STUDIES ON THE NUTRITIVE VALUE OF THE MEAT OF SEA CUCUMBER (*STICHOPUS JAPONICUS* SELENKA)

VI. Nitrogen Distribution and Kind of Chemical Components of the Extractives of the Meat and Skin Parts of *Stichopus japonicus*

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1. Nitrogen distribution in the hydrolyzate of the extractive

It is necessary to know the nitrogen distribution of the extractive of meat and skin parts of *Stichopus japonicus* for the sake of the determination of the tasteful matter.

The authors have undertaken to ascertain the nitrogen distribution of nonprotein in the hot water extractive of the meat part and skin part of *Stichopus japonicus*.

In this experiment, each chemical component in the extractive has not been separated.

(1) Preparation of sample

The bodies of *Stichopus japonicus* were divided into meat part and skin part as shown in Fig. 1, previous paper.[4] One hundred and fifty grams of meat and skin parts respectively were extracted with about 500 cc of hot water at 45°–50°C for one hour. Hot water soluble protein in each extractive was precipitated by 20% trichloracetic acid solution, and was filtered. Each filtrate was employed.

(2) Experimental method

Each filtrate was concentrated to about 100cc. Peptide-like substance* which is formed during the concentration of the filtrate was again filtered, and then the filtrate was hydrolyzed with 20% HCl solution for 48 hours. Nitrogen distribution in the hydrolyzate was determined by Van Slyke's method. The amounts of total nitrogen in meat and skin parts were estimated by the usual method.

(3) Experimental results

Results obtained are shown in Table 1.

As seen in Table 1, soluble nitrogen in the meat extractive which was hydrolyzed by 20% HCl solution for about 48 hours is about 0.06 g per 100 g of meat, and there is no difference found between meat part and skin part.

The ratio of amide nitrogen to total nitrogen is 3–5%, and the ratio of humine nitrogen to total nitrogen is 13–17%. The percentage contained in the skin part is larger than that in meat part. The ratio of the amount of histidine nitrogen to the amount of total nitrogen is the largest in the fraction of basic nitrogen showing 20–24%; this component is important to the taste of *Stichopus japonicus*.

* This substance was detected by paper chromatography after the filtration of it. The Biuret reaction showed light violet color. Consequeutly, the substance was determined to be not an amino acid.

Table 1. Nitrogen distribution of extracts from meat and surface skin of *Stichopus japonicus*

Fraction \ Sample	Extract of meat part		Extract of surface skin part	
	g in 100 g of sample	Per cent to total-N of extract	g in 100 g of sample	Per cent to total-N of extract
Total-N of extract	0.067	100	0.058	100
20 % HCl insoluble-N	0.003	4.48	0.002	3.45
20 % HCl soluble-N	0.064	95.52	0.056	96.55
Amide-N	0.002	2.98	0.003	5.17
Humine-N	0.009	13.41	0.010	17.21
Basic total-N	0.025	37.35	0.023	39.70
Arginine-N	0.011	16.40	0.008	13.80
Histidine-N	0.014	20.09	0.014	24.15
Lysine-N	0.0004	0.598	0.001	1.72
Cystine-N	0.0003	0.448	0.0003	0.517
Basic amino-N	0.008	11.94	0.008	13.80
Mono acid total-N	0.027	40.3	0.022	37.97
Mono amino acid amino-N	0.010	14.9	0.010	17.24
Mono amino acid non-amino-N	0.017	25.4	0.012	20.73
Total	0.066	98.52	0.060	103.5
Remarks	Total-N of meat part is 1.02 %		Total-N of surface skin part is 0.997 %	

The absolute amounts of total nitrogen of the meat and the meat extractive are smaller than those found in other fish meat. That is to say, the amount of total nitrogen of extractive of meat and skin parts is about 60 mg%, respectively, and this quantity is only a fraction of the quantity of total nitrogen of extract of red flesh fish meat, bonito and tuna[1], or squid meat.[2] The quantity of total nitrogen of extract of *Stichopus japonicus* meat is a quarter of that of white flesh fish meat of tetradon fish, ayu-fish, flat fish, etc. Similar facts are seen not only in the absolute amount of total nitrogen of the extract of the meat of *Stichopus japonicus*, but also in the ratio of the amount of total nitrogen of extractive of the meat part or the skin part to the amount of total nitrogen in meat part or skin part.

