STUDIES ON THE MUSCLE MEAT OF *PARALITHODES CAMTSCHATICA* (TIL.)-(I)

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In Japan, the Alaska king crab (*Paralithodes camtschatica TIL.*) is packed in Okhotsk Sea and Bering Sea waters. The crab industry in Japan is an industry of economic importance.

In the processing of the canned crab, there have been many troubles. Among them, difficult problems, such as "The formation of struvites[1]", "The browning of the canned crab meat[2]", and "The blue meat in the canned crab[3]" have been recently solved respectively. However, "The changes of crab meat by freezing in winter[4]" has not been solved. To reach a solution of problem, the muscle meat of the crab must be studied histologically and chemically.

The chemical composition of the canned crab was studied by many investigators[5], but few fundamental studies concerning the properties of the raw crab meat have been reported.

The present authors have undertaken composite fundamental studies on the muscle meat of *Paralithodes camtschatica*, and they will offer in this paper the results obtained.

Before going further the authors wish to acknowledge with the thanks the assistance rendered in this investigation through a grant in aid for developmental scientific research from the Ministry of Education.

I. CHEMICAL COMPOSITION OF CRAB MEAT (*PARALITHODES CAMTSCHATICA*)

As raw material for the canned crab, soft-shell crab (mainly in spring) and hard-shell crab (hard crab) (in autumn) are used.

Seasonal changes in the chemical composition of the parts of shoulder and leg flesh of both sorts of crab were studied.

(1) Experimental method

Shoulder and leg flesh were respectively taken from soft-shell crab which was caught in spring and hard crab caught in autumn. Those materials were crushed and the chemical composition was estimated as usual.

(2) Experimental results and consideration

The results obtained are shown in Table 1.

As seen in Table 1, generally speaking, the amount of water-content of *Paralithodes camtschatica* is 80~82%, crude protein (Total N×6.25) is 14~17%, pure protein is 8~11%, crude fat is 0.2~0.4%, and the ash is 1~2%. Comparing those compositions with fish flesh, there is no difference in the amounts of crude protein and ash between

Table 1. Seasonal and partial change of proximate components of *Paralithodes camtschatica* flesh

Parts / Season / Components	Leg flesh				Shoulder flesh			
	Soft-shell Crab (Spring)		Hard Crab (Autumn)		Soft-shell Crab (Spring)		Hard Crab (Autumn)	
	in raw flesh (%)	in dried matter (%)	in raw flesh (%)	in dried matter (%)	in raw flesh (%)	in dried matter (%)	in raw flesh (%)	in dried matter (%)
Water-content	82.19	0.0	80.84	0.0	82.82	0.0	80.07	0.0
Crude protein (Total-N×6.25)	14.75	82.7	16.96	88.5	14.56	84.7	17.03	85.3
Pure protein	11.13	62.6	10.31	53.8	9.56	55.6	8.50	42.6
Crude fat	0.41	2.3	0.33	1.7	0.23	1.3	0.17	0.9
Ash	1.33	7.5	1.40	7.3	1.32	7.7	1.56	7.8

crab and fish flesh, but the water-content of the crab flesh is larger than that of fish; contrarily the amount of crude fat of the former is less than that of the latter.

Okamoto[6] has estimated the chemical composition of commercial canned crab (*Paralithodes camtschatica*), and compared it with that of the meat of usual meat creatures, poultry and fish. According to his observations, the amount of water-content of the crab meat was larger than that of the others, and contrarily the amount of crude fat was less than in the others meats.

As seen in Table 1, the amounts of water-content and the crude fat of soft-shell crab flesh are considerably larger than those of the hard crab, but contrarily the amounts of crude protein and ash of the former are less than those of the latter. Therefore, the amount of non-protein nitrogen (extractive nitrogen) of the hard crab is larger than that of the soft-shell crab. In fact, the hard crab flesh has good taste while the soft-shell crab is watery and insufficiently palatable.

Shimoda[7] has observed that the chemical composition of crab flesh, particularly in crude fat and ash is changed remarkably by peeling, and at the peeling time the amount of ash becomes the least. Shimoda's result agrees with the present author's observation.

As for the differences of the parts of crab body, there is no remarkable difference in the amounts of water-content, crude protein and ash between the leg and the shoulder flesh, but the amounts of pure protein and crude fat of the leg flesh are larger than those of the shoulder flesh, therefore the amount of non-protein nitrogen of the former is considered to be smaller than that of the latter.

Kondo *et al.*[8] and Okuda[9] *et al.* have studied the nitrogen distribution of the muscle meat of *Paralithodes camtschatica* and amino acids composition of the meat protein. Matsui[10] has studied the differences of chemical composition of crab meat and mineral

matter between male and female.

The present authors did not re-examine those results, but according to the results obtained as above stated, the following conclusions may be stated. (1) *Praralithodes camtschatica* flesh is more rich in the amounts of hot water-soluble protein nitrogen and non-protein nitrogen and amino acids, such as lysine, arginine, cystine, than the fish flesh. (2) Male crab flesh is lower in the amounts of water-content, mineral matters (particularly, iron and sulfur), non-albumin nitrogen and free amino acids than the female crab flesh. (3) Crab flesh contains a larger amount of calcium than fish flesh. This may have intimate relation with the formation of the crust of the crab.

II. HISTOLOGICAL OBSERVATION OF *PARALITHODES CAMTSCHATICA* FLESH

(1) Experimental method

Leg flesh of fresh *Paralithodes camtschatica* was histologically observed. Leg flesh with crust was cut in lengths of 1 *cm* at a cannery. Several pieces of the cut leg flesh were soaked in Bouin's solution in a bottle, and the bottle was brought to the laboratory. The leg meat fixed with Bouin's solution was removed from the crust. The removed meat was dehydrated by alcohol as usual, and finally imbedded with paraffin. Thus treated meat was sliced in 10 μ thin pieces by a microtome. Each slice was dyed by Delafields' haematoxyline staining method and imbedded in balsam to make a permanent preparate. As contrast, *suketo*-cod (Alaska pollack) (*Theragra chalcogramma PALLAS*) flesh was treated in the same way as the crab flesh[11].

