CHEMICAL STUDIES ON THE MEAT OF "SUKETODARA" (*THERAGRA CHALCOGRAMMA*)- I

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INTRODUCTION

Theragra chalcogramma (PALLAS) is called "Suketodara" or "Sukesodara" in Japan. In Korea it is called "Mentai". It is called "Alaska pollack" in English.

This is a useful fish belonging to the Gadidae as do cod also. Typical genera of the Gadidae are *Gadus* and *Theragra*.

Theragra chalcogramma is distinguished from the ordinary cod (*Gadus macrocephalus*) by having more slender body, the lower jaw is longer than the upper, the barbel on the chin is much smaller than on the cod and the caudal fin is more or less emaciated posteriorly. The body height is about 1/6 of the length; the snout is sharply pointed; in the large mouth the maxilaries extend to below the middle of the eye; the large eye is placed on the head of about 4 inches.

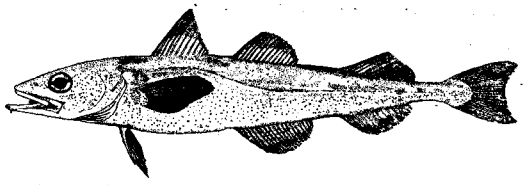


Fig. 1. "Suketodara" (*Theragra chalcogramma*)

The anus opens below between the first and second dorsal fins. The ventral fins are filamentous distally and their tips reach to the anus. The body color is brownish in the back part, white in belly part; the two interrupted horizontal dark bars on the side are irregularly bordered. The total average length reaches to about 600 mm.

The "Suketodara" occurs in the waters of Japan Sea from Yamaguchi Prefecture northward to the Bering Sea and off Korea; it is scarce on the Pacific coast of Japan. Differing from cod (*Gadus macrocephalus*) the "Suketodara" lives in the middle layers of waters at about 200 m depth; in the Bering Sea they swim near the surface of the water; very frequently they are devoured by the fur-seals.

The spawning season of "Suketodara" extends from December to February in the waters off Korea, and February to April in waters near Hokkaido and elsewhere. The separate, pelagic eggs are spherical and each measures 1.35 to 1.45 mm in diameter; the egg membrane is smooth, the yolk has no cleavage and the oil-globule is absent. The larvae just before hatching are colored by only black chromatophores which are uniformly scattered over the body. The fishing season of this fish lasts from December to February, and the fishing gears include set-line, gill-net and small-trawl. The fish is utilized as cod is, and the salted eggs ("Momijiko") of this fish are especially appreciated; in Korea this fish, being one of the important aquatic products, is dried after being frozen.

The catching of this fish totaled 280 thousand tons in 1958, with 90% of them being caught in Hokkaido.

The skeletal meat is utilized as fresh raw fish (for human consumption), eggs are salted and the liver is utilized as raw material for liver oil in Hokkaido.

With increase in the demand for fish pastes (e.g. "Kamaboko" or fish sausage and fish ham), requiring a great amount of raw material, the use of "Suketodara" is being developed with keen interest as a raw material for such products.

A small amount of the catch has been utilized as raw material of fish pastes, but as the freshness of this fish meat falls easily, the elasticity of the paste products ("Ashi" in Japanese) is lost remarkably, therefore the use of this fish is not looked upon with favor. The loss of elasticity has been explained recently from the view point of protein chemistry. But there are many unsolved problems remaining today in respect to the chemistry of the use of this fish.

As described in this paper the present authors have tried to clarify some little known properties of this fish meat in order to utilize it.

They have tried to study the fish from the points of view of general chemical analysis, histology and protein chemistry.

I. GENERAL CHEMICAL ANALYSIS OF "SUKETODARA" MEAT

In order to know the chemical constituents of the "Suketodara" meat, the general chemical composition and distribution of nitrogens and kinds of amino acids were determined.

1. Seasonal variation in general chemical composition

In general, the chemical composition varies remarkably by seasons of spawning. The "Suketodara" disappears from sight for some seasons in Hokkaido. So the authors have estimated monthly the general chemical composition of "Suketodara" meat from October to March of the succeeding.

(1) Experimental method

Fresh "Suketodara" caught in waters near Hakodate, Hokkaido, was used as the sample. The fish was filleted, and the meat was removed from the skin. The meat was homogenized and used as sample. Items estimated were water-content, protein, fat and ash.

(2) Results

The results obtained are shown in Table 1.

As seen in Table 1, the amount of water-content was maximum in December with decrease afterward, then showed the minimum in March of the next year in the present experimental period. The difference in the amount of water-content between the maximum

Table 1. Seasonal variation of proximate components of "Suketodara" (*Theragra chalcogramma*) meat

Items / Date	Water content (%)	Crude fat (%)	Crude ash (%)	Pure-protein (N×6.25) (%)	Non-protein -N (%)	Total-N (%)	Crude protein (%)
25/Oct. (1953)	81.18	0.68 (3.61)	1.09 (5.79)	14.13 (75.1)	0.09 (0.48)	2.35 (12.48)	14.69 (78.0)
5/Nov.	81.15	0.73 (3.87)	1.21 (6.42)	14.75 (78.3)	0.18 (0.96)	2.54 (13.46)	15.88 (84.3)
9/Dec.	82.11	0.70 (3.91)	1.11 (6.21)	13.38 (74.8)	0.35 (1.96)	2.49 (13.91)	15.56 (87.0)
18/Jan. (1954)	81.06	0.53 (2.80)	1.03 (5.44)	13.69 (72.2)	0.33 (1.74)	2.52 (13.30)	15.73 (83.1)
10/Feb.	80.71	0.84 (4.36)	1.18 (6.12)	15.06 (78.0)	0.24 (1.24)	2.65 (13.73)	16.55 (85.9)
16/Mar.	80.31	0.93 (4.72)	1.23 (6.25)	16.75 (85.1)	0.20 (1.01)	2.88 (14.61)	18.00 (91.5)

Note : Number in parenthesis shows the percentage of each component per dried matter

and the minimum was about 2 %.

