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Title	STUDIES ON POST-MORTEM CHANGES IN THE CHEMICAL CONSTITUTION OF THE MEAT OF SEA CUCUMBER (STICHOPUS JAPONICUS SELENKA) : III. The Putrefaction of the Meat of Stichopus japonicus
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STUDIES ON POST-MORTEM CHANGES IN THE CHEMICAL CONSTITUTION OF THE MEAT OF SEA CUCUMBER (*STICHOPUS JAPONICUS* SELENKA)

III. The Putrefaction of the Meat of *Stichopus japonicus*

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The chemical components of the meat of *Stichopus japonicus* are different from fish meat muscle in the larger amount of water content and the smaller total amount of nitrogen content. This subject has been treated in an earlier paper, article II.[1] The larger amount of water content in the meat of *Stichopus japonicus* is supposed to have intimate relation with the putrefaction of the meat.

In order to ascertain the type of putrefaction, the velocity of bacterial decomposition was estimated by calculating the velocity constant (the volatile base producing velocity constant), temperature constant and temperature coefficient.

1. Velocity of bacterial decomposition

(1) Sample

Bodies of *Stichopus japonicus*, which were caught in the sea near Hakodate were eviscerated, and crushed. The crushed meat was employed for the experiment.

(2) Experimental method

The crushed meat was put in separate bottles with wide mouths which were plugged with cotton. The material was left in aerobic condition at various temperatures: 16°, 28°, 35° and 45°C (± 2°C respectively). On the other hand, samples of the crushed meat were also left in anaerobic condition at 19° and 35°C respectively. Anaerobic condition was secured by leaving several bottles with sample in a vacuum glass vessel, in which pyrogalic acid and sodium hydroxide were added.

The skin part of *Stichopus japonicus* as shown in Fig. 1 in the previous experiment,[2] was also crushed and left in aerobic condition at 28°C.

The samples were taken up at definite time intervals, and employed for the following various estimations. Determination of pH: By glass electrode meter. Volatile base nitrogen: By Weber and Wilson's method using 10 g of the sample.

(3) Experimental results

Results obtained are shown in Table 1– Table 6 and Fig. 1– Fig. 3.

Table 1. Chemical changes and organoleptic inspection of *Stichopus japonicus* meat during aerobic putrefaction at 16°C

Item \ Time (hrs)	0	20	44	68	115	141
Volatile base-N(mg%)	7.06	6.39	12.92	16.72	35.04	32.36
Amino-N (mg%)	158.8	214.4	259.3	214.4	185.2	92.6
pH	6.1	5.9	6.2	6.25	6.3	6.4
$HgCl_2$-reaction A soln.	−	−	−	−	−	−
$HgCl_2$-reaction B soln.	−	−	±	±	±	±
Organoleptic test	freshness fishy smell	〃	slight stale	stale	putrefactive odor	〃

Table 2. In the case of aerobic putrefaction at 28°C

Item / Time (hrs)	Surface skin part: Volatile base-N (mg%)	Surface skin part: pH	Meat part: Volatile base-N (mg%)	Meat part: Amino-N	Meat part: pH	Meat part: $HgCl_2$-reaction A soln.	Meat part: $HgCl_2$-reaction B soln.	Organoleptic test
0	8.4	5.8	64	66.6	5.8	−	−	freshness fishy smell
20	15.1	6.2	24.4	56.8	6.0	−	±	
27	15.1	6.5	23.0		6.1	−	±	
42	23.0	6.5		66.6	6.2	−	±	
48	16.6	6.6	15.3		6.2	−	±	
50			38.4	56.4		−	±	putrefactive odor
65			52.8		6.3	−	±	
69	56.3	6.7		51.3		−	±	
72			38.4			−	±	
92	70.3	6.9	105.6	94.8	6.0	−	±	