(2) Experimental results

Observing preparates of crab and *suketo*-cod made as above stated under a microscope, the writers obtained photographs as shown in Figs. 1 and 2. Both photographs show the longitudinal section in relation to the direction of muscular fibre. The histological difference between the crab and the fish is observed by the illustrating diagrams as shown in Figs. 3

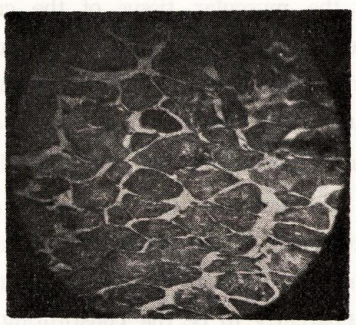

Fig. 1. Microphotograph of the leg flesh tissue of *Paralithodes camtschatica* (×70)

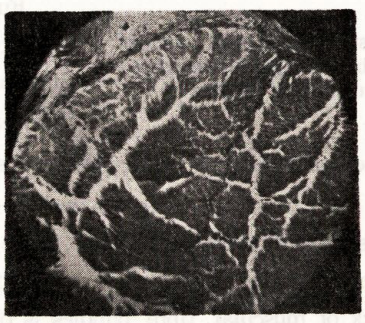

Fig. 2. Microphotograph of the flesh tissue of *Theragra chalcogramma* (×70)

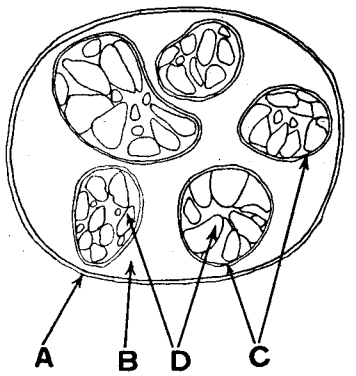

Fig. 3. Illustrating diagram of the leg flesh tissue of *Paralithodes camtschatica*

A: Surface skin part (epithelial tissue)
B: Interstice in inside of A (gelatinous tissue)
C: Blocks of muscular fibre bundles
D: Muscular fibre bundles

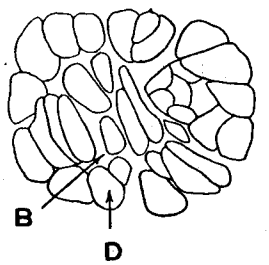

Fig. 4. Illustrating diagram of the flesh tissue of *Theragra chalcogramma*

B: Interstice among each muscular fibre bundles (gelatinous tissue)
D: Muscular fibre bundles

and 4.

In Figs. 1 and 2, the part of muscular fibre is observed to have dyed to pink brown color, and the parts of nucleus of muscular fibre, of nucleus of gelatinous fibre and of the wandering cells of the other pigment cells or lymph cells are observed to have dyed to blue color.

As seen in Fig. 3, which is a diagram illustrating the crab flesh, in the leg flesh, blocks (C) of muscular fibre bundles are observed to be surrounded by gelatinous tissues (B) which consist of comparatively clear and thick layer.

On the other hand, as seen in Fig. 4 which is an illustrative diagram of *suketo*-cod flesh, many muscular fibre bundles distributed closely, among which there are gelatinous fibres which form thin layers.

The epidermis (A) of the crab which is dyed to pink brown color as well as muscle tissue is extended narrow in form; it is epithelial tissue having gelatinous fibre-like constitution in which many nuclei gather. In the most outside of the part of (C) which is considered to be the block of muscular fibre bundles and is situated facing toward the gelatinous fibre (B), there are numerous, pretty thin and long nuclei which are like to muscular fibre substances in the epithelial tissue. Those substances are considered to be sarcolemma which surrounds muscle fibre bundles (D) and are partly extended.

Further, in the muscular tissue of the crab, the presence of the gelatinous fibre surrounding each muscular fibre bundle is indistinct, and it is difficult to distinguish from each muscular fibre. However, in the *suketo*-cod flesh, the comparatively clear gelatinous fibres are observed among the muscular fibre bundles. As above stated, in the *suketo*-cod flesh the muscular fibre bundle are observed closely associated among gelatinous tissue, on the contrary, in the crab flesh, the muscular fibre bundles make blocks; and each block is distributed coarsely in the gelatinous tissue. The block of muscular fibre bundles is observed apparently to be the constituting unit of crab flesh tissue. But, there is no difference in histological constitution between the crab and *suketo*-cod. In the crab flesh, gelatinous tissue is only seen to divide muscular fibre

bundles into several blocks. When the crab flesh which has the tissue constitution as above described is boiled, the gelatinous fibres change to gelatine substance and the blocks of muscular fibre bundles are considered to separate in the form of thick fibre. In fact, in the processing of the canned crab, after the crab flesh is boiled, the thick fibre of muscular tissue easily separated. At the boiling, body fluid in gelatinous tissue in the muscle flesh will flow out in large quantity. The thickness of muscular fibre bundles of male flesh is larger than the female, and the distribution of the muscle fibre bundles of the former is coarse and the arrangement is complicated and strong, but that of the latter is close and the arrangement is regular.

The above observations may supply the particular fundamental knowledge needed to solve the troubles of "frozen meat" of the raw material of the canned crab in winter.

III. SOLUBILITY OF NITROGENOUS SUBSTANCE OF *PARALITHODES CAMTSCHATICA* FLESH BY VARIOUS SOLUTIONS

From the experimental results as above described, the muscular tissue of ***Paralithodes camtschatica*** is considered to be easily separatable as units of the block of muscular fibre. Therefore the body fluid components which are present in the gaps among each blocks of muscular fibre are believed to flow out easily.

In the present experiment, the respective solublities of nitrogenous substances by water, NaCl solution, acid and alkali solutions were examined.

1. Solubility by water of nitrogenous substance of the crab flesh

In order to know the solubility of the nitrogenous substance of leg flesh of the crab (male), the leg flesh was treated according to Matsumoto's method[12] as shown in Scheme 1, and the amounts of the nitrogen in fractions E_1, E_2, and E_3 were estimated.

