)	Transmission (%)	Histamine (mg/100g)
1	0	76.5	4	49	10.7
2	30	63.1	4	35	16.2
3	60	48.8	2	28	9.9
4	90	36.5	2	32	8.8
5	120	22.1	2	55	5.5
6	150	18.6	2	71	2.3

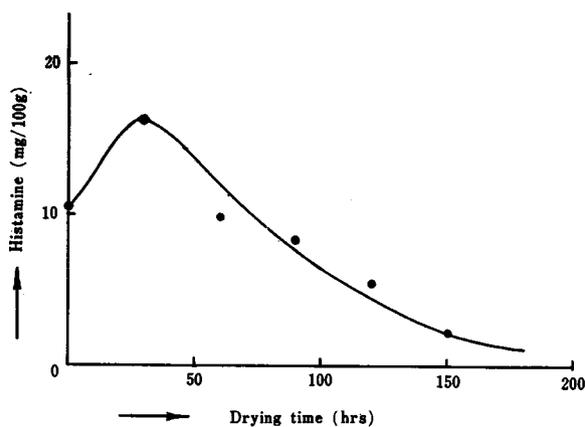


Fig. 4. Relation between the drying time and histamine content in the meat of mackerel

Discussion

The mackerel meat was, 30 hours later, half dried with 16.2 mg/100 g (the largest through the entire investigation) of histamine and 63.1% of water content. It may be considered that since an antiseptic substance was added to this mackerel meat there was no endoenzyme action caused by bacteria.

Kimata reports on the quantity of histamine under various conditions during autolysis that in red muscle fishes and similar fishes a small quantity of histamine is produced by the enzyme in the meat. It is proper to consider that the best hydrogen ion concentration is pH 3.5~4.5 and that there is no effect from the viewpoint of the freshness of the sample. In the investigations of codfish and mackerel, a comparison was made between the quantities of histamine and water in the white muscle fish and in the red muscle fish. It turned out that the white muscle fish had far smaller quantities of histamine than the red muscle fish: at first, the water content was 73.4% and the histamine was found in very small quantity. Ninety hours later, the histamine was 4.3 mg/100 g. This shows

that the quantity of histamine decreased as time went on. In other words, in the case of the white muscle fish the histamine would not effect humans even when the meat is only half dried, namely when autolytic enzyme is in action. When refrigerated Atka mackerel is dried up without any antiseptic substance, the transition of its histamine is similarity to that of codfish, but the quantity of its histamine is far larger. Forty-eight hours later, the Atka mackerel had 667 mg/100g of histamine. This proves that a considerable amount of histamine is produced by the action of decarboxylase caused by saprogenous bacilli. From what has hitherto been described, it is considered that fish meat, especially, red muscle fish meat, when being dried, will be poisonous because of the effect of the bacteria on the meat and decarboxylase in the meat unless it goes through a complete process of drying.

Summary

1. Investigations were made into the formation of histamine during the drying of codfish and mackerel, in which the quantities of water and histamine were compared.

2. When the water content of the meat was 50~70%, the quantity of histamine increased; then according to the decrease of moisture, the histamine content was found to diminish gradually as storage continued.

3. Atka mackerel, when kept at 20°C and dried through the processes of autolysis and decomposition, increased in histamine during the process of drying. However, the quantity of histamine decreased to an extreme degree at the end of the drying.

References

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- 2) Igarashi, H. (1946). "Ptomaine of Fishes" Published by Hakodate Municipal City.