Table 3. In the case of aerobic putrefaction at 35°C

Item \ Time (hrs)	0	9	23	32	44	71	92
Volatile base-N (mg%)	8.4	19.7	29.7	37.7	70.7	83.4	163.3
pH	6.05		5.7	5.8	5.95	4.9	4.3
Organoleptic test		slight putrefactive odor		strong putrefactive odor			

Table 4. In the case of aerobic putrefaction at 45°C

Item \ Time (hrs)	0	18	24	32	40
Volatile base-N (mg%)	8.4	37.7	41.7	56.3	95.6
pH	6.06	5.85	5.4	5.55	5.8
Organoleptic test		putrefactive odor			

Table 5. In the case of anaerobic putrefaction at 19°C

Time (hrs) \ Item	Volatile base-N (mg%)	pH
0	5.7	6.25
41	19.0	6.0
44	20.3	6.05
48	20.7	5.95
68	25.0	6.0
90	28.4	6.05
119	33.0	6.15
144	45.7	6.2
162	33.7	5.85
167	49.7	6.0

Table 6. In the case of anaerobic putrefaction at 35°C

Time (hrs) \ Item	Volatile base-N (mg%)	pH
0	8.4	6.05
19	17.7	6.1
26	17.7	5.4
38	21.0	5.7
69	41.7	5.15
95	73.1	5.4

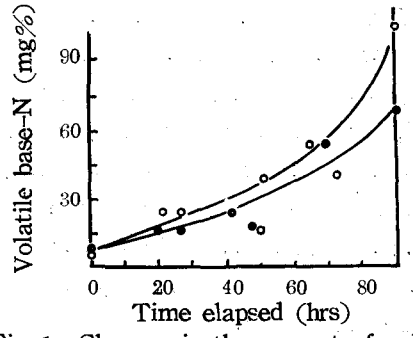

Fig. 1. Changes in the amount of volatile base nitrogen during aerobic putrefaction at 28°C

○——○ Volatile base-N (surface skin part)
●——● Volatile base-N (meat part)

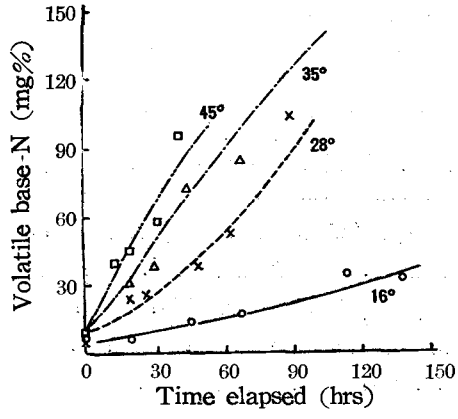

Fig. 2. Changes in the amounts of volatile base nitrogen during aerobic putrefaction at various temperatures

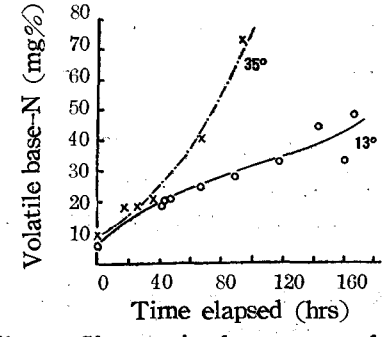

Fig. 3. Changes in the amount of volatile base nitrogen during anaerobic putrefaction at 35° and 13°C

As seen in the Tables and Figs., the higher the leaving temperature, the larger the amount of volatile base nitrogen is. That is to say, the velocity of bacterial decomposition of the meat of *Stichopus japonicus* becomes more rapid with the rising of temperature within the range of present experiment temperatures. The value of pH decreased temporarily with the prolongation of leaving time at temperatures above 35°C, but the value increased without

temporarily decreasing at lower temperatures. In the previous experimental results,[3] the value of pH of the meat of *Stichopus japonicus* in rigor mortis decreased temporarily and then increased again.