The results obtained are shown in Table 2 comparing with the respective results in meats of *suketo*-cod[11], Atka mackerel[13], sea-cucumber[14] and of sea-ear[15].

As seen in Table 2, when the crab flesh was extracted by water of 10-fold volume, about 48% of the total nitrogen dissolved out in the fraction-E_1. And when the water-extract was dialysed in tap water for a week, the precipitated protein (R_1) was yielded. In the remaining liquid (E_2) of the inner part of the dialysis, about 10% of the total nitrogen was present. About 38% of the total nitrogen corresponding to the difference between fractions E_1 and E_2 was considered to be the amount of nitrogen in the precipitated protein (R_1). When this precipitated protein (R_1) was extracted by 0.2 % NaCl solution, there was only about 3.5 % of the total nitrogen in the extracted solution (Fraction-E_3); the larger part of the precipitated protein (R_1) may be denatured in inactive state. In this case, the solution extracted by water in Fraction-E_1 did not show streaming birefringence, and of course, it contained non-protein nitrogen. From the result obtained, the greater part of the total nitrogen in the crab flesh is known to be water-soluble nitrogenous

Scheme 1. Extraction of water-soluble protein of *Paralithodes camtschatica* flesh

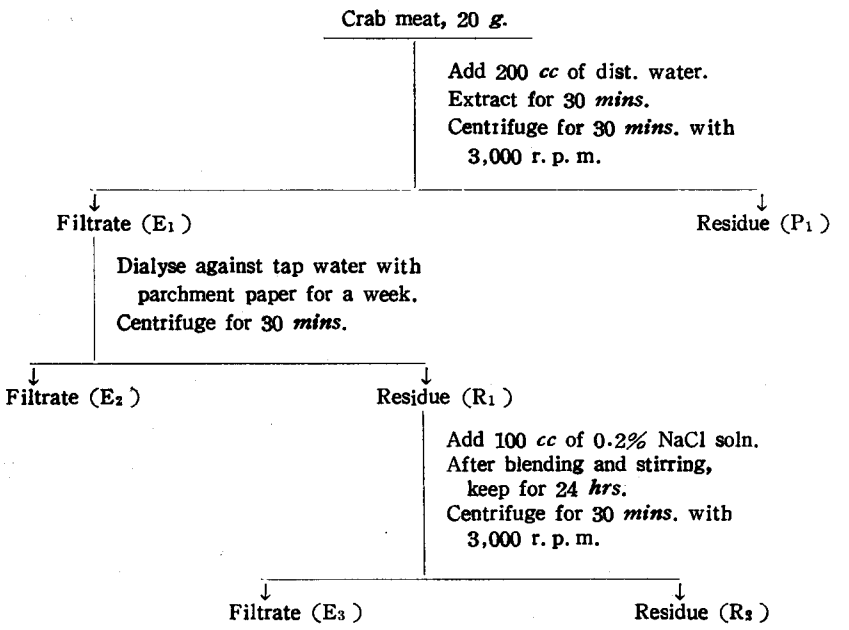

Table 2. Comparative data on the amount of water-soluble protein of *Paralithodes camtschatica* meat with the respective results in meats of various species of fish (Numerals: % for total-N in flesh)

Fraction / Fishes	E_1	E_2	E_3
Crab flesh	47.9	9.64	3.5
Atka mackerel flesh	27.8	7.20	3.5
Suketo-cod flesh	30.6	3.40	2.1
Sea-cucumber flesh	6.45	1.91	0.6
Sea-ear flesh	21.9	6.90	2.5

substance. It is remarkable that the amount of the water-soluble nitrogenous substance in the crab is much greater than that of Atka mackerel, *suketo*-cod and sea-ear flesh. The dissolving out of the extractive itself from the tissue is considered to depend on the constitution of the muscular tissue, therefore the characteristics of the crab tissue described in previous Experiment II have remarkable influence, largely on the dissolving of the nitrogenous substance.

2. Solubility of *Paralithodes camtschatica* flesh by NaCl solution

To the crushed and homogenized raw leg flseh of fresh *Paralithodes camtschatica* (male) was added 10-fold volume of NaCl solution of various concentrations; the preparate was left at 20°C for 24 hours, and then centrifuged. The liquid separated by a centrifugal

machine was estimated for the determination of the amount of nitrogen. The estimated amount of nitrogen in the extracted liquid was calculated in percentage against the total amount of nitrogen in the crab flesh.

The same experiment was carried out for the crab meat boiled at 100°C for 20 minutes.

The results obtained are shown in Fig. 5.

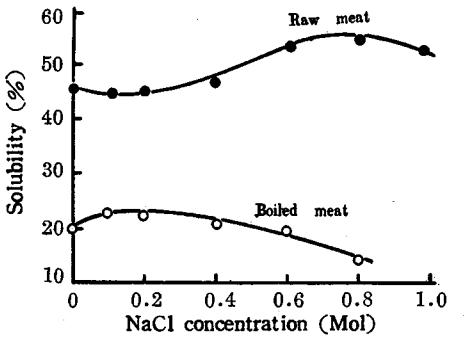

Fig. 5. Solubility of the nitrogenous substance of *Paralithodes camtschatica* flesh in NaCl solutions of various concentration

As seen in Fig. 5, the largest amount of nitrogen was extracted from the raw crab meat by 0.7 Mol (about 4 %) NaCl solution. This is about 55 % of the total amount of nitrogen. This is larger than the maximum solubility by water, 48%. If the loss of the dissolved nitrogen has relation with the taste, it is unbeneficial that the crab flesh be washed or boiled with sea water. From the boiled crab meat, the percentage of the amount of nitrogen dissolved by 0.2 Mol NaCl solution in relation to the total nitrogen was only about 25 %. This small value of solubility may be due to the heat coagulation of the protein of the crab flesh.

3. Solubility of *Paralithodes camtschatica* flesh by acid or alkali solution

To the crushed and homogenized crab (male) leg flesh was added 10-fold volume of acid solutions, such as HCl, H_2SO_4, CH_3COOH or alkali solution, such as NaOH, of various concentrations; the material was left at 20°C for 24 hours, and then centrifuged. The liquid separated by a centrifugal machine was used for the determination of the amount of nitrogen. The amount of nitrogen in the extracted liquid was calculated for the percentage relation to the total nitrogen in the crab flesh. The results obtained are shown in Fig. 6.

