# STUDIES ON POST-MORTEM CHANGES IN THE CHEMICAL CONSTITUTION OF THE MEAT OF SEA CUCUMBER (*STICHOPUS JAPONICUS* SELENKA)

## IV. Determination of the Freshness of the Meat of *Stichopus japonicus* and the Limit of the Freshness for Use as the Raw Material of Canned Food

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The meat of *Stichopus japonicus* has been clearly demonstrated to be less decomposable than other fish meat in the previous article III.[1] However, when bodies of *Stichopus japonicus* are used as the raw material of the canned food, it is very important to know the degree of freshness, the limit of the freshness for the raw material and the point of incipient putrefaction.

The authors have tried to throw some light upon those matters by estimating the amount of volatile base nitrogen. Simultaneously, $HgCl_2$-reaction (Amano's method)[2] and also bacterial counts were determined. In view of the previous experimental results, the changes in the freshness, corresponding to the amount of volatile base nitrogen of 20 mg%, were observed in detail.

(1) Sample

Living bodies of *Stichopus japonicus* which were caught in the sea near Hakodate were eviscerated by splitting the ventral side, and were left as they were in flat enamelled iron dishes at $16° \pm 3°C$ and $25° \pm 1°C$ respectively. The freshness of the meat falls with the lapse of the time.

(2) Experimental method

Several bodies which were left as such were taken after the lapse of definite interval hours, and used for the estimation of the amount of volatile base nitrogen, amino acid nitrogen and for the determination of pH values and $HgCl_2$-reaction. For the bacterial counts in the meat, a part of the body remaining was taken in steril condition and crushed in the Waring Blendor, and then was employed.

(i) The amount of volatile base nitrogen was estimated by Weber and Wilson's method.

(ii) The amount of amino acid nitrogen was estimated by Pope and Stevens' method.

(iii) The $HgCl_2$-reaction was determined by Amano's method.

(iv) The pH value was determined by glass electrode meter.

(v) Bacterial count was made by the decimal dilution method.

(3) Experimental results and discussion

The amounts of volatile base nitrogen and amino acid nitrogen at intervals of definite hours' leaving are shown in Tables 1 and 2. In those Tables the values of pH, result of $HgCl_2$-reaction, bacterial counts, organoleptic observation and kind of the detected organic bases are also recorded.

Figs. 1 and 2 show the changes of the amount of volatile base nitrogen and detected organic bases, and of the bacterial counts of *Stichopus japonicus* meat during the aerobic deterioration at $16° \pm 3°C$.

Fig. 3 shows the change of the bacterial counts during the aerobic deterioration at 25°C. The changes in the amounts of volatile base nitrogen and detected organic bases during the deterioration at 25°C have been shown in Fig. 3 in the previous article III.[1]

Table 1 Chemical changes in the meat of *Stichopus japonicus* ($16° \pm 3°C$)

| Time (hrs) | Volatile base-N (mg%) | Amino -N (mg%) | pH | $HgCl_2$-reaction A soln. | $HgCl_2$-reaction B soln. | Bacterial counts | Organoleptic test | Organic bases detected |
|---|---|---|---|---|---|---|---|---|
| 0 | 1.9 | 29.9 | 6.2 | − | ± | $16\times10^5$ | in rigor mortis | arginine |
| 24 | 3.2 | 28.8 | 5.7 | − | ± | $92\times10^5$ | fresh smell | |
| 48 | 13.1 | 27.2 | 6.4 | ± | + | $27\times10^6$ | end period of rigor mortis, | |
| 58 | 13.1 | 43.5 | 7.0 | − | ± | $20\times10^6$ | viscous in surface skin, fresh smell, edible | |
| 71 | 14.8 | 95.2 | 6.7 | − | ± | $98\times10^5$ | softening, fishy smell, unedible | arginine |
| 76 | 14.5 | 57.1 | 6.6 | − | ± | $27\times10^7$ | good smell, notable viscidation | arginine, indol putrescine |
| 91 | 18.4 | 23.0 | 7.2 | − | ± | $20\times10^7$ | ill smell, 〃 〃 | indol |
| 94 | 20.4 | 32.6 | 6.3 | − | ± | | perfect viscidation, muddy stink | arcaine, arginine, putrescine |
| 97 | 18.4 | 32.6 | 6.6 | − | ± | | | |
| 99 | 24.4 | 29.9 | 6.8 | − | ± | $60\times10^7$ | | |
| 103 | 30.3 | 46.2 | 7.2 | − | ± | | | indol, arcaine, agmatine arginine, tyrosine, putrescine |