The ratio of the amount of total nitrogen of extract of the meat of *Stichopus japonicus* to the amount of total nitrogen thereof is about 6%. On the other hand, the similar percentages of tetradon fish and flat fish are about 10%. The light taste of

Stichopus japonicus may be owing to those facts. Simidu[3] has said that the activity and the heavy taste of red flesh fish may be owing to the large amount of basic nitrogen (diamino acid nitrogen). In fact, red flesh fish meat does have a larger amount of diamino nitrogen than monoamino nitrogen in the extractive. The amounts of diamino nitrogen and monoamino nitrogen in the extractive of the meat of *Stichopus japonicus* are almost equal.

The absolute amounts of monoamino nitrogen and diamino nitrogen in the extractive of the *Stichopus japonicus* meat are smaller than those in other fish. The light taste of the meat of *Stichopus japonicus* may be also due to this fact.

The amount of humine nitrogen in the extract of the *Stichopus japonicus* meat is remarkably large in the meat part as well as in the skin part.

2. The kind of chemical components detected in the extracts of meat and skin parts by paper chromatography

In the experiment described just above, the amounts of chemical components in the extract of meat and skin parts of *Stichopus japonicus* are found to be inferior to those of other fish meat as estimated by Van Slyke's method.

The authors have tried to investigate the kind of chemical components. The extract of meat and skin parts were respectively concentrated and subjected to two-dimensional paper chromatography.

(1) Sample

The sample was prepared by the same method as described in the previous article 1.

(2) Experimental method

Small quantities of the concentrated extract of meat and skin parts respectively were employed for two-dimensional paper chromatography. Adsorbed chemical components were tested by ninhydrin, Pauli, Sakaguchi and Xanthoprotein reactions and by Dragendorf's reagent.

(3) Results

Fig. 1 shows a two-dimensional paper chromatogram which exhibits the kind of chemical components in the extract of meat part of *Stichopus japonicus*. The first run was made with the phenol containing 10% water and second run with a mixture of lutidine, aniline and water (65 : 7.2 : 28). The spots were tested by 1% ninhydrin butanol solution.

As seen in Fig. 1, amino acids which have been found in fish meat are also nearly all found in the extract of meat part of *Stichopus japonicus*.

Fig. 2 shows the paper chromatogram of the extract of the skin part which was obtained by the same method. No difference in the kinds of amino acids in the meat parts and skin parts was detected.

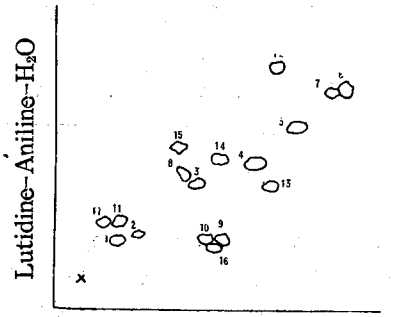


Fig. 1. Paper chromatograms of amino acids of extract from *Stichopus japonicus* meat

1. Aspartic acid
2. Glutamic acid
3. Glycine
4. Alanine
5. Valine
6. Phenylalanine
7. Leucine
8. Serine
9. Arginine
10. Lysine
11. Cystine
12. Tyrosine
13. Arcaine
14. Threonine
15. Betaine
16. Ornithine
17. Adenine

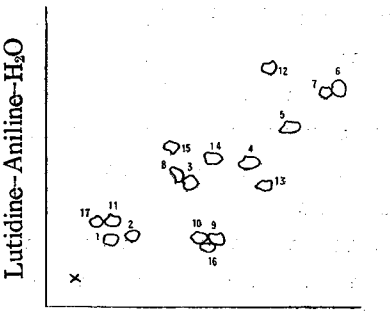


Fig. 2. Paper chromatograms of amino acids of extract from surface skin of *Stichopus japonicus*

1. Aspartic acid
2. Glutamic acid
3. Glycine
4. Alanine
5. Valine
6. Phenylalanine
7. Leucine
8. Serine
9. Arginine
10. Lysine
11. Cystine
12. Tyrosine
13. Arcaine
14. Threonine
15. Betaine
16. Ornithine
17. Adenine

From the extract of meat part and skin part of *Stichopus japonicus* by means of paper chromatography, there were found: (1) aspartic acid (2) glutamic acid (3) glycine (4) alanine (5) valine (6) phenylalanine (7) leucine (8) serine (9) arginine (10) lysine (11) cystine (12) tyrosine (13) arcaine (14) threonine (15) betaine (16) ornithine and (17) adenine, etc. That is to say, the chemical components found in both the meat and skin parts are almost the same.