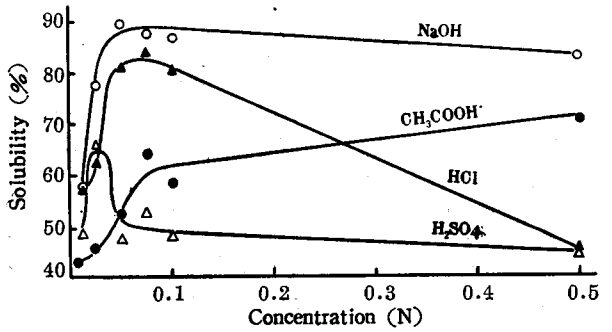

Fig. 6. Solubility of the nitrogenous substance of *Paralithodes camtschatica* flesh in acid or alkali solutions of various concentration

As seen in Fig. 6, in the case of extraction by HCl solution, the largest solubility was shown by the concentration of 0.05~0.075 N. This was about 83% of the total nitrogen. Above 0.075 N of concentration, the solubility decrease, and in 0.5 N

HCl solution the solubility decreased to about 46%; this was the same value as the maximum solubility by water. In the case of H_2SO_4 solution, the largest solubility was shown by the concentration near 0.025 N. This was about 65 % of the total nitrogen. Above 0.025 N concentration, the solubility decreased remarkably. Above 0.1 N concentration, the solubility showed below 50 % of the dissolved nitrogen in relation to the total nitrogen. On the other hand, in the case of acetic acid solution, differing from the mineral acid solution, such as HCl or H_2SO_4, the solubility of the nitrogenous substance in the crab flesh increased remarkably until 0.1 N concentration, and even above 0.1 N, the solubility of the nitrogen gradually increased.

From the results obtained, the amounts of the extracted nitrogen differed according to the differences of pH values of extracting solutions. As seen in the strong acid, such as HCl or H_2SO_4 solutions, in the lower pH value solutions the crab flesh coagulates, and the solubility brought about by those acid solutions may decrease. As seen in Fig. 6, in the extraction by alkali solutions, above 0.5 N solution about 90 % of the total nitrogen in the raw crab flesh dissolved out. That is to say, the solubility of the protein by the alkali solution was the largest.

IV. ISOELECTRIC POINT OF FLESH PROTEIN OF *PARALITHODES CAMTSCHATICA*

In order to learn futher about the characteristics of the flesh proteins of ***Paralithodes camtschatica***, the isoelectric points of the proteins extracted by water or NaCl solution were determined by the estimation of the protein precipitated by the solutions of various pH values and by relative viscosity measurement. The isoelectric reaction of the crab flesh was also observed from the solubilities of the nitrogenous substances in the buffer solutions of various pH values.

1. Isoelectric point of flesh protein of the crab

Fresh raw leg flesh of the crab was crushed and homogenized. To 300 *g* of the crushed flesh was added 500 *cc* of distilled water and material shaken for one hour. After the shaking, the upper liquor was separated by a centrifugal separator (3,000 r.p.m.) for 20 minutes. The separated liquor was half saturated with ammonium sulfate and was left over one night at a cold place. The precipitated protein here yielded, was dialysed in running water for a week. Thus the water-soluble protein in the wet matter was obtained. On the other hand, to the residue which was obtained in the extraction by water, was added 500 *cc* of 0.5 M. NaCl solution. This mixed solution was shaken and the upper liquor was separated by a centrifugal machine. The separated liquor was 2/3 saturated by ammonium sulfate. The precipitated protein was dialysed, and thus NaCl solution-soluble protein in the wet matter was obtained.

To each 10 *g* portion of the two kinds of prepared proteins of wet matter was added

250 *cc* of 0.025 N NaOH solution separately and preparates were dissolved. After leaving them over one night, the undissolved part was filtered through a pulp layer. The filtrates were used as samples of the protein solution. Each 10 *cc* portion of the samples of the protein solution was added respectively to 30 *cc* of the buffer solution of various pH values in measuring flasks and then dist. water was added to bring the total volume up to 50 *cc*. After leaving the solution over one night, the precipitated protein was filtered. The amount of nitrogen in the precipitated protein was estimated by micro-Kjeldahl method. The pH values at which the maximum amount of nitrogen is determined was considered to be the isoelectric point of the protein.

The relative viscosity of the filtrate as above stated was measured by Ostwald's viscosimeter. The pH value which shows the minimum value of relative viscosity was also considered to be the isoelectric point of the protein. The results obtained are shown in Figs. 7 and 8.

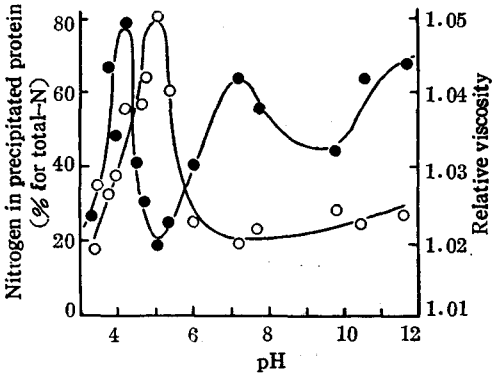

Fig. 7. Isoelectric point of water-soluble protein in *Paralithodes camtschatica* flesh

○—○ "pH-Nitrogen" curve
●—● "pH-Relative viscosity" curve

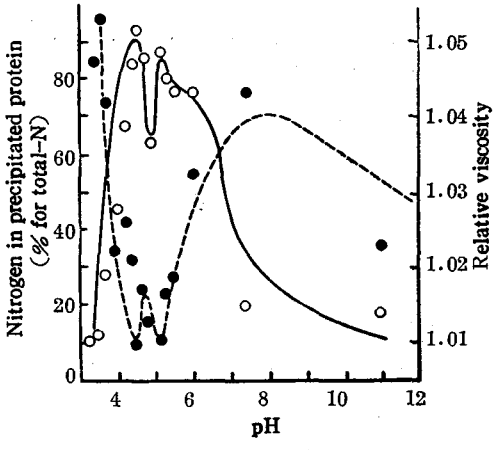

Fig. 8. Isoelectric point of NaCl solution-soluble protein in *Paralithodes camtschatica* flesh

○—○ "pH-Nitrogen" curve
●···● "pH-Relative viscosity" curve

As seen in Fig. 7 which shows the solubilities of water-soluble protein in solution of various pH values, the maximum amount of protein was precipitated at near pH 5.0. The minimum relative viscosity was also shown at near pH 5.0. That is, the isoelectric point of the water-soluble protein is to be at pH 5.0.