Table 2 Chemical changes in the meat of *Stichopus japonicus* ($25° \pm 1°C$)

| Time (hrs) | Volatile base-N (mg%) | Amino -N (mg%) | pH | $HgCl_2$-reaction A soln. | $HgCl_2$-reaction B soln. | Bacterial counts | Organoleptic test | Organic bases detected |
|---|---|---|---|---|---|---|---|---|
| 0 | 3.2 | 40.8 | 6.5 | ± | ± | $4\times10^6$ | in rigor mortis | |
| 19 | 9.6 | 32.6 | 6.2 | − | + | $9\times10^6$ | 〃 〃, fresh smell | arginine, tyrosine, histidine |
| 27 | 18.5 | 35.4 | 5.8 | − | ± | $10\times10^6$ | no fishy smell, unedible | indol, arcaine, agmatine, tyrosine, histidine, arginine |
| 42 | 31.7 | 81.5 | 5.9 | − | ± | $18\times10^6$ | viscous in surface part, ill smell | 〃 〃 〃 〃 |
| 50 | 39.7 | 34.0 | 6.4 | − | ± | $12\times10^6$ | notable viscidation, stimulus | arcaine, agmatine, tyrosine, arginine, histidine |
| 72 | 76.2 | 35.4 | 7.0 | − | ± | $20\times10^6$ | discolored in surface part, $H_2S$ smell | indol, tyrosine, histidine |
| 95 | 139.2 | 40.8 | 7.5 | ± | + | $36\times10^6$ | 〃 〃 | |
| 115 | 125.8 | 27.2 | 8.4 | ± | + | $20\times10^6$ | notable viscous | |

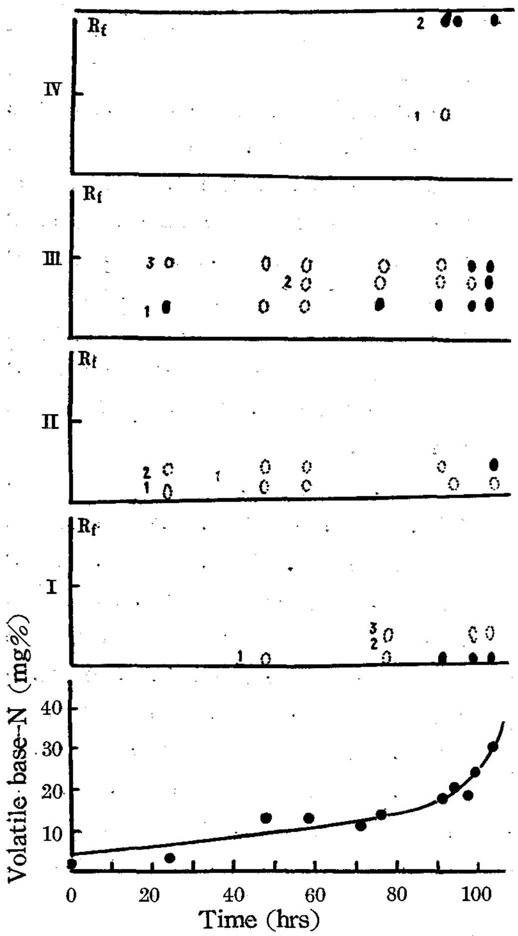


Fig. 1. Changes in the amount of volatile base nitrogen and detected organic bases of *Stichopus japonicus* meat during aerobic deterioration at 16° ±3°C