Among those chemical components, tyrosine was again found in the two-dimentional paper chromatograms of the extracts of meat and skin parts respectively, of which the first run was with 1N ammonium saturated butanol and second run with phenol containing 10% water, and developed spots were tested by Pauli reaction. In those chromatograms, the spot of histidine was not confirmed by ninhydrin and p-bromaniline.

Arginine and arcaine also were again confirmed in the two-dimensional paper chromatograms of the extract of both meat and skin parts of which the first run was made with a mixed solution of butanol, acetic acid, pyridine and water (4 : 1 : 1 : 2) and second run with 10% ammonium saturated butanol. The developed spots were tested by the Sakaguchi reaction.

Tyrosine, phenylalanine also were again confirmed in the two-dimensional paper chromatograms of the extractives of both parts of which the first run was carried out with a mixture of lutidine and aniline, the second run with phenol containing 10% water, and the adsorbed spots were tested by Xanthoprotein reaction.

Glycine, alanine, betaine, arginine, adenine which have been previously found, and creatinine newly were confirmed in the extract of meat part in the two-dimensional paper

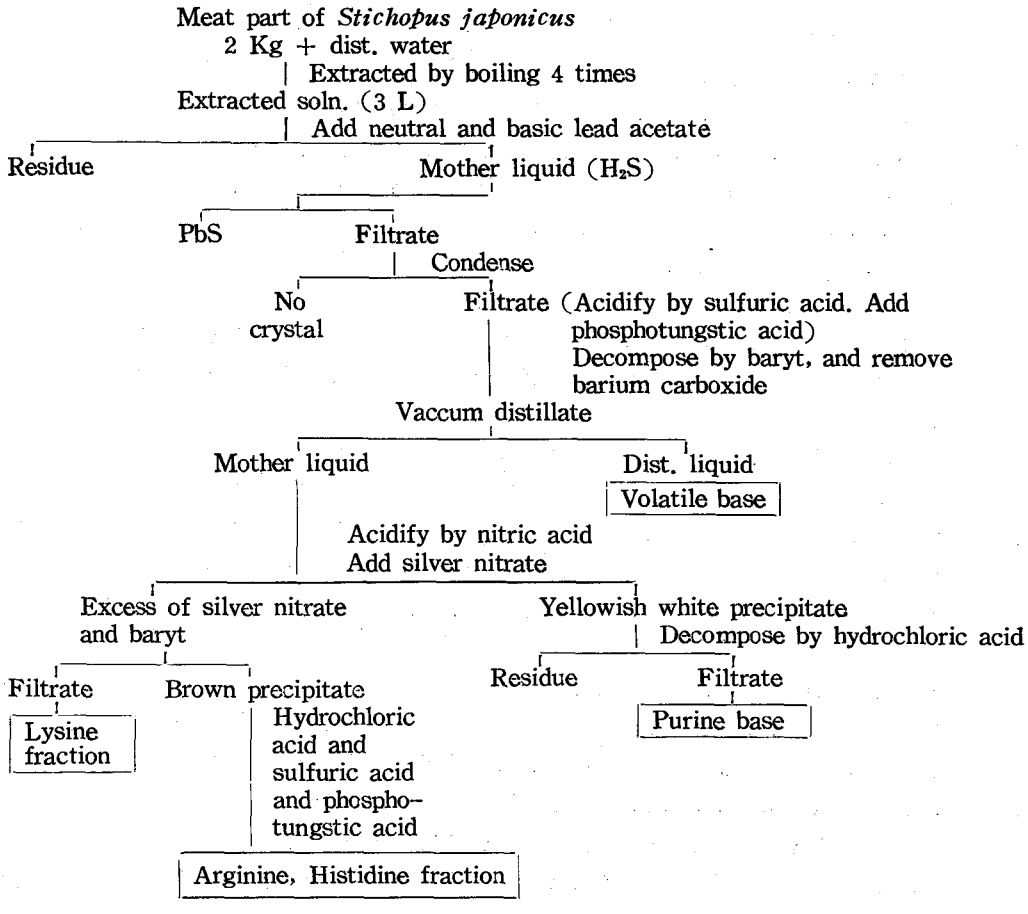
chromatogram of which the first run was made with a mixture of n-butanol, ethanol and water (4 : 1 : 2) and second run with a mixture of n-butanol, acetic acid and water (4 : 1 : 2). The developed spots were tested by Dragendorf's reagent. Creatinine was found in a partition chromatogram of which the spots were tested by Jaffé's reaction. But creatine was not confirmed.

From the results obtained as described above aspartic acid, glutamic acid, glycine, alanine, valine, phenylalanine, leucine, serine, arginine, lysine, cystine, tyrosine, arcaine, threonine, betaine, ornithine, adenine, creatinine were found to exist in the meat and skin parts of *Stichopus japonicus*. Those components are almost the same as those in the extracts of fish meat.