As seen in Fig. 8, the isoelectric point of the NaCl solution-soluble protein of the crab is to be at pH 4.5 and 5.1. That is to say, it may be assumed that there are two kinds of proteins in the NaCl solution-soluble protein.

2. Isoelectric reaction of the crab flesh

In this experiment, the isoelectric reaction of the crab flesh itself was estimated by

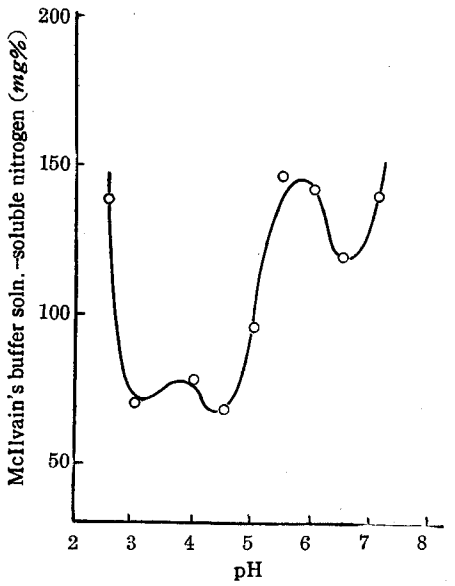

Fig. 9. Isoelectric reaction of *Paralithodes camtschatica* flesh

determining the dissolved amount of nitrogen from the soaked crab flesh in the buffer solutions having various pH values.

To each 10 *g* portion of the crushed and homogenized crab leg flesh was added McIlvain's buffer solution having various pH values bringing the total volume up to 100 *cc*. After leaving the mixed solution over one night, the upper liquor was centrifuged. To each 10 *cc* portion of the separated liquor were used for the determination of nitrogen. The results obtained are shown in Fig. 9.

As seen in Fig. 9, the minimum dissolved amount of nitrogen was at neer pH 4.5. That is to say, the crab flesh has the characteristic of being insoluble at this pH value. This is agrees with the finding that the isoelectric points of the crab flesh proteins are at pH 4.5~5.0.

V. ELECTROPHORETIC STUDIES ON THE PROTEINS OF *PARALITHODES CAMTSCHATICA* FLESH

It was assumed from the previous experiment, that there are two kinds of proteins in the crab flesh, from the results showing two pH values of 5.1 and 4.5 as the isoelectric points of the NaCl solution-soluble protein.

In this experiment, the NaCl solution-soluble protein which was prepared in the previous experiment, was studied electrophoretically. About 5 *g* of the NaCl solution-soluble protein was dissolved in phosphate buffer solution of which ionic strength, I, was 0.35 (prepared by means of the buffer solution of M/5 Na_2HPO_4 and M/5 KH_2PO_4), and the total volume was made up to 20 *cc*; the material was left over one night in a cold place. The dissolved solution was dialysed in 50 *cc* of the same buffer solution for 48 hours. Inner solution of the dialysis was centrifuged, and the obtained upper clear liquor was used for the electrophoresis. This liquor had pH 8.4 value, and its electric conductivity was 59.5 Ohm. The electrophoresis was examined by an H. T. type Tiselius' apparatus. The size of the electrophoretic cell is $2\times15\times50mm$ (area of the section is 0.3 cm^2). The potential grade was 5.75 *volts/cm* and the electric current was 16 mA. The temperature of the cell was

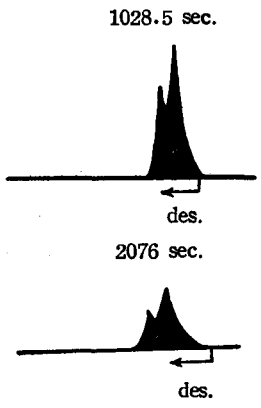

Fig. 10. Electropheretic pattern of NaCl solution-soluble protein in *Paralithodes camtschatica* flesh

maintained at 1~2°C during the electrophoretic examination.

Results obtained are shown in Fig. 10.

As seen in Fig. 10, NaCl solution-soluble protein is composed of a mixture of two components, of which the mobilities were -1.02 and-1.39 $\times 10^{-5}$ *cm²/volt. sec.* respectively, from the descending boundary.

VI. THE PHENOMENON OF STREAMING BIREFRINGENCE OF THE EXTRACTS FROM *PARALITHODES CAMTSCHATICA* FLESH

As noted in the previously reported experimental results (III), a larger amount of nitrogen flowed out in the water extract or NaCl solution-extract of *Paralithodes camtschatica* flesh than in corresponding extracts of fish flesh. The nitrogenous substance which was extracted out, contains water-soluble proteins, such as myogen, myoalbumin and globulin-X, likewise NaCl solution-soluble proteins, such as myosins (The word "myosins" was used here to include both myosin or L-myosin and actomyosin). The possibility must also be considered of the presence of non-protein nitrogen, such as meat-extractive nitrogen in addition to the proteins above mentioned.

It is known that if a sample of the solution of the fibrous protein, myosins, is placed between two crossed Nicol prisms, and is made to flow, a light field is observed; this is the phenomenon of streaming birefringence (S.B.)[16]. On the other hand, the water-soluble proteins (sarcoplasmic), such as myogen, myoalbumin and glogulin-X did not show S.B. The myosins are insoluble in water, but soluble in dil. salt solutions. However, according to Migita *et al.*,[17] the water extract of squid meat (*Ommastrephes sloani pacificus*) distinctly shows the phenomenon of S. B. The reason why the water extract of squid meat shows S.B., is the dissolution of the myosins in the dispersed state for some unknown reason. This is one of the characteristics of the squid meat protein. According to Okada *et al.*,[18] the phenomenon that water extracts of squid meat show S.B. is characteristic to the mollusca, whilst water-soluble protein of shrimp meat in the crustacea does not show S.B. On the basis of those experimental results, in *Paralithodes camtschatica* flesh also, it is considered that the myosin-like protein does not flow out in the water extract.