In this experiment. if the sample is examined after a short time material, the temporary decreasing of the value of pH may be observed.

As seen in Fig. 1, the meat part seems to be more decomposable than skin part.

From Figs. 2 and 3, the relation between the storing time (hrs) "t" and the values of "log y/A–y" corresponding to the storing time, was plotted as shown in Fig. 4, similarly to what was done in a previous study on crab meat.[4] Here, "y" is the increasing amount of volatile base, "A" is the maximum amount of volatile base nitrogen produced, but the value "A" in this experiment can not be found so the value of "A" was considered to be the value of "y" after the storing time of 40 hrs, e. g. 18 mg% at 16°C, 38 mg% at 28°, 70 mg% at 35°, 90 mg% at 45° aerobically, and 20 mg% at 19°, 70 mg% at 35°C anaerobically, respectively. "t" is time (hrs) from the starting point of the experiment until the estimation of the amount of volatile base nitrogen. The relation between "t" and the values of "log y/A-y" is linear. The value of the bacterial decomposition velocity constant "K" is shown as the degree of the declination of the straight line.

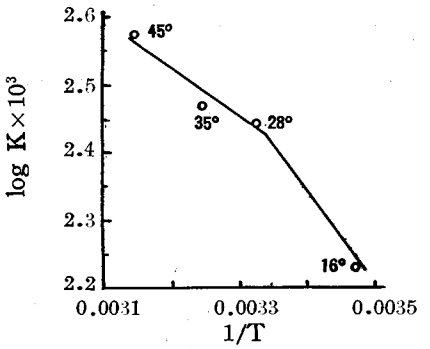

Fig. 4. Relation between the values of "log K×10³" and "1/T"

Here, the values of "K" are shown in Table 7.

Table 7. "K" values of *Stichopus japonicus* meat at various temperatures

Temp. (°C) / Leaving condition	16°	19°	28°	35°	45°
Aerobic	17	—	28	29	37
Anaerobic	—	6	—	34	—

As seen in Table 7, the value of K × 10³ was 17-37 in the range of 16°-45°C aerobically, and 6 at 19°, 34 at 35°C anaerobically, respectively. Below 30°C, the anaerobic decomposition occurs later than the aerobic decomposition. At higher temperatures, there is no difference between them.

The values of temperature constant (B) and temperature coefficient (Q_{10}) which were calculated from a similar equation to the one used in the previous study on crab meat,[4] are shown in Table 8.

Table 8. "Q_{10}" and "B" values of *Stichopus japonicus* meat

Leaving condition	Temp. (°C)	Q_{10}	B
Aerobic	16 ~ 28°C	1.5	6500
	28 ~ 45°C	1.2	34300
Anaerobic	19 ~ 35°C	3.1	19900

As seen in Table 8, in the aerobic storing the values of "Q_{10}" are 1.2–1.5 in the range of 16°–45°C; in the anaerobic storing the value was 3.1 in the range of 19°–35°C.

From those results, the meat of *Stichopus japonicus* in anaerobic condition is seen to be less decomposable than in aerobic condition, but if the decomposition once starts, the meat in anaerobic storing is more decomposable than in aerobic storing corresponding to the difference of storing temperature.

The values of the bacterial decomposition velocity constant "K", temperature constant (B), and temperature coefficient (Q_{10}) of the meat of *Stichopus japonicus* are compared with corresponding values for other fish, crab and squid meat, e. g. crab meat (*Erimacrus isenbeckii*), Atka mackerel (*Plourogrammus azous*),[5] mackerel (*Scomber japonicus*),[6] Saury (*Cololabis saira*),[7] squid (*Ommastrephes sloani pacificus*),[8] caught in autumn, which were investigated previously by the senior author.

The respective values for comparison are shown in Table 9 and Table 10.