The amount of crude fat in raw meat showed minimum in January, but increased remarkably from February to March. The increased amount was 0.5 ~ 0.97 %.

The amount of ash was larger in November and March than in other months.

The total amount of nitrogen was considerably small in December. The amount of protein-nitrogen increased or decreased proportionally with the total amount of nitrogen. The maximum amount of protein-nitrogen was shown in March and the minimum in December.

The spawning season of the "Suketodara" is considered to be from January to April. The samples which were used by the authors in February and March were completely matured before the spawning. Therefore, the amounts of protein, fat and ash in those seasons were large while the water-content was less than in samples in other months. From the results showing the percentage of each chemical component per dried matter, the same conclusion as that stated above was also reached.

2. Differences of chemical compositions according to various factors

In general, the chemical components of fish meat are different owing to various factors, e.g. age, sex, size, etc.

Here, the authors have estimated the amounts of the general components in the "Suketodara" meat which vary with those factors.

(1) Experimental method

The items estimated were the same as those described in the previous experiment.

(2) Results

Results obtained are shown in Tables 2 ~ 4.

Table 2. Difference of proximate components of "Suketodara" (*Theragra chalcogramma*) meat by age

Items / Kinds	Water content (%)	Crude fat (%)	Crude ash (%)	Total-N (%)	Pure protein-N (%)	Crude protein (%)	Pure protein (%)
Young	83.01	0.49 (2.88)	1.08 (6.36)	2.45 (14.42)	2.05 (12.08)	15.31 (90.2)	12.81 (75.4)
Adult	80.31	0.93 (4.72)	1.23 (6.24)	2.80 (14.22)	2.50 (12.70)	17.50 (88.9)	15.61 (79.3)

Note : Number in parenthesis shows the percentage of each component per dried matter

Table 3. Difference of proximate components of "Suketodara" (*Theragra chalcogramma*) meat by sex

Items / Sex	Water content (%)	Crude fat (%)	Crude ash (%)	Total-N (%)	Pure protein-N (%)	Crude protein (%)	Pure protein (%)
Male	81.71	0.64 (3.50)	1.03 (5.64)	2.49 (13.61)	2.14 (11.70)	15.56 (85.2)	13.36 (73.0)
Female	80.31	0.93 (4.72)	1.23 (6.24)	2.80 (14.22)	2.50 (12.70)	17.50 (88.9)	15.61 (79.3)

Note : Number in parenthesis shows the percentage of each component per dried matter

Table 4. Difference of proximate components of "Suketodara" (*Theragra chalcogramma*) meat by size

Items / Size	Water content (%)	Crude fat (%)	Crude ash (%)	Total-N (%)	Pure protein-N (%)	Crude protein (%)	Pure protein (%)
L=45 *cm* W=910 *g*	81.15	0.73 (3.87)	1.21 (6.43)	2.54 (13.48)	2.36 (12.50)	15.88 (84.2)	14.75 (78.3)
L=62 *cm* W=1,850 *g*	80.71	0.84 (4.35)	1.18 (6.12)	2.65 (13.72)	2.41 (12.50)	16.56 (85.8)	15.06 (78.0)

Note : Number in parenthesis shows the percentage of each component per dried matter

As seen in Table 2, the amount of water-content in the raw sample of young fish meat was larger than in that of adult fish meat. On the contrary, all the amounts of other components in young fish meat were smaller than those of adult fish meat. Especially, it is noticeable that the amounts of fat and pure protein in adult fish meat were larger than those of young fish meat, when those amounts were compared with each other in the percentage to the dried matter of samples. From those data, it is clear that there are some differences in the amounts of chemical components according to age.

As seen in Table 3, there were differences between amounts of water-content, fat and protein. The amount of water-content in the raw female fish meat is less than that of male fish of almost the same age. The amounts of fat and protein in the female were larger than those in the male. The relations noted above were the same when comparison was made in the percentage to the dried matter of samples. These findings may be due to the accumulation of those components by female fish before the spawning.

As seen in Table 4, there are some differences of chemical components between the large sized fish (62 cm in length, 1,850 g weight) and the small sized fish (45 cm in length, 910 g weight). Both fishes were female. The amount of water-content in the raw sample of the larger fish was much smaller than that of the smaller fish. On the contrary, the amount of fat in the larger fish meat was much greater than that in the meat of the smaller fish.

Generally, the smaller fish is considered to be young, so the difference by size is also considered to be difference by age. At any rate, there are some difference between the larger fish or adult fish and the smaller or young fish.

3. Nitrogen distribution and amino acids composition of "Suketodara" meat and water-extractives

(1) Nitrogen distribution of raw meat of "Suketodara"

1) Experimental method

Fresh "Suketodara" was filleted and the raw meat removed from the skin, was homogenized; nitrogen distribution was determined by Van Slyke's method.

2) Results

The results of the determination of nitrogen distribution of the raw meat are shown in Table 5.

As seen in Table 5, about 29 % of the total nitrogen is basic amino-total nitrogen, about 53 % is mono amino-total nitrogen, and about 12 % is amide-N.

In basic nitrogen, of which about 10 % was arginine-N and lysine-N.

(2) Nitrogen distribution of water-extractives of "Suketodara" meat

1) Experimental method

Fresh "Suketodara" was filleted and the raw meat removed from the skin, was homogenized. Three hundred cc of distilled water was added to 100 g of the homogenized meat and the mixture was heated at 45° ~ 50°C with agitation for one hour. Trichloracetic acid was added to the extracted solution to precipitate protein. The precipitated protein was removed by filtration, and the separated solution was evaporated in vacuum. Thus the water-extractive was obtained. This extractive was used for determination of nitrogen distribution, after hydrolysis by 20 % HCl solution.