In the present experiments, in order to gather qualitative data on the dissolution

phenomenon, the authors have examined the phenomenon of S.B. in water extract and KCl solution extract of the crab flesh.

(1) Experimental method

Fresh raw leg flesh of the crab was crushed and homogenized, and the repeated extractions were carried out by the same method as described in Tanikawa's study[14] with 10-fold volume of dist. water or Weber's solution (a mixture of 0.6 Mol KCl, 0.04 Mol $NaHCO_3$ and 0.01 Mol Na_2CO_3 solutions). Each extract was used for the examination of S.B. At the same time, the amount of dissolved nitrogen in the 1st extraction was measured.

(2) Experimental results and consideration

The results obtained are shown in Table 3

Table 3. Phenomenon of streaming birefringence and nitrogen contents in both water and Weber's extracted solutions of *Paralithodes camtschatica* flesh (Numerals: % for total-N in flesh)

No. of extraction	Water extracted soln.		Weber's extracted soln.	
	S.B.	Nitrogen content (%)	S.B.	Nitrogen content (%)
1	—	31	++++	41
2	—	—	+	—
3	—	—	—	—
4	—	—	—	—
5~10	—	—	—	—

As seen in Table 3, when the crab meat was extracted repeatedly with 1.5-fold volume of dist. water, in the 1st extraction about 30 % of the total amount of nitrogen in the crab meat was dissolved out, but the water extract did not show S.B. On the other hand, when the crab meat was extracted by Weber's solution as well as by the use of water, in the 1st extraction about 40 % of the total nitrogen in the crab meat was dissolved out, and the extracts show S. B. comparatively clearly. But after the second extraction, the extracts have decreased in their showing of S. B. After the third extraction, the extract did not show S. B. at all. Those obtained results are the same as in the extraction of fish flesh such as Atka mackerel[13] and carp[18]. From those results, it is considered that the crab flesh as well as the fish flesh, myosins did not dissolve out in dispersed state.

Ⅶ. THE SWELLING OF THE FLESH OF *PARALITHODES CAMTSCHATICA*

In the processing of canned crab, the crab flesh is often treated with neutral salts (*e. g.*, NaCl) or acids during the processes of water-washing and bleaching. In this process, the crab flesh protein shows the swelling corresponding to the water holding affinity of the protein. This phenomenon of swelling affects the yield of the canned crab.

In this experiment, examination has been made of the phenomenon of the swelling of

the flesh of *Paralithodes camtschatica* immersed in various salt or acids solutions, and of the relation between the degree of swelling and the pH value of the immersed solution.

1. The swelling of the crab flesh immersed in salt solutions of various concentration

(1) Experimental method

Blocks of the raw meat (about 2 cm^3) were taken from the fresh leg meat of the crab. After having been weighed, each block was immersed in 50 *cc* of the following various salt solutions in a cold place for 24 hrs. After taking up, adsorbed water on the surface of the blocks was wiped off with a filter paper, and the weight of the block after the immersion was measured. The ratio of the weight (W) of the block meat after immersion to the weight (W_o) of meat before immersion was calculated as the degree of the swelling (S) of the block of meat ($S=W/W_o$). Salt solutions employed were NaCl, KCl, $CaCl_2$, $MgCl_2$, KI, K_2SO_4, KCNS, KNO_3, $NaNO_3$, CH_3COONa, $C_3H_4OH(COONa)_3$, NH_4Cl, Na_2CO_3 and Na_3PO_4 solutions of 0.1~2.0 Mol, and $Na_2B_4O_7$ solution of 0.0025~0.5 Mol. As control, dist. water was also employed.

(2) Experimental results

The results obtained are shown in Figs. 11~13.

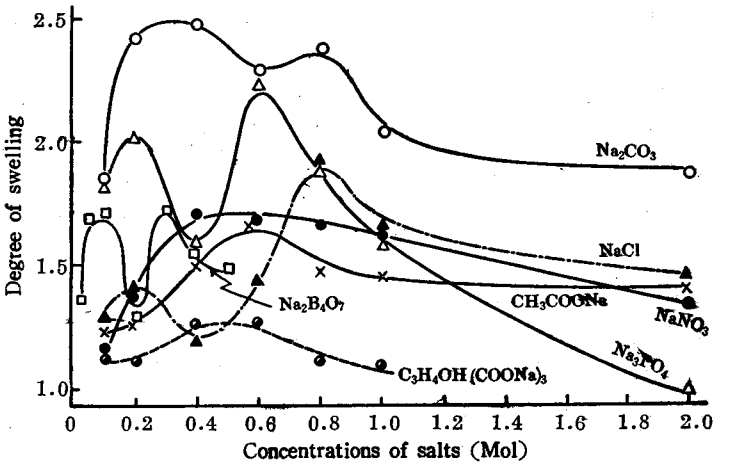

Fig. 11. Swelling curves of *Paralithodes camtschatica* flesh immersed in salt solutions of various concentration (in the case of varying anions upon Na^+)

Fig. 11 shows the influence of varying anions upon Na^+, Fig. 12 shows the influence of varying anions upon K^+, and Fig. 13 shows the influence of varying cations upon Cl^-.

As seen in Fig. 11, excepting $NaNO_3$ solution, the curve showing the relation between the degree of swelling and the concentration of solutions has two peaks which are shown at near 0.2 and 0.6~0.8 Mol. (the swelling curve of the flesh block immersed in solution of $Na_2B_4O_7$ shows two peaks which are situated at lower concentrations, 0.1 and 0.3 Mol).