As seen in Tables 9 and 10, the bacterial decomposition velocity constant "K", of squid meat is the largest among them, mackerel, crab, Atka mackerel are next to squid meat in that order. The meat of *Stichopus japonicus* is the smallest. That is to say, the meat of *Stichopus japonicus* is less decomposable. This is perhaps owing to the fact that the meat of *Stichopus japonicus* has a small amount of meat extractive and the "meat" consists principally of connective tissue, collagen, as stated lately in "Studies on the proteins of the meat of Sea Cucumber (*Stichopus japonicus SELENKA*)".[9]

Table 9. Comparative value of "K" in *Stichopus japonicus* and other fish meat

Species \ Temp. (°C)	14°–16°	19°–21°	24°–28°	33°–37°	45°±2°
Sea cucumber meat (Aerobic)	17	—	28	29	37
Sea cucumber meat (Anaerobic)	—	6	—	34	—
Crab meat (Aerobic)	—	—	74	91	—
Crab meat (Anaerobic)	—	—	71	—	—
Atka mackerel meat (Aerobic)	—	22	36	60	—
Mackerel (〃)	30	—	99	114	134
Saury (〃)	27	—	30	48	59
Squid (〃)	—	77	103	151	—

Table 10. Comparative values of "Q_{10}" and "B" in *Stichopus japonicus* and other fish meat

Materials	Range of temp.	Q_{10}	B
Sea cucumber meat			
Aerobic	16°~28°	1.5	7×10^3
	28°~45°	1.2	34×10^3
Anaerobic	19°~35°	3.1	20×10^3
Crab meat (Aerobic)	4°~35°	1.2	3×10^3
Atka mackerel	over 25°C	1.5	8×10^3
(〃)	below 25°C	3.5	21×10^3
Mackerel	over 25°C	1.2	3×10^3
(〃)	below 25°C	3.6	22×10^3
Saury (〃)	below 40°C	1.3	5×10^3
Squid (Autumn) (〃)	below 40°C	1.6	9×10^3

The values of temperature constant (B) and temperature coefficient (Q_{10}) of mackerel meat are the largest amongst all the meats of fish, crab and squid. Atka mackerel, squid, *Stichopus japonicus*, saury and crab meat are next in order.

2. Increase and decline of the production of organic bases accompanying the bacterial decomposition of the meat of *Stichopus japonicus*

When the meat of *Stichopus japonicus* is stored at 25°C, the amount of volatile base nitrogen incearses with the lapse of time.

During the bacterial decomposition, the organic bases were detected.

(1) Sample

The sample employed in this experiment was the same as in article I.[1)]

(2) Experimental method

The crushed meat was left aerobically at 25°C, and taken up for the experiment.

The estimation of the amount of volatile base nitrogen was done by Weber and Wilson's method. Organic bases were detected by paper chromatography. The preparation of samples, developing solvents, and revealing reagents were as follows.

a) Volatile bases: Each 30 g of the crushed meat which was taken at definite time intervals, was put in a flask. Five cc of 1 N HCl was put in a receiver which was kept to soak in the lowest end of the cooler which was connected with the flask. Five cc of 10 % NaOH was added to the sample and distilled by steam. When 50 cc of the distillate was obtained (after 45 minutes' distillation), the distillation was stopped. The distillate was transferred into an evaporating dish, evaporated to dryness on a water bath, and the excess of HCl was driven off. Then, hydrochloride of volatile bases obtained was diluted with 0.5 cc of water and employed for the chromatography. As solvents a mixture of n-butanol, acetic acid and water (3 : 1 : 2) was employed in one dimensional (ascending) chromatography. Developed spots were revealed by spraying of

0.1 % ninhydrin-n-butanol solution.

b) Non volatile bases: Ten g quantity of the crushed meat was taken and adjusted to below pH 3.0, diluted with 50 cc of 95% alcohol and left for about 2 hours. The precipitated matter was separated by centrifugal separator. As the freshness of the sample falls, the larger becomes the amount of precipitatd matter, and it takes a long time to separate the precipitated matter. After the transparent solution thus obtained was concentrated to 1 cc it was employed for paper chromatography.