Table 5. Nitrogen distribution of the meat of "Suketodara" (*Theragra chalcogramma*)

Fishes / Fraction	"Suketodara" (Alaska pollack) % in total-N	"Hokke" (Atka mackerel) % in total-N	"Surume-ika" (squid) % in total-N
Total-N	100.00	100.00	100.00
20% HCl insoluble-N	1.41	1.15	—
20% HCl soluble-N	98.59	98.85	—
Amide-N	11.82	5.36	4.09
Humine-N	3.90	5.14	3.33
Basic total-N	28.71	26.13	39.70
Arginine-N	10.52	8.65	20.00
Histidine-N	6.73	8.71	8.74
Lysine-N	10.40	7.85	10.07
Cystine-N	1.06	0.91	0.92
Basic amino-N	16.34	13.83	18.90
Mono amino-acid total-N	52.56	56.56	52.80
Mono amino-acid amino-N	51.31	46.22	43.00

2) Results

Results of investigation of the nitrogen distribution are shown in Table 6.

Table 6. Nitrogen distribution of the water-extractive from "Suketodara" (*Theragra chalcogramma*) meat

Fishes / Fraction	"Suketodara" (Alaska pollack) % in total-N	"Hokke" (Atka mackerel) % in total-N	"Surume-ika" (squid) % in total-N
Total-N	100.00	100.00	100.00
20% HCl insoluble-N	0.88	0.79	—
20% HCl soluble-N	99.12	99.21	—
Amide-N	8.69	0.13	2.85
Humine-N	24.90	26.21	15.71
Basic total-N	31.09	32.49	40.04
Arginine-N	12.19	24.63	—
Histidine-N	—	—	—
Lysine-N	—	—	—
Cystine-N	0.85	1.57	2.44
Basic amino-N	5.19	7.13	—
Mono amino-acid total-N	32.12	38.41	41.19
Mono amino-acid amino-N	19.08	35.12	9.44

As seen in Table 6, about 31 % of the total amount of extractive nitrogen (non-

protein-N in the extractive obtained from the fish meat by use of hot water) was basic amino-total nitrogen, and almost the same amount (about 32 %) was mono amino-total nitrogen. In basic amino-total nitrogen, arginine-N showed about 12 %, but other form of nitrogen could not be estimated, because the total amount of nitrogen in form of bases was too small.

About 25 % of the total nitrogen of the extractive was humine nitrogen.

(3) Amino acids of the "Suketodara" meat and the water-extractive

1) Experimental method

The HCl-hydrolyzates of the raw "Suketodara" meat and the water-extractive were used for two dimensional paper chromatography. The developing reagents were phenol with 10 % water, and a mixed solution of lutidine, anilin and water (65 : 7 : 28); the revealing reagent was ninhydrin-butanol solution. Revealed amino acids were identified.

2) Results

In the raw "Suketodara" meat, aspartic acid, glutamic acid, glycine, alanine, arginine, lysine, ornithine, cystine, threonine, tyrosine, leucine, histidine were identified. The kinds of amino acids identified in the water-extractive of the "Suketodara" meat were almost the same as those of the raw "Suketodara" meat.

Consequently, the nitrogen distribution and the amino acids composition of the "Suketodara" meat can be stated to be almost the same as those of other fish meats[1].

II. DECOMPOSITION OF THE "SUKETODARA" MEAT

It has been known that when the freshness of "Suketodara" meat falls, the elasticity of the fish paste products ("Ashi" of "Kamaboko") decreases[2].

The reason for this phenomenon begins recently to understand from the increased knowledge in the field of protein chemistry. But, here, it is important to know the velocity of decomposition of the "Suketodara" meat for solving the problems of employment of this fish for various products.

The authors have tried to calculate the decomposition velocity constant, temperature coefficient and temperature constant from the data obtained when the "Suketodara" meat was left and allowed to decompose.

1. Changes of the amounts of volatile basic nitrogen when the "Suketodara" meat is left

(1) Experimental method

From fresh "Suketodara" body, head and viscera were removed. Each two carcasses were left as they are at 35° ± 1°C, 25° ± 1°C and 10° ± 1°C, respectively. And the fish meat was allowed to decompose aerobically. At certain definite intervals of leaving time, a part of each fish body was taken off; on such portions the amount of

volatile basic nitrogen (V.B.-N) was estimated by Weber and Wilson's method. From the amounts of V.B.-N estimated, the relations among the amount of V.B.-N produced, leaving time and leaving temperature were determined.

(2) Results

At the respective leaving temperatures, the variations in amount of V.B.-N in the fish meat were as shown in Fig. 2.

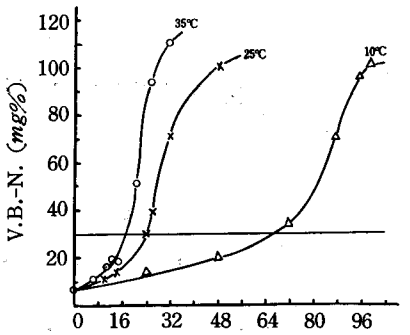


Fig. 2. Changes in the amount of volatile basic nitrogen during the leaving of carcasses of the "Suketodara" (*Theragra chalcogramma*) at various temperatures

When carcasses were left at higher temperature, the amount of V.B.-N increased rapidly. On the contrary, at comparatively lower temperature, the amount of V.B.-N increased slowly.

2. Calculations of decomposition velocity coefficient (K), temperature coefficient (Q_{10}) and temperature constant (B)

From data obtained as shown in Fig. 2, the relation between "y" and "$\log\frac{y}{A-y}$ (here, "y" is the producing amount of V.B.-N at each time "t" of the estimation, "A" is the maximum amount of V.B.-N produced) was calculated and graphed in Fig. 3.

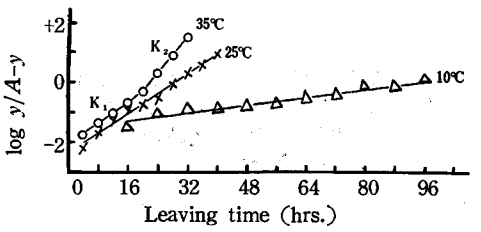


Fig. 3. Relation between the value of $\log\frac{y}{A-y}$ and time "t"

As seen in Fig. 3, the relation between "$\log\frac{y}{A-y}$" and "t" is linear.

Therefore, the following equation is obtained after Kimata[3].

$$\log\frac{y}{A-y}=Kt+C \quad \cdots\cdots(1)$$

Here, C is a constant.

