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Citation	北海道大學水産學部研究彙報, 6(1), 37-41
Issue Date	1955-05
Doc URL	https://hdl.handle.net/2115/22912
Type	departmental bulletin paper
File Information	6(1)_P37-41.pdf



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

V. Nitrogen Distribution and Amino Acid Composition of
Protein of Meat of *Stichopus japonicus*

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In order to ascertain the nutritive value of foods, the nitrogen distribution and amino acid composition of the protein in the foods should be determined. This is true because there are nutritive-complete proteins which have special important amino acids and nutritive incomplete which are lacking in the important amino acids. It is fully accepted that the proteins of cattle meat, chicken meat and fish meat are nutritive-complete.

The authors have tried to investigate the nitrogen composition of the so called meat (edible part) of *Stichopus japonicus* in order to determine whether it contains nutritive-complete protein or not.

1. Nitrogen distribution and amino acid composition of protein of raw fresh meat

(1) Sample

Raw fresh *Stichopus japonicus* which was caught in the sea near Hakodate was employed. The bodies of *Stichopus japonicus* were eviscerated and were separated into two parts, meat part and skin part, as described previously.⁷⁾ Each part of the bodies was cut with a knife and crushed in a glass mortar respectively and employed as samples.

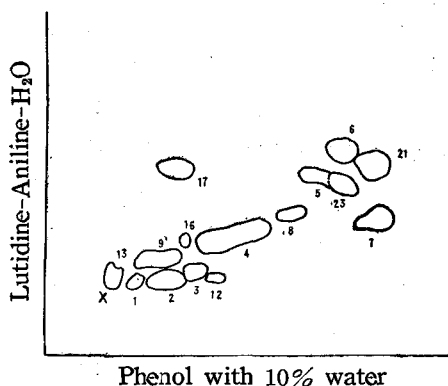


Fig. 1 Paper chromatograms of amino acids detected in spring *Stichopus japonicus* meat (hydrolysis with 20 %HCl)

- | | |
|-------------------|------------------------|
| 1. Aspartic acid | 9. Serine |
| 2. Glutamic acid | 12. Ornithine |
| 3. Glycine | 13. Cystine |
| 4. Alanine | 16. Threonine |
| 5. Valine | 17. 3,5-dijodotyrosine |
| 6. Phenylalanine | 21. Leucine |
| 7. Proline | 23. Methionine |
| 8. Hydroxyproline | |

Three to five gram quantities of the prepared samples of each part were hydrolyzed with 120 cc of 20% HCl solution for 48 hours, and then the nitrogen distribution of protein of samples was investigated by the use of Van Slyke's method.

At the same time small quantities of each of the hydrolyzates were employed to determine amino acid composition by two-dimensional paper chromatography. Each hydrolyzate was concentrated on a boiling water bath, and then was spotted on a

corner of a square filter paper. First run (ascending) was done with phenol containing 10% water; after drying a second run was made with the mixed solution of lutidine, aniline and water (65 : 7.2 : 28) in the right-angled direction. After drying of the dampened paper, adsorbed amino acids were tested by 1% ninhydrin butanol solution, and the kinds of amino acid were determined by the values of R_f of the colored maps.

(2) Experimental results and discussion

Results obtained are shown as Table 1, Fig. 2 and Fig. 3.

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

Fraction	Sample	Meat part		Surface skin part	
		g in 100 g of sample	Per cent to total-N	g in 100 g of sample	Per cent to total-N
Total-N		1.020	100.00	0.997	100.00
20% HCl insoluble-N		0.049	4.80	0.053	5.35
20% HCl soluble-N		0.971	95.20	0.944	94.69
Amide-N		0.035	3.43	0.025	2.53
Humine-N		0.115	11.47	0.175	17.55
Basic total-N		0.228	22.40	0.216	21.65
Arginine-N		0.114	11.16	0.104	10.48
Histidine-N		0.095	9.43	0.086	8.61
Lysine-N		0.008	0.82	0.019	1.87
Cystine-N		0.009	0.93	0.007	0.69
Basic amino-N		0.078	7.65	0.080	8.04
Mono amino acid total-N		0.579	56.70	0.534	53.60
Mono amino acid amino-N		0.430	42.15	0.449	45.00
Mono amino acid non amino-N		0.149	14.55	0.085	8.60
Total *		1.008	98.80	1.003	100.60