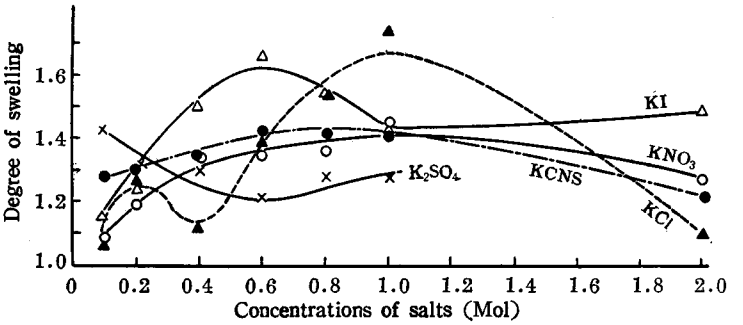

Fig. 12. Swelling curves of ***Paralithodes camtschatica*** flesh immersed in salt solutions of various concentration (in the case of varying anions upon K^+)

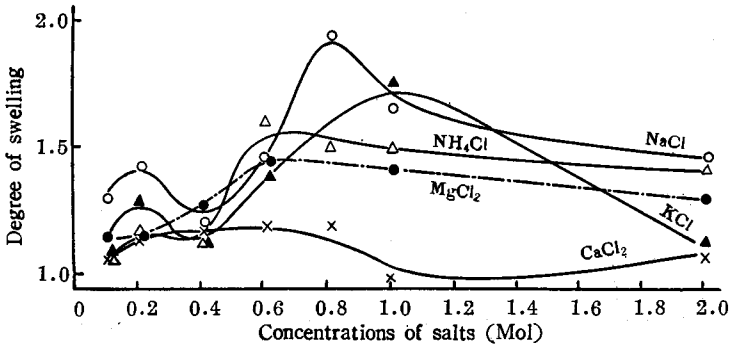

Fig. 13. Swelling curves of ***Paralithodes camtschatica*** flesh immersed in salt solutions of various concentration (in the case of varying cations upon Cl^-)

Among the alkali salt, the degree of the swelling caused by Na_2CO_3 was the largest. On the other hand, the curves of the degree of swelling of the block flesh immersed in $NaNo_3$ and organic salts, such as CH_3COONa and C_3H_4OH $(COONa)_3$ have each only one peek which is situated at 0.4~0.6 Mol. The degree of swelling caused by organic salts is smaller than that by the inorganic salts.

As seen in Fig. 12, the swelling curve brought about by KCl in potassium salts shows two peaks at near 0.2 and 1.0 Mol similar to NaCl, but other potassium salts, such as KI, KNO_3 and KCNS, show only one peak which is situated at a point near 0.6 Mol or 1.0 Mol. The curve of swelling caused by K_2SO_4 is considered to have a maximum point at below 0.1 Mol, but the degree of swelling decreased when concentration was above 0.1 Mol.

As seen in Fig. 13, comparing between salts having Cl^-, one sees that the curves of the swelling brought about by the salts having monovalent cations, such as Na^+, K^+ and NH_4^+ show two peaks which are situated at near 0.2 Mol and 0.8~1.0 Mol, but the

curves of salts having bivalent cations, such as Mg^{++} and Ca^{++} show only one peak which is situated at near 0.6 Mol; further, the inclination of the latter curve was gentle.

From those results, when the crab flesh is immersed in salt solutions having concentration up to a strength of 2 Mol, it is noticeable that the cruves of swelling caused by monovalent neutral salts, such as NaCl, KCl, NH_4Cl, or by alkali Na-salts, such as Na_3PO_4, $Na_2B_4O_7$, Na_2CO_3 have two peaks.

It is an interesting fact that the swelling curve of the crab flesh has two peaks. In senior author's laboratory, the swelling of *suketo*-cod[11] (*Theragra chalcogramma PALLAS*), Atka mackerel[13] (*Pleurogrammus azonus JORDAN et METS*), sea-cucumber[14] (*Stichopus japonicus SELENKA*) and sea-ear[15] (*Haliotis* (*Sulculus*) *kamtchatkana JONAS*) by various salt solutions has been observed in addition to work done in this experiment. Among those marine animals, the swelling curve of sea-cucumber meat showed two peaks like the crab flesh, while the cruves made by other meats did not show two peaks, but only one in the range between 0.4~1.0 Mol. The fact that the swelling of the crab flesh resembles that of the sea-cucumber meat and is different from the fish flesh is considered to be interpretable as follows. As observed from the previous experiment, II, the tissue constitution of the crab leg flesh is made up of blocks of muscular fibre bundles, surrounded by a gelatinous tissue (connective tissue), and epithelial tissue envelopes the internal tissues. Here it is necessary to consider the effects of swelling both by the muscular tissue protein and by the insoluble proteins of the connective tissue, such as collagen and elastin.

Accorbing to Takahashi[19], the swelling curve of shark skin, in which the amount of the water-content is 70~80% and of collagen is about 13%, shows the maximum at 1% concentration of NaCl solution (about 0.2 Mol). The swelling of the shark skin is considered to be affected by the swelling of collagen tissue. Likewise, the flesh of the sea-cucumber also consists mainly of a net work of collagen fibre, of which the amount of collagen is three-fold that of the fish flesh. The collagen fibres of the connective tissue in the crab flesh which surround the blocks of muscular tissue bundles are also considered to swell primarily at immersion in the various salt solutions, and to give the first peak on the curve of swelling.

From the consideration above stated, in the flesh of crab or sea-cucumber, one of the peaks of the curves occurring at lower concentration of the salt solutions, at near 0.2 Mol, is considered to be followed by the swelling of the insoluble proteins (collagen and elastin) in the connective tissue. With the increasing of the concentration of salt solution, the degree of swelling decreases once because of the osmotic dehydrating effect, and with further increasing of the concentration, the swelling of the muscle proteins occurs at higher concentration of 0.8~1.0 Mol, and shows the other peak of the swelling.

2. The influence of the hydrogen ion concentration on the degree of the swelling of the crab flesh

(1) Experimental method

Blocks of crab meat (about 2 cm^3) were taken from the raw leg flesh as samples. pH of the immersing solution was adjusted by means of N/10 HCl and N/10 NaOH. The degree of swelling was estimated by the same method as described above.

(2) Experimental result

Results obtained are shown in Fig. 14. In this case, the pH values are recorded as the pH values of each solution after the immersion of the crab meat blocks.