In this equation of the monomolecular autocatalytic reaction, the value of "K" is decomposition velocity coefficient (V.B.-N producing velocity coefficient).

From equation (1), the values of "K" at various leaving temperatures were calculated as shown in Table 7.

Table 7. Value of "K" of "Suketodara" (*Theragra chalcogramma*) meat at various leaving temperatures

Temp. (°C)	$K \times 10^3$
35°	$K_1 = 89$, $K_2 = 101$
25°	79
10°	28

As seen in Table 7, the higher the leaving temperature, the larger the value of "K". At 35°C leaving temperature, that the value of K_1 is less than K_2, may be due to the fact that the decomposition of the meat is slow at initial stage, but becomes rapid

from the middle stage.

After Ōya[4], from equation (2) of Arrhenius' fundamental equation, temperature constant (B) and temperature coefficient (Q_{10}) were derived as follows:

$$\log K + \frac{B}{R} \cdot \log e \frac{1}{T} = \text{const.} \quad \cdots\cdots (2)$$

$$B = \frac{a}{\log e} \cdot R = \frac{a}{0.4343} \cdot 1.985 \quad \cdots\cdots (3)$$

$$\text{here, } a = \frac{B}{R} \cdot \log e$$

$$\log Q_{10} = \frac{B}{R} \cdot \log e \left(\frac{1}{T} - \frac{1}{T+10} \right) = a \cdot \frac{10}{T(T+10)} \quad \cdots\cdots (4)$$

Here, "K" is the decomposition velocity coefficient at T°K; R is gas constant and it is 1.985 cal.

Here, the values of $\log K \times 10^3$ at respective temperatures were plotted on ordinate and the reciprocal values of the absolute temperature of the estimation, $\frac{1}{T}$, were plotted on abscissa; Fig. 4 was obtaind.

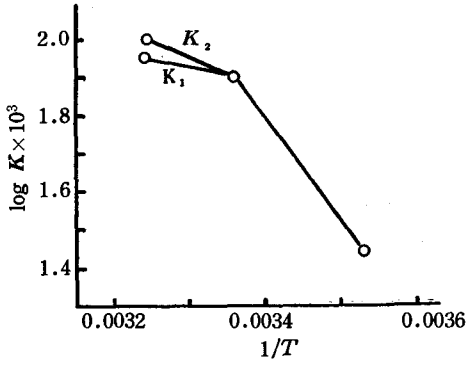


Fig. 4. Relation between the value of "log K" and "$\frac{1}{T}$"

As seen in Fig. 4, the relation is linear in the range between two different temperatures. The degree of the inclination of the straight lines is shown as "a" value in equations (3) and (4). Here the values of "B" and "Q_{10}" are obtained at each range of temperatures as shown in Table 8.

Table 8. Values of temperature coefficient, "Q_{10}", and temperature constant, "B", of "Suketodara" (*Theragra chalcogramma*) meat

Temp. range	Q_{10}	B
10° ~ 25°C	2.2	11,900
25° ~ 35°C K_1	1.3	4,100
25° ~ 35°C K_2	1.0	1,800

The value of "K" of "Suketodara" meat at various temperatures is compared with those of other marine creatures obtained by Tanikawa[5] as shown in Table 9.

As seen in Table 9, the "Suketodara" meat is easily decomposable even at low temperature.

The values of "Q_{10}" and "B" of "Suketodara" meat at 25°C in comparison with those of other marine creatures are shown in Table 10.

As seen in Table 10, at comparatively higher storing temperature, "Suketodara" meat is more easily decomposable than other fish meat.

Thus the "Suketodara" meat is decomposable, so care should be taken in the handling

Table 9. Values of "*K*" in "Suketodara" (*Theragra chalcogramma*) meat compared with those of other marine creatures ($K \times 10^3$)

Fishes \ Temp. range	10°C	14° ~ 16°C	24° ~ 28°C	33° ~ 37°C
Sea cucumber[5a]	—	17	28	29
crab[5b]	—	—	74	91
Atka mackerel[5c]	—	22	36	60
mackerel[5d]	—	30	99	114
saury[5e]	—	27	30	48
squid[5f]	—	—	103	151
"Suketodara"	28	—	79	89 101

Table 10. Vaules of "Q_{10}" and "*B*" of "Suketodara" (*Theragra chalcogramma*) meat and those of other fish meat

Fishes	Temp. range	Q_{10}	*B*
Atka mackerel[5c]	above 25°C	1.5	8×10^3
	below 25°C	3.5	21×10^3
mackerel[5d]	above 25°C	1.2	3×10^3
	below 25°C	3.6	22×10^3
"Suketodara"	above 25°C	1.3	4×10^3
	below 25°C	2.2	12×10^3

of this fish. Before the processing, it must be stored at low temperature.

3. Calculation of initial decomposition velocity coefficient

It is rather important to know the initial decomposing value of "*K*".

Next, the authors have determined the relation between the storage time and amount of V.B.-N produced until that amount reaches to 30 mg% (incipient putrefaction). (Ref. Fig. 2).

Up to the value of 30 mg%, the curve showing the relation between the storing time and amount of V.B.-N produced is almost hyperbolic. For example, it requires 17 hours at 35°C, 25 hours at 25°C and 68 hours at 10°C for the amout of V.B.-N to reach 30 mg%. But when the amount of V.B.-N in "Suketodara" meat reaches 30 mg%, the meat smells putrefactive, so the limit of the freshness should be set at a rather less amount of the V.B.-N (the authors consider it is 20 mg%).

As follows, the authors have devised an equation (5) applicable for estimating the beginning of incipient putrefaction.

$$V = pt^2 + b \quad \cdots\cdots (5)$$

Here, "*V*" is the amount of V.B.-N in the fish meat after "*t*" hours, "*b*" is a constant (in this case it is the amount of V.B.-N, when the storing time is 0); "*p*" is the coefficient of the velocity of decomposition in the initial stage.

The values of "*p*" obtained from equation (5) are compared with those from other fish meat as shown in Table 11.