* (20% HCl insoluble-N) + (Amide-N) + (Humine-N) + (Basic total-N) + (Mono amino acid total-N)

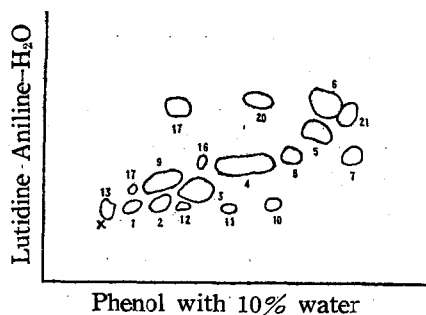


Fig. 2 Paper chromatograms of amino acids detected in surface skin part of spring *Stichopus japonicus* (hydrolysis with 20% HCl)

- | | | |
|------------------|-------------------|------------------------|
| 1. Aspartic acid | 7. Proline | 13. Cystine |
| 2. Glutamic acid | 8. Hydroxyproline | 16. Threonine |
| 3. Glycine | 9. Serine | 17. 3,5-dijodotyrosine |
| 4. Alanine | 10. Arginine | 20. Tyrosine |
| 5. Valine | 11. Lysine | 21. Leusine |
| 6. Phenylalanine | 12. Ornithine | |

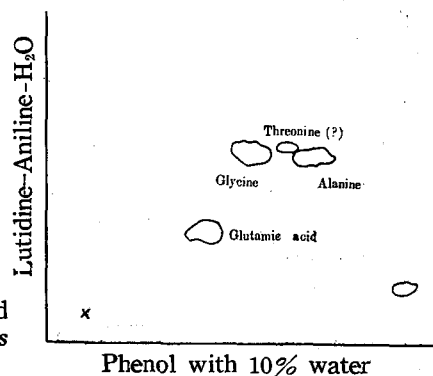


Fig. 3 Paper chromatograms of free amino acids detected in fresh *Stichopus japonicus* meat

As seen in Table 1, the amount of total nitrogen in 100 g of the meat part of *Stichopus japonicus* is larger than that of the skin part. On the other hand, the amount of HCl-insoluble nitrogen of the meat part is smaller than that of skin part. The amount of amide nitrogen of the meat part is larger than that of the skin part having a difference of about 1%. The amount of humine nitrogen of the meat is smaller than that of the skin part with a difference of 6%. The amount of basic nitrogen of the meat part is as large as that of the skin part; the amounts of the both parts indicate 21~22%. The amount of arginine nitrogen of the meat and skin parts indicate respectively 10~11% being the largest quantity among the basic nitrogens. The amount of histidine nitrogen which indicates 8~9% is next in quantity to the amount of arginine nitrogen. The amount of mono-amino nitrogen is 57% for the meat part and 54% for the skin part.

To compare the nitrogen distribution of the meat of *Stichopus japonicus* with other fish meat, the differences are shown as Table 2.

Table 2. Comparative nitrogen distribution in various fish and shell fish meat (calc. as 100 of total-N)

Sample	Squid (dried)	Octopus	Sea-bream	Sardine	Atka mackerel	<i>Stichopus japonicus</i>
Researcher	Igarashi & Shirai ¹⁾	Sekine, Sumi & Hara ²⁾	Hatagoshi ³⁾	Fujikawa & Naganuma ⁴⁾	Fujii ⁵⁾	Tanikawa & Ishiko
Year	1953	1920	1932	1936	1953	1953
Total-N (dry matter)	12.60	15.59	16.57	13.31	15.98	9.53
Amide-N	4.09	4.33	8.14*	6.44	5.36	3.43
Humine-N	3.33	1.45	1.81	1.22	5.14	11.47
Basic total-N	39.70	32.00	28.16		26.13	22.40
Arginine-N	20.00	17.10	11.34	11.86	8.65	11.16
Histidine-N	8.74	4.90	0.80	0.41	8.71	9.43
Lysine-N	10.07	9.37	16.19	14.97	7.85	0.82
Cystine-N	0.92	0.57	0.30	0.20	0.91	0.93
Mono amino acid total-N	52.80	59.94	59.94		56.56	56.70
Mono amino acid amino-N	43.00	58.50	59.22	61.38	46.22	42.15
Total	99.92	97.28	98.53		98.85	98.80
Remarks	Based on 100 of hydrochloric acid soluble-N, containing extract	Containing extract	Values below the mark * were calculated to the base on 100 of hydrochloric acid soluble-N (6.47%). Lacking extract.	Lacking extract	Containing extract	Containing extract

The amount of total nitrogen in the meat of *Stichopus japonicus* is about 1%, which is much smaller than the 3% total nitrogen of other fish meat. That is to say, the amount of protein in the meat of *Stichopus japonicus* may be smaller than in other fish meat. However, the ratio of the amounts of various fractions of nitrogen to the amount of the total nitrogen in the meat of *Stichopus japonicus* is almost the same as in other fish meat, excepting a few fractions.