I. Volatile amines
Spot 1. putrescine 2. cadaverine 3. agmatine

II. Non-volatile amines (Diazo)
Spot 1. hastidine 2. tyrosine

III. Non-volatile amines (Sakaguchi)
Spot 1. arginine 2. agmatine 3. arcaine

IV. Indol fraction (Ehrlich)
Spot 1. tryptophan 2. Indol

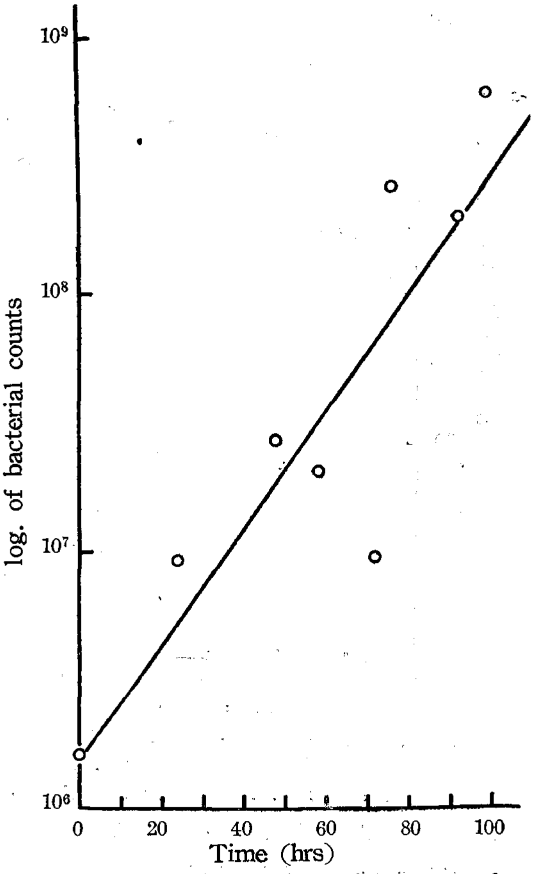


Fig. 2. Changes in the bacterial counts of *Stichopus japonicus* meat during aerobic deterioration at 16° ± 3°C

As seen in Fig. 2, in the case of storing at 16°±3°C, the number of bacteria increased from the initial period of the storing. The number of bacteria amounted to $6\times 10^8$ at 99 hours' storing. After this storing time, the amount of volatile base nitrogen was 24.4 mg%; this storing time corresponds to the initial stage which shows the rapid increasing of the amount of volatile base nitrogen.

In the storing at 25°±1°C, the number of bacteria indicated maximum at 50 hours' storing, thereafter the number did not increase. During this storing time, the amount of volatile base nitrogen increased rapidly following the period of rapid increase in number

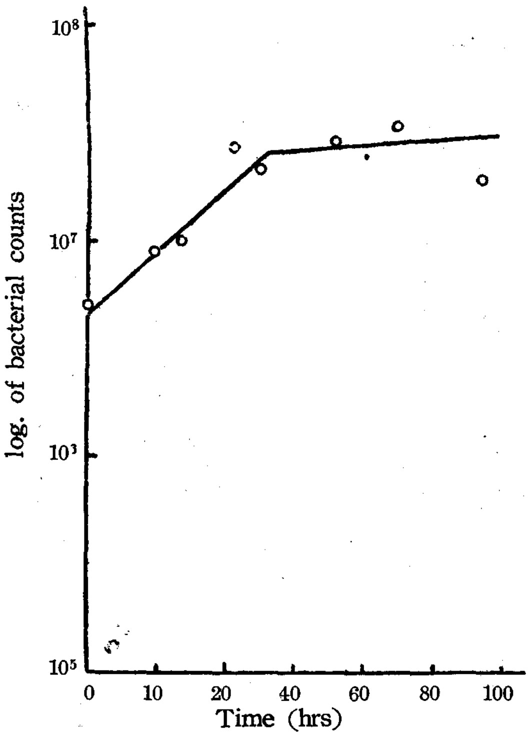


Fig. 3. Changes in the bacterial counts of *Stichopus japonicus* meat during aerobic deterioration at 25°±1°C

of bacteria.

Shewan[3] has studied on the putrefaction of haddock and dogfish, and Fieger & Friloux[4] have studied on the same subject in the case of Gulf shrimp. They have observed the relation between the amounts of volatile base nitrogen or the amino acid nitrogen and the number of bacteria. According to their results, the amount of volatile base nitrogen showed rapid increase after the number of bacteria reached to equilibrium via the lag period.