Table 11. Values of "*p*" of "Suketodara" (*Theragra chalcogramma*) meat compared with corresponding values of other fish meat

Temp. \ Fishes	salmon	squid	"Suketodara"
35° C	0.367	0.082	0.075
25° C	0.023	0.031	0.034
10° C	0.013 (15° C)	0.003	0.006

As seen in Table 11, as the value of "*p*" is larger at 25° or 10°C than that of other fish meat, the "Suketodara" meat is seen to be easily decomposable in the initial stage.

4. Change of histological aspects during the falling of freshness of the "Suketodara" meat

When the fresheness of the "Suketodara" meat falls, the chemical properties of the meat change. This is accompanied by histological change. Here the authors have attempted to observe the histological aspect during the falling of the freshness of the "Suketodara" meat.

(1) Experimental method

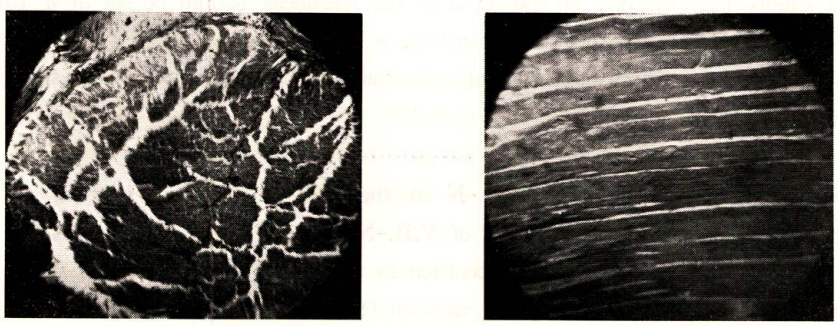
i) For the making of histological preparates as samples, fresh "Suketodara" meat and unfresh fish meat, of which V.B.-N value was 50 mg% were used. From both sorts of fish bodies, a block of a part of back muscle meat ($0.5\times0.5\times1.0cm^3$) was taken out. Those blocks were fixed with Bouin's solution. Then they were dehydrated with alcohol as usual, and finally imbedded in paraffin. Thus treated meat was sliced in 10μ (or 15μ) thicknesses by a microtome. Each slice was dyed by Delafields' haematoxyline staining method and imbedded in balsam to make a parmanent preparate.

ii) Microscopic observation

Microscopic observations were photographed as shown in Fig. 5-1, Fig. 5-2 and Fig. 5-3.

Fig. 5-1 and Fig. 5-2 show photographs of fresh "Suketodara" meat; Fig 5-3 shows the model chart of the same fresh meat.

Figs. 6-1, 6-2 and 6-3 show the results on unfresh meat.



Figs. 5-1,2. Section of fresh "Suketodara" (*Theragra chalcogramma*) meat muscle

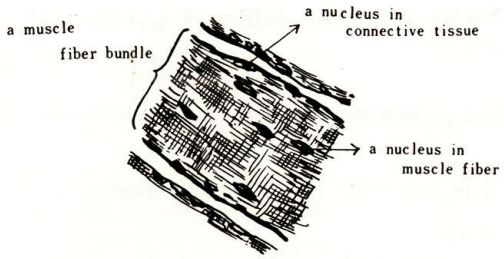
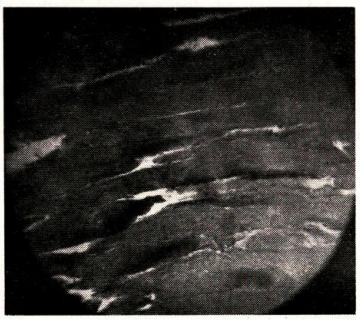


Fig. 5-3. Model chart of fresh "Suketodara" (*Theragra chalcogramma*) meat muscle

As to fresh "Suketodara" meat, observations were made from two directions parallel or perpendicular to muscle fiber bundles. A muscle fiber bundle consists of several hundred muscle fibers which were bound twisting. This muscle fiber is one unit of the cell, and is composed of myofibril. A muscle fiber is surrounded by connective tissue having



Figs. 6-1,2. Section of unfresh "Suketodara" (*Theragra chalcogramma*) meat muscle

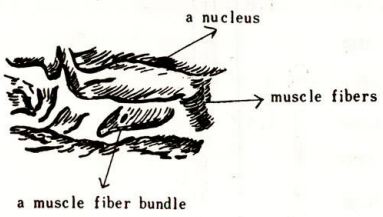


Fig. 6-3. Model chart of unfresh"Suketodara" (*Theragra chalcogramma*) meat muscle

nuclei. Many muscle fibers are enveloped by a muscle sheath (sarcolemma) making a muscle fiber bundle. Meat muscle is composed of transversely striated muscle, so nuclei are seen in meat muscle tissues. There are spaces between the muscle fiber bundles which are filled with water, body fluid, blood vessels and lymphatic vessels.

As to unfresh "Suketodara" meat, different histological observations from those of fresh "Suketodara" meat were obtained. The meat became opaque. Striae became not clear. Cell nuclei in the connective tissues were remarkably deformed. This is considered due to the phenomena caused by karyorrhexis, karyolysis and pycnosis. At every part the muscle sheaths dissolved, and they are put one upon another. This may be due to the deformation of connective tissues which are binding muscle fibers. For these phenomena Kawabata[6] has given the name "waxy degeneration" of muscle fiber. The degrees of the deformation of tissues differs with the leaving time and temperature. Concomitant with the falling of the freshness of the meat and the deforming of the muscle bundles, fluid matter escapes from the inter-spaces, and the proteins of the meat may denaturalize.

5. Change in the amount of bound water during the falling of freshness of "Suketodara" meat

The degree of the denaturation of the meat protein may be judged by estimating the amount of bound water in the meat.

The authors have estimated the amount of bound water by the vapour tension method.

(1) Experimental method

Fresh "Suketodara" bodies were left in ice box (10° ± 2°C) and the samples of meat having various degrees of freshness were obtained as shown in Table 12.