The developing reagent was 1 N NH_4OH saturated n-butanol. The spot was developed by ascending method of one dimensional paper chromatograpy. The developed spots were revealed by Diazo reaction. Histidine and tyrosine derivatives were detected. Another spot was developed by a mixture of n-butanol, acetic acid, pyridine and water (4 : 1 : 1 : 2), and revealed by Sakaguchi reaction; chemical components containing guanidine radical were detected.

c) Detection of indol and phenols: Fifty grams of the crushed meat was taken and dil. sulfuric acid was added to acidify for the congo-red test paper, and then the solution was distilled out 2/3 of the original volume. Ten grams of NaCl was dissolved in the distillate, and 30 cc of ether was added and shaken. The fraction of indol and phenols is obtained in the ether layer. The ether layer was separated from the water layer. To the separated ether layer sodium sulfate was added to dehydrate. Ether was removed at lower temperature, and the residue was employed for chromatography. As the developing solvent, water saturated n-butanol was used. The spot was developed ascending by one dimensional chromatography. The developed spot of phenols was detected by Millon reaction, and spot of indol was revealed by Ehrlich's reagent (HCl solution of *p*-dimethylaminobenzaldehyde).

(3) Experimental results

The relation between the amount of volatile base nitrogen produced in the meat and the kind of organic bases produced at definite intervals of time is shown in Fig. 5.

In the curve of the production of volatile base nitrogen in the meat which was stored aerobically at 25°C, agmatine in the fraction of volatile bases was detected, also histidine and tyrosine were revealed by Diazo reaction. Arginine and agmatine-like substance were detected at 20 hours' storing (at that time, the amount of volatile base nitrogen was 9.6 mg%). Putrescine and cadaverine in the fraction of volatile base were observed, agmatine was also detected and histidine and tyrosine were detected by Diazo reaction; while arginine, agmatine and arcaine were detected by Sakaguchi reaction, respectively, after 27 hours' storing (at that time, the amount of volatile base nitrogen was 18.5 mg%).

The production of histidine and tyrosine continued to 72 hours' storing after the leaving of the meat (at that time, the amount of volatile base nitrogen was 76.2 mg%).

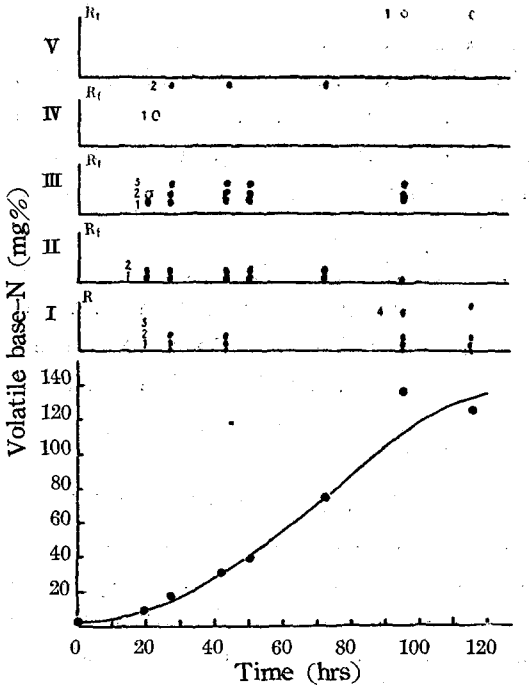

Fig. 5. Changes in the amount of volatile base nitrogen and detected organic bases of *Stichopus japonicus* meat during aerobic deterioration at 25°C

I. Volatile amines
Spot 1. putrescine 2. cadaverine 3. agmatine 4. iso-amylamine (?)
II. Non-volatile amines (Diazo)
Spot 1. histidine 2. tyrosine
III. Non-volatile amines (Sakaguchi)
Spot 1. arginine 2. agmatine 3. arcaine
IV. Tryptophan derivatives
Spot 1. tryptophan 2. Indol
V. Phenol derivatives
Spot 1. cresol

The production of arginine, agmatine and arcaine continued to 95 hours (at that time, the amount of volatile base nitrogen was 139.2 mg%).