The authors' result which was obtained in the relation between the amount of volatile base nitrogen and the number of bacteria agreed with the results of Shewan, and Fieger & Friloux. The pH value of the meat decreased temporarily to the acidic side, but it increased in company with the increasing of the amount of volatile base nitrogen at 30 hours' storing. The amount of volatile base nitrogen of the meat increased rapidly after the period of rigor mortis. This is perhaps due to the decomposition by bacteria of amino acids or peptides in free state.

Observing the relation between the amount of volatile base nitrogen and the kind of organic bases detected, it differs dependant upon the storing temperature. Agmatine was detected when the amount of volatile base nitrogen reached to about 9.6 mg% and indol was detected when the amount of volatile base nitrogen reached about 20 mg% (after 90 hours' storing at 16°±3°C or after 27 hours' storing at 25°±1°C.) Putrescine was also detected at the point of production of the amount of volatile base nitrogen of about 20 mg%. Cadaverine-like substance was detected when the amount of volatile base nitrogen reached to 25 mg%.

Observing the $HgCl_2$-reaction of the meat of *Stichopus japonicus*, A-solution indicated (−) and B-solution indicated (±) at the initial period of the storing. When the pH value of meat shifted to the alkali side, A-solution indicated (±), B-solution indicated (+). The $HgCl_2$-reaction of the meat shows contrary to that for fish meat. That is to say, the reaction of the meat extractive of *Stichopus japonicus* is sharp for

B-solution (acidified $HgCl_2$ solution with acetic acid), but it is not sharp for A-solution ($HgCl_2$ solution without acetic acid).

That fact that in the fresh meat of *Stichopus japonicus*, B-solution is positive, may be due to the presence of some substance which is stable toward the $HgCl_2$ solution, but is not stable toward acetic acid. If this substance is supposed to be some protein, it may be a mucin which is precipitated by acetic acid.

Taking into consideration the results as above obtained, the mucous matter began to increase on the surface of skin of *Stichopus japonicus* within a short time after rigor mortis, at which time the amount of volatile base nitrogen indicated 10 mg%, and the body was yet edible.

With the decline in the freshness of the meat, at the time when the amount of volatile base nitrogen reached about 20 mg%, indol and putrescine were detected, and the fishy smell of the body changed to a bad smell and the meat became unedible. This point is considered to represent the incipient putrefaction of the meat. The incipient putrefaction of fish meat is at 30 mg% in amount of volatile base nitrogen,[5] but it is better to consider that point of the meat of *Stichopus japonicus* to be at 20 mg% of the volatile base nitrogen, from the results obtained as above stated.

In case *Stichopus japonicus* is prepared for the canned boiled meat, when raw material is used of which the amount of volatile base nitrogen is over 10 mg%, the canned food becomes of bad quality, that is to say, the limit of the freshness of *Stichopus japonicus* for the raw material of canned food is considered to be at about 10 mg% of volatile base nitrogen.

Accordingly, it is important to know the time at which the meat of bodies of *Stichopus japonicus* after death will reach to 10 mg% in the amount of volatile base nitrogen. This time, "$t_{10}$" is different from the storing temperature. From Figs. 2 and 5 in the previous article III and from Fig. 1 in this article, when the meat is left aerobically at various temperatures, the values of "$t_{10}$" are as shown below in Table 3 and Fig. 4.

According to the results obtained with the meat of crab[6] (*Erimacrus isenbeckii*), saury[7] (*Cololabis saira*), mackerel[8] (*Scomber japonicus*), Atka mackerel[9] (*Pleurogrammus azonus*) and squid[10] (*Ommastrephes sloani pacificus*) by the senior author, the relation between the freshness of the meat and the storing temperatures is shown as the following equation (1).