Table 12. The samples used in the experiment of hydration of decomposed "Suketodara" (*Theragra chalcogramma*) meat

Items \ Leaving times	0 day	3 days	6 days
Water content(%)	81.18	81.80	81.52
pH	6.2	6.6	6.8
Volatil basic-N (mg%)	5.34	50.06	83.34
Total-N (%)	2.35	2.51	2.68
Pure protein-N (%)	2.26	2.25	1.72
Water soluble-N (% in total-N)	37.2	34.2	27.9
0.5 M NaCl-soluble-N (% in total-N)	46.0	36.4	34.4

Here, "g" is grams of water per gram of the dried matter, and "p" is the vapour pressure of water containd in sample at the estimating temperature "t°C", and "p_0" is the same of pure water at the same temperature. As seen in Fig. 7, with the falling of freshness the curve moved to the left. This shows the increase of the relative vapour pressure (p/p_0) in the entire range of water-content "g". That is to say, the curve shows the increase of water-activity "a" of the water contained in the sample. The amount of bound water is different more or less according to the properties of the kinds of proteins, the presence of salts, estimating temperatures, etc. But it is generally considered that the water of which the water-activity "a" is 0.6 ~ 0.8, is a colloid-chemical bound water[7]. In Fig. 7, the differences between curves (A) and (B), or (B) and (C) show the decrease in the amount of bound water with the falling of the

The samples above described were employed for the estimation of the amount of bound water by means of the vapour tension method[7]; the water-content "g" —relative vapour pressure (p/p_0) curve was obtained.

(2) Results

The experimental results are shown in Fig. 7.

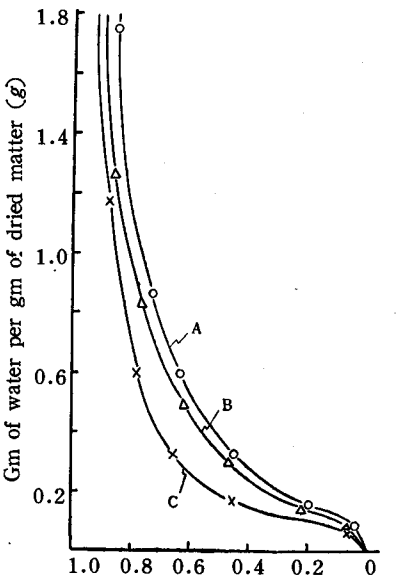


Fig. 7. Water-content (g) — relative vapour pressure (p/p_0) curves of fresh and unfresh "Suketodara" (*Theragra chalcogramma*) meat

freshness of the "Suketodara" meat. Here the value of the difference, $\Delta\bar{F}$, between the differential molal free energy of pure water and that of water in the sample at the same temperature is calculated by the following equation, according to Lewis and Randall[8].

$$\Delta\bar{F} = F^\circ - \bar{F} = -RT\ln p/p_0 \quad \cdots\cdots\cdots\cdots \quad (6)$$

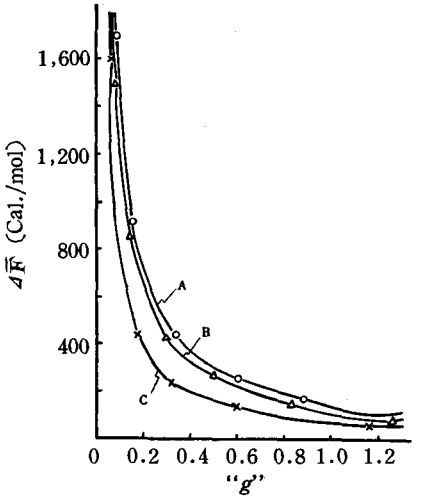


Fig. 8. Relation between the values of "*g*" and $\Delta\bar{F}$ of fresh and unfresh "Suketodara" (*Theragra chalcogramma*) meat

Here, F° is the differential molal free energy of pure water at T°K, $\bar{F}$ is that of water in the sample at the same temperature and R is gas constant.

Fig. 8 shows the relation between the values of "*g*" and $\Delta\bar{F}$.

As seen in Fig. 8, the value of $\Delta\bar{F}$ at a definite amount of water-content increased with the falling of the freshness of sample meat used. This shows the decrease of affinity of water to the meat protein with falling of the freshness, in which case the denaturation of the meat protein occurs.

III. CHEMICAL PROPERTIES OF "SUKETODARA" MEAT PROTEIN

1. Dissolution of "Suketodara" meat

It is important to know the solubility of the "Suketodara" meat by water, acid or alkaline solutions, because when this fish meat is used as the raw material of various products, especially for fish paste ("Kamaboko" or "Chikuwa"), the solubility influences the quality of the fish paste.

Next, the authors undertook to determine the solubility by various solutions.

(1) Experimental method

After fresh "Suketodara" had been filleted, the meat was removed from the skin and homogenized.

To 10 g of the homogenized meat, 200 cc of dist. water was added and the solution was stirred for 30 minutes, then centrifuged (3,000 r.p.m. for 30 minutes). An upper transparent liquid was obtaind. Five cc of the liquid was used for the estimation of the amount of dissolved nitrogen by Kjeldahl's method.

The amount of nitrogen dissolved out by water was 30.6 % of the total amount of nitrogen in sample meat.

Next, similarly, the homogenized "Suketodara" meat was used for the estimation of

the solubilities of the meat by various salt, alkaline or acid solutions.

With each 10 g samples of the homogenized meat, 50 cc each of NaCl, KCl, NaOH, H_2SO_4, HCl and CH_3COOH solutions having various concentration was put into tall glass bottles, respectively. The bottles were shaken frequently and were left for 24 hours.

After the leaving, the upper transparent liquid was obtained by centrifugation. Five *cc* of the liquid was used for the estimation of amount of dissolved nitrogen.

The solubities (S) were calculated by the following equation (7)

$$S = \frac{a \times 100}{\text{total amount of nitrogen in 1 g of the sample}} (\%) \quad \cdots\cdots\cdots\cdots (7)$$

Here, "*a*" is the amount of nitrogen in 5 cc of the upper transparent liquid. The amount of nitrogen in 1 g of the sample was 24.45 mg.