A similar spot to that of indol which was revealed after 27 hours' storing, continued to be detectable after 72 hours storing (at that time, the amount of volatile base nitrogen was 76.2 mg%). Spot in the fraction of phenols which was revealed in yellowish green by Millon's reagent after 95 hours' storing and 115 hours' storing seemed to be *p*-cresol (at that time, the amounts of volatile base nitrogen were 139.2 mg% and 125.8 mg% respectively).

Spot in the fraction of volatile bases which was revealed after 95 hours' storing and of which the value of Rf was 0.60, was judged to be iso-amylamine (Rf=0.62) or secondary amylamine(Rf=0.55). It was not ascertained whether the spot is the former or the latter.

Spot in the fraction of volatile bases which was revealed after 115 hours' storing (at that time, the amount of volatile base nitrogen was 125.8 mg%), of which the Rf value was 0.69, seemed perhaps to be not β-phenethylamine, but isoamylamnie.

In the authors' experiment on the distribution of amino acid nitrogen in the meat of *Stichopus japonicus*, the amount of nitrogen in the fraction of arginine was estimated to be twice that in other fractions.

In the organic bases produced, the amount of guanidine compounds became larger with the falling of the freshness of the meat, but histamine which is produced from histidine, tyramine which is produced from tyrosine, tryptamine, iso-butylamie, and β-phenethylamine were not detected.

Ornithine which is a decomposed interim product from arginine was not detected,

but putrescine which is a decomposed end product was detected, so the presence of ornithine may be admitted.

The presence of agmatine was also detected in the course of the putrefaction of the meat. Both decompositions of arginine perhaps occurred at the same time.

Arcaine which was detected in the meat extractive was also detected in this experiment.

But the amount of arcaine was not ascertained, so it is difficult to judge whether arcaine is a decomposition product or not.

As decomposition products of tyrosine, besides the production of tyramine, the course of production of phenol through oxyphenylacetic acid is expected. In the present experiment, a spot like that of *p*-cresol was also observed. However, it is still open to question whether *p*-cresol or phenol may be produced by bacterial action.

Indol, skatol and indol acetic acid are produced from tryptophan, at the putrefaction of the meat. Although the production of indol was observed, the presence of tryptophan was not detected.

Discussion

Summarising up the results obtained, the process of the decomposition of the meat of *Stichopus japonicus* was similar to that of fish meat in the kind of decomposition products and in the variation of the amount of the products. But the meat of *Stichopus japonicus* is more difficult to decompose than fish meat. This is considered to be due to the small amount of the meat extract, moreover the histological characteristic of the tissue may exert some influence. The meat of *Stichopus japonicus* consists of a collagen fiber network, in which there is contained a large amount of water bound with various kinds of protein.[9]

According to Simidu,[10] there are differences in the decomposition velocity in accordance with the differences of the physical properties (e. g. hardness of the meat). The tissue of the meat of *Stichopus japonicus* which is different histologically from fish meat consists principally of insoluble scleroprotein (structural proteins), therefore, there seems to be difference in the decomposition velocity.

Summary

(1) Aerobic and anaerobic bacterial decomposition of *Stichopus japonicus* meat were discussed in comparison with the same process in fish meat.

(2) It was ascertained that the meat of *Stichopus japonicus* is more difficult to decompose than fish meat. One of the causes of such difference in the velocity of bacterial decomposition was considered to be the histological characteristic.

(3) The variation of the amounts of amino acid and organic bases in the process of the bacterial decomposition was examined, and the results obtained were discussed.

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