3. Chemical components in the basic fraction

In the previous article 2 of this paper, chemical components in the extractive of

Scheme 1. Fractionation of the extract of meat part of *Stichopus japonicus*



meat and skin parts of *Stichopus japonicus* were detected by two-dimensional paper chromatography, but it was difficult to make a full analysis of all the spots adsorbed in the filter paper. Therefore a determination of the basic fraction was separately undertaken in the following manner. The sample was analyzed by two-dimensional paper chromatography.

(1) Preparation of sample

The extract of meat part of sea cucumber was fractionated in the following manner.

(2) Experimental results

Chemical components in the volatile base fraction were ascertained by partition paper chromatography and visible maps were treated with ninhydrin and Dragendorf's reagents, but the Rf values of the maps were not determined. Chemical components in purine base fraction were determined by partition paper chromatography of which the first run was made with butanol acetic acid and adsorbed spots were tested by nitrate solution containing 0.25 M mercury nitrate. Adenine was detected, but hypoxanthine, xanthine, guanine were not found.

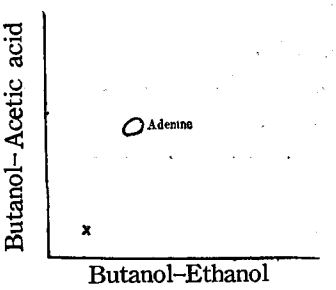


Fig. 3. Paper chromatograms showing purine base fraction

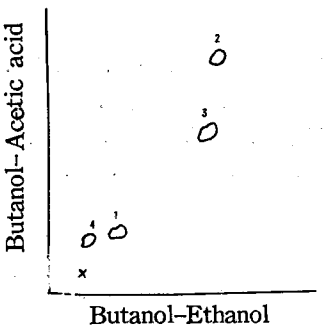


Fig. 4. Paper chromatograms showing arginine and histidine fraction

1. Arginine 2. Arcaine 3. Creatinine 4. Ornithine

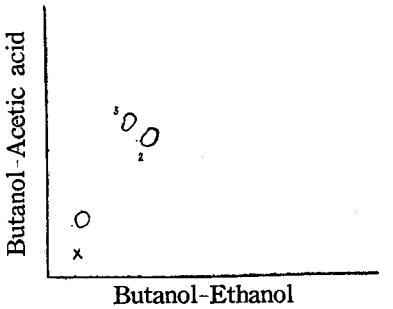


Fig. 5. Paper chromatograms showing lysine fraction

1. Lysine 2. Betaine 3. Choline

In the arginine and histidine fraction, methylguanidine, which is often found in animal bodies, was not detected by the ninhydrin reaction, Sakaguchi reaction nor Jaffé's reaction in the same chromatography.

In the lysine fraction, choline was detected with Dragendorf's reagent and ninhydrin reaction by the same chromatography.

A map in the chromatogram in the fraction of volatile base which is positive for Dragendorf's reagent is supposed to

represent trimethylamine, because choline, glycine and betaine were clearly detected as above described. But the point is questionable.

As one may see from the above described results, adenine, arginine, arcaine, creatinine, ornithine, choline, betaine, lysine were detected in the fraction of volatile base.

Summary

The nitrogen distribution of the extracts of meat and skin parts of *Stichopus japonicus* is inferior quantitatively to that of fish meat. The absolute amount of total nitrogen in the extract of *Stichopus japonicus* is inferior to that of fish meat. Consequently the meat of *Stichopus japonicus* is light in taste.

The sorts of chemical components of extract of meat and skin parts of *Stichopus japonicus* were detected to be as follows: aspartic acid, glutamic acid, glycine, alanine, valine, phenylalanine, leucine, serine, arginine, lysine, cystine, tyrosine, arcaine, threonine, betaine, ornithine, adenine, creatinine, choline.

Those components are almost the same as those of fish meat.

Literature cited

1) Simidu, W. (1948). *Bull. Jap. Soc. Sci. Fish.* **15** (1), 35.

2) Yoshimura, K. & Kubo, S. (1952). *Bull. Fac. Fish., Hokkaido Univ.* **3** (3), 205.

3) Simidu, W. *et al.* (1953). *Bull. Jap. Soc. Sci. Fish.* **19** (8), 871.

4) Tanikawa, E., Akiba, M. & Yoshitani, S. (1955). *Bull. Fac. Fish., Hokkaido Univ.* **5** (4), 341.