(2) Results

Results obtained are shown in Fig. 9.

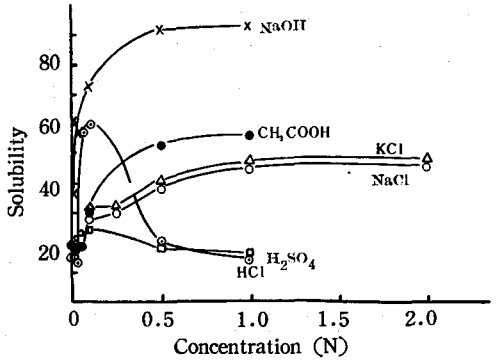


Fig. 9. Solubility of "Suketodara" (*Theragra chalcogramma*) meat by various salt, alkaline or acid solutions

The amount of nitrogen in the "Suketodara" dissolved by water was 30.6 % as described above.

As seen in Fig. 9, the solubility of the meat by NaCl solution differs according to the concentration, but it ranged 30 ~ 45 %. The solubility by KCl solution has almost the same tendency with NaCl, but somewhat larger.

On the other hand, the solubility by NaOH solution is large, 90 %. Differently, the solubilities by acids vary with the kind. For example, there is remarkable difference between hydrochloric acid and sulfuric acid. Okada *et al.*[9] or Fujii[1] have explained this difference as follows: hydrochloric acid acts on proteins as a mono-basic acid, while sulfuric acid acts as a di-basic acid. The difference in the case of the "Suketodara" meat also can be explained in the same manner.

The solubility of the "Suketodara" meat in acetic acid increased in accordance with the increase of the concentration.

In general, the solubility by alkaline solution did not decrease with the increase of the concentration below 1 N, differing from acid solution. The reason why such a difference exists may be explaind as follows: in acid solution in high concentration the protein of the fish meat coagulates, contrarily in alkaline solution the meat protein is hydrolyzed. From the results obtained, the "Suketodara" meat can be said to be dissolved by water or NaCl solution, when the meat is soaking in water or NaCl solution during the processing of the fish paste ("Kamaboko" or "Chikuwa").

2. Isoelectric points of protein of the "Suketodara" meat

The "Suketodara" meat consists of a few kinds of protein, therefore the solubility of the meat by various solvents differs.

Next, the authors have tried to determine the isoelectric points of proteins which are prepared from extracting meat by means of water or NaCl solution.

(1) Experimental method

Fresh "Suketodara" was filleted and the meat was removed from the skin, and then homogenized. The homogenized meat was extracted by water or 0.6M NaCl solution. Thus water soluble protein or NaCl soluble protein solutions were obtained. Each 10 cc of those water soluble or NaCl soluble protein solutions was added to each 40 cc of the buffer solutions having various pH values which were prepared from N/25 NaOH and N/25 HCl solutions. After the solutions had been left for 24 hours, the resultant precipitate was filtered. The pH value and viscosity of the filtrate were determined. The relative viscosity (η/η_0) was measured by Ostwald's viscosimeter. The pH value of the solution which indicates the minimum relative viscosity is regarded as the isoelectric point of the protein. On the other hand, the total amount of nitrogen in the precipitated substance noted above was estimated, and the pH value of which the maximum amount of nitrogen, was determined and regarded as the isoelectric point of the protein. Comparing the isoelectric points which were determined by the viscosity method or by the method of the estimation of the total amount of nitrogen in the precipitated substance, the two isoelectric points were found to be in agreement as shown in Figs. 10 and 11.

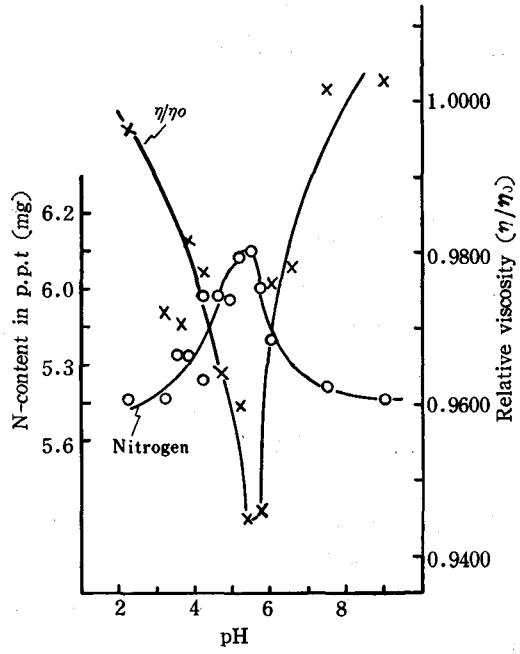


Fig. 10. Isoelectric point of water soluble protein of "Suketodara" (*Theragra chalcogramma*) meat

(2) Results

As seen in Figs. 10 and 11, the isoelectric point of water soluble protein of the "Suketodara" meat was pH 5.2 ~ 5.4, and that of NaCl solution soluble protein was pH 5.0 ~ 5.2. The reason why the isoelectric point has some range is due to the fact that the protein is not a single component, but consists of several components.

The isoelectric point is different according to the kinds of fish or constituent proteins. Tadokoro[10] has examined cod, sardine or flat fish meats, and

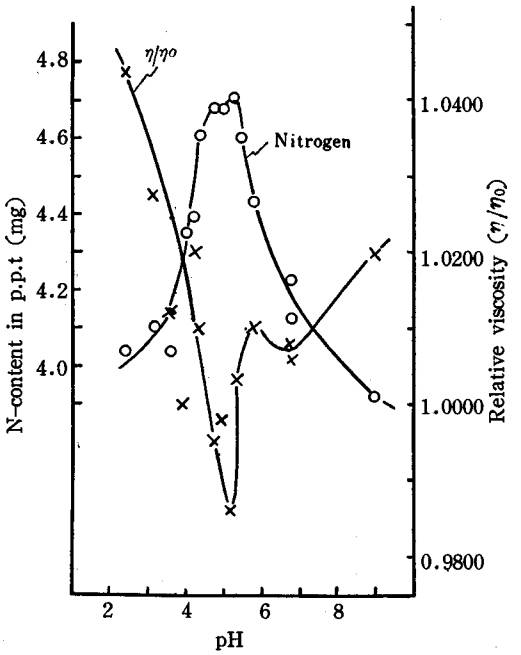


Fig. 11. Isoelectric point of 0.6M NaCl solution-soluble protein of "Suketodara" (*Theragra chalcogramma*) meat

Takagi[11] has also examined "Nibe" (*Nibea mitsukurii*) meat; they made it clear that the isoelectric point of fish meat protein is different by the kinds of fish constituent protein and method of preparation of the protein.