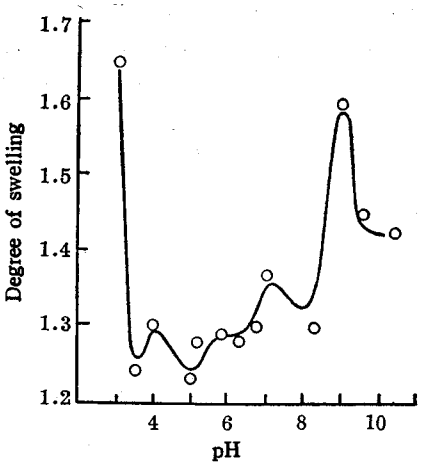

Fig. 14. The influence of the hydrogen ion concentration on the degree of swelling of *Paralithodes camtschatica* flesh

As seen in Fig. 14, the curve of swelling of crab meat shows characteristically, (1) the minimum degree of swelling was at near pH 5, (2) below pH 4, the degree of swelling increased remarkably, (3) the maximum degree of swelling was at near pH 9. Among those facts, the fact (1) is affirmed from the result of the previous experiment, V, in which the isoelectric reaction of the crab meat was shown at near pH 5. Facts (2) and (3) obtained are considered to agree with the point that the degree of swelling of tissues consisting largely of collagen fibre, such as cowhide or shark skin, shows the peaks at pH 2.5 and pH 12.0[19,20].

In sea-cucumber meat[14], the curve of swellig shows two peaks at pH 2.0 and 10.0 while in fish flesh, it is well known that the curve of swelling has comparatively high degree on both acid and alkali sides. This is perhaps owing to the presence of more or less amount of acidic amino acids (asparatic acid, glutamic acid) or basic amino acids (arginine, lysine). According to Okada *et al.*[21] or to the present authors' results which were obtained in carp, Atka mackerel, *suketo*-cod or sea-ear meats, it was also observed that the larger degree of swelling occurred at acidic side or alkali side of the isoelectric point of the fish flesh. However, in the curve of the swelling of those fish fleshes, there was no distinct peak such as was seen in the acidic or alkali sides in collagen or keratine fibres[22]. The swelling curve of crab flesh resembles the curves of shark skin or collagen fibres, but is different indistinctly from that of fish flesh.

VIII. HEAT COAGULATION OF THE FLESH OF *PARALITHODES CAMTSCHATICA*

In the processing of canned crab, the cooking procedure is important in order to harden

the crab flesh, to make easy the removal of flesh from the crust, and further to prevent the autolytic action of the flesh. The cooking procedure is carried out after the removal of the carapace when the crab is landed at the cannery. In this procedure, the leg flesh is boiled with the crust in sea water. It can not be overlooked that this procedure affects the yielding ratio of the number of cases of cans to the number of raw crabs used.

Therefore it must be ascertained how the kinds of boiling water, number of repetitions of cooking, relationships of cooking temperature to time and the freshness of the raw material affect the qualities of the canned crab flesh product. At the cooking, the crab flesh protein shows the phenomenon of heat coagulation accompanied with dehydration or adsorption of water. In this experiment, the authors have examined the phenomena of heat coagulation, dehydration and adsorption of water in the cooking of crab flesh protein.

1. The phenomenon of dehydration or adsorption in the cooking of crab flesh

(1) Relation between the cooking time and the phenomenon of dehydration or adsorption

When crab flesh is cooked in water or salt solution, the flesh coagulates and is dehydrated. Here, when the crab flesh is cooked in dist. water having various temperatures, the dehydrating ratio of crab flesh was estimated with the lengthening of the cooking time.

Blocks of raw leg flesh sized 1 cm^3 were taken, and weighed (W_o); they were immersed in dist. water of 40°, 50°, 60°, 70° and 100°±2°C for 5~20 minutes. After taking up the blocks immersed, the adsorbed water on the surface of the blocks was wiped off with a filter paper, and the weight after boiling was measured (W). The dehydrating ratio (D) was calculated from the following equation.

$$D=\frac{W-W_o}{W_o}\times 100\ (\%) \quad \cdots\cdots\cdots\cdots \quad (1)$$

In this equation, when W is less than W_o, the value of D shows the minus sign, and it represents dehydration, and when W is larger than W_o, the value of D shows the plus sign and it stands for adsorption.

The results obtained are shown in Fig. 15.

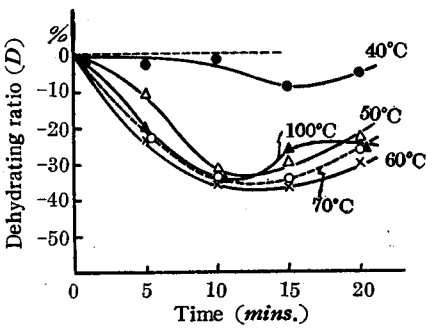

Fig. 15. Dehydrating curves of leg flesh of *Paralithodes camtschatica* in distilled water having various temperatures

As seen in Fig. 15, the crab flesh is dehydrated by boiling. At boiling of 40°C for 15 minutes, or in the range of 50°~100°C for 10 minutes, the dehydrating ratios, D, increased to the maximum. When boiling was for more than that time, adsorption was shown, and the value of D decreased. This may be because, after the coagulation of the flesh protein becomes constant, the amount of water adsorbed by the coagulated flesh protein becomes larger than the dissolved

amount of the soluble components. But within the range of boiling temperatures used in this experiment, the ratio of the dehydration, D, showed the maximum (about 38 %) at 60°C, and the ratio decreased when boiling temperatures were above 60°C. When the crab flesh was immersed in hot water at various different temperatures, the ratio of the dehydration increases or decreases as a boundary of some temperature. The increase in dehydrating ratio is owing to the heat contraction or decrease of the dried matter by the dissolving of the soluble components. The decrease of dehydration is a kind of hydration owing to the difficulty of passage of water from the inner part of the flesh to the outside due to the rapid heat coagulation of the surface protein of the meat block in the boiling.

Next, the shoulder flesh of the crab was examined as well as the leg flesh, and