Table 3

| Temp. ($\theta$°C) | $t_{10}$ (hrs) |
|---|---|
| 16 | 45 |
| 25 | 15 |
| 28 | 10 |
| 35 | 4 |
| 45 | 2 |

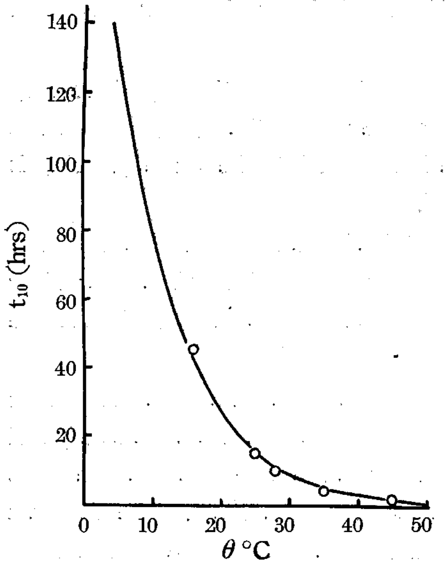


Fig. 4. Relation between the values of "$t_{10}$" and temperature "t"

$$t_{10\theta}=\alpha-\beta\theta \quad \ldots\ldots\ldots\ldots (1)$$

Here, $t_{10\theta}$ is the maximum time in which the freshness of the meat reaches to the limit (10 mg% of the amount of volatile nitrogen) for the raw material at the temperature of $\theta°$ C; $\alpha$ and $\beta$ are the constants.

The relation between the values of "log $t_{10\theta}$" and the storing temperature of "$\theta°$ C" is linear as shown in Fig. 5. This straight line is manifested by equation (2).

$$\log t_{10\theta}=2.322-0.0452\theta \quad \ldots\ldots\ldots\ldots (2)$$

The relation between the values of $t_{10\theta}$ and the storing temperatures ($\theta$°C) is shown in Fig. 6 as a particular functional scale.

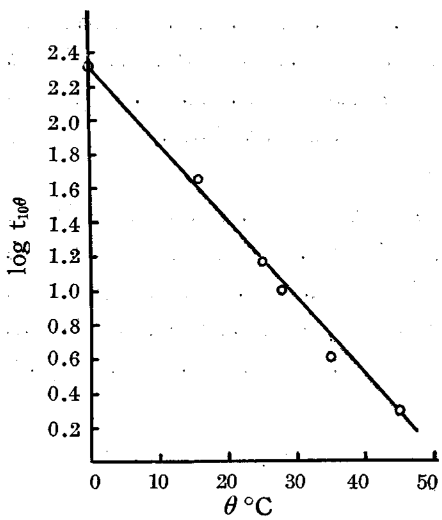


Fig. 5. Relation between the values of "log $t_{10\theta}$" and temperature "t"

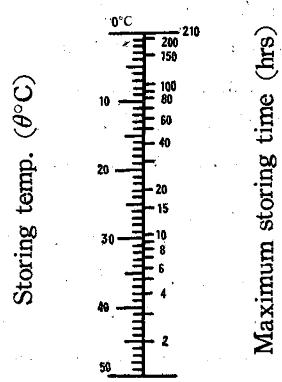


Fig. 6. Functional scale of *Stichopus japonicus* meat to show the relation between the maximum storing time and storing temperature

In Fig. 6, it may be seen that if the room temperature of the cannery is 10°C, the meat of *Stichopus japonicus* must be handled and packed in cans and the sterilization started within 74 hours; if at 20°C, the maximum time is 27 hours; and if at 30°C, the maximum time is 9 hours.

## Summary

(1) The change in the freshness of the meat of *Stichopus japonicus* was observed by estimation of the amounts of volatile base nitrogen and amino acid nitrogen, by the $HgCl_2$-reaction (Amano's method), by the values of pH and by the bacterial counts. The estimation of the amount of volatile base nitrogen is considered to be the most suitable for the measurement of the freshness of the meat of *Stichopus japonicus.*

(2) The increase and the decrease of the amount of organic bases were observed in company with the estimation of the amount of volatile base nitrogen.

(3) When the amount of volatile base nitrogen reaches to 20 mg% in the meat of *Stichopus japonicus*, it is considered to be in the state of incipient putrefaction, and the limit of freshness of the raw material safe for use is suggested to be 10 mg% of the amount of volatile base nitrogen.

(4) When the limit of the freshness of meat for edible or for raw material for canned food being decided to be 10 mg% in the amount of volatile base nitrogen, the change of the limit of leaving time of the meat according to the environmental temperatures was examined. The relation between the limit of leaving time and the environmental temperatures has been manifested by a functional scale.


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