The "Suketodara" meat is also constituted of several proteins.

3. The phenomenon of flow birefringence of the extractive of the "Suketodara" meat

Among several kinds of proteins in the "Suketodara" meat, "myosins" (including actomyosin, L-myosin and actin) are considered to have an intimate relation with the quality of the fish paste ("Kamaboko") product.

The authors undertook to observed the flow birefringence in the solutions of water soluble protein, Weber's solution-soluble and NaCl solution-soluble proteins by the use of the same apparatus employed in studying the extracts of *Stichopus japonicus*[12].

(1) Experimental method

Fresh "Suketodara" was filleted, the meat was removed from the skin and homogenized. The homogenized meat was used for the sample. To 20 g of the sample meat, 200 cc of distilled water was added. After 30 minutes' stirring of the mixture, it was centrifuged. The flow birefringence of the upper clear liquor thus obtained was observed. The total amount of nitrogen in the same upper clear liquor was estimated, and the dissolved ratio (%) of the nitrogen to the total nitrogen in the raw meat was presented as the solubility. To the residue of the 1st extraction, 200 cc of distilled water was added and the 2nd extraction was carried out. Intensity of birefringence and the total amount of nitrogen were estimated for the 2nd extract. The extractions were continued until the 5th extraction. Extraction was also done by the use of Weber's solution (which consists of KCl, $NaHCO_3$ and Na_2CO_3 in the proportion of 0.6, 0.04 and 0.01 Mol respectively). Thus the dissolution of myosins was detected.

(2) Results

Results obtained are shown in Tables 13 and 14.

Table 13. Flow birefringence and amount of nitrogen dissolved in water extracted solution of "Suketodara" (*Theragra chalcogramma*) meat and appearance of the sediment

No. of extraction	Soluble-N (% in total-N)	S. B.	Appearance of sediment
S_1	26.45	−	
S_2	9.35	−	Did not effuse by decantation
S_3	7.42	−	Softening of meat, upper layer of sediment flowed out by decantation
S_4	4.92	−	Further softening, ditto
S_5	Trace	−	Ditto
Residue	46.90		

Table 14. Flow birefringence and amount of nitrogen dissolved in the extract of "Suketodara" (*Theragra chalcogramma*) meat obtained by use of Weber's solution and appearance of the sediment

No. of extraction	Soluble-N (% in total-N)	S. B.	Appearance of sediment
S_1	37.74	−	
S_2	12.76	−	Did not effuse by decantation
S_3	5.83	−	Softening of meat, did not effuse
S_4	Trace	−	Slight coagulation of meat
S_5	Trace	−	Ditto, coagulation of meat
Residue	40.60		

In the case of the 1st extraction of the "Suketodara" meat with water, no pattern of flow birefringence appeared. After the 5th extraction, the residue was extracted with Weber's solution. But this extract also did not show flow birefringence. On the other hand, in the extraction of the meat with Weber's solution, even in the case of the 1st extraction flow birefringence was not seen. After the 5th extraction, the residue was extracted with the same solution. But this extract also did not show flow birefringence. In the case of the extraction with 0.6 Mol NaCl solution, every extraction also showed no pattern of flow birefringence.

That is to say, the extractions of "Suketodara" meat with water, 0.6 M NaCl solution or Weber's solution all showed no pattern of flow birefringence. But the amount of nitrogen in the 1st extraction with Weber's solution was larger than that in the 1st extraction with water. After the 2nd extraction with Weber's solution or water, the amount of nitrogen dissolved out became small. In the extraction of the meat with Weber's solution, the amount of nitrogen dissolved out showed only trace after the 4th extraction.

According to Okada *et al*[13], the extracted solution of fish meat muscle with 0.6 Mol KCl solution shows flow birefringence (S.B. +).

This is due to the reason that myosins (including actomyosin, L-myosin and actin)

dissolve easily into salt solution, and the fact that those filamentous molecule coordinate in one definite direction by flowing. On the contrary, the extracted solution of fish meat muscle with water does not show flow birefringence. (However, there are some exceptions. For example, the water-extraction of squid or sea cucumber meat shows flow birefringence because of their characteristics of histological properties or proteins[12)13)]).

In the present experiments, considering that the amount of nitrogen dissolved with 0.6 Mol NaCl solution or Weber's solution was larger than that with water, it is suggested that myosins which are soluble by salts solutions shall be dissolved out. Therefore, the fact that 0.6 Mol NaCl or Weber's extracted solution did not show flow birefringence is considered to be peculiar. This is considered explicable as follows: the amount of the dissolved nitrogen from the "Suketodara" meat with salts solutions might be too small to show flow birefringence by use of the apparatus employed of which the accuracy was limited. Furthermore, peculiarities of myosins in the "Suketodara" meat, for example the quantitative ratio of actomyosin, L-myosin and actin, are also considered to be involved in the explanation. According to Okada *et al.*[13)], when the 0.6 M KCl extracted solution of fishes meat, or fishes meat themselves are frozen, the showing of flow birefringence disappears in a comparatively short time. This may be explained by the denaturation of the protein by freezing.

As examined in the previous experiment, Article II above, the freshness of the "Suketodara" meat falls easily, therefore the denaturation of meat protein of the "Suketodara" also occurs easily. So the elasticity of the fish paste products becomes small. Considering together those facts with the peculiarities of the "Suketodara" meat which are observed in the phenomenon of flow birefringence, it is especially important to examine those peculiarities of this fish meat in relation to the properties of myosins in the meat.

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