The amounts of basic nitrogen and mono-amino nitrogen of the *Stichopus japonicus* meat are small, but the amount of arginine nitrogen is comparatively large.

In addition, the meat of *Stichopus japonicus* contain lysine and other amino acids which are nutritive-important amino acids. They were also detected by the paper chromatography as later stated.

That is to say, the protein of the meat of *Stichopus japonicus* is lacking in some amino acids which are contained in the fish meat protein.

The meat of *Stichopus japonicus* consists mainly of connective tissue which is collagen and a small amount of other proteins as previously stated. Collagen does not contain cystine, cysteine and tryptophan.

In the present experiment, the meat part was found not to contain tryptophan by paper chromatography, as it was the HCl hydrolyzate.

It is remarkable that the meat of *Stichopus japonicus* contains a large amount of humine nitrogen differing from other fish meat. The significance of this fact can not be interpreted on the basis of data obtained by the present experiments.

The kinds of amino acids in the meat and skin parts which have been detected by paper chromatography are seen as in Fig. 2 and Fig. 3. Those amino acids are aspartic acid, glutamic acid, glycine, alanine, valine, phenylalanine, proline, hydroxyproline, serine, arginine, lysine, ornithine, cystine, threonine, 3-5 diiodotyrosine, tyrosine, leucine and methionine. To state it again, almost all the amino acids are contained in the meat and skin parts of *Stichopus japonicus*.

2. The kind of amino acids in free state in the meat of *Stichopus japonicus*

The free amino acids in the meat of *Stichopus japonicus* were detected by paper chromatography.

(1) Sample

To 10 g of crushed raw meat of *Stichopus japonicus* was added the same volume of dist. water and then mixed homogeneously and left for 30 minutes at room temperature. Protein in the mixture was removed by 5 cc of 20% trichloroacetic acid solution.

Free amino acid solution obtained thus was concentrated to 0.5 cc on a water bath of 45°-50°C.

(2) Running and coloring in the paper chromatography

The kind of amino acids was detected by the same two-dimensional paper

chromatography as described above.

(3) Results and discussion

Results obtained are shown in Fig. 4.

As seen in Fig. 4, the kinds of amino acids in free state which were detected in the meat of *Stichopus japonicus* are glycine, alanine, glutamic acid, arginine, and threonine-like substance.

Arginine which was found in large quantity in the hydrolyzate by Van Slyke's method is present in free state in living bodies of *Stichopus japonicus*.

Threonine has been detected in the hydrolyzate of the meat by Hatagoshi⁹⁾.

Threonine-like substance was also detected by paper chromatography in free state. But the map of the threonine-like substance appeared near the maps of glycine and alanine and the map of alanine was spread, therefore the map of threonine was not accurately determined.

Histidine, proline, valine, leucine and lysine which have been detected by paper chromatography in free state by other authors were not ascertained in this experiment.

Summary

The ratio of the nitrogen distribution of meat and skin parts of *Stichopus japonicus* was almost equal to that of fish meat except that the amount of humine nitrogen of the former is larger than that of the latter. The amounts of various nitrogen compounds of the former are inferior quantitatively to those of the latter.

The kinds of amino acids of hydrolyzed protein of *Stichopus japonicus* which were detected by partition paper chromatography were as follows: aspartic acid, glutamic acid, glycine, alanine, valine, phenylalanine, proline, hydroxyproline, serine, arginine, lysine, ornithine, cystine, threonine, 3-5 diiodotyrosine, tyrosine, leucine, methionine. However tryptophan is lacking in the *Stichopus japonicus* meat and skin parts.

The kinds of amino acid in free state were detected as follows: glycine, alanine, glutamic acid, arginine and threonine-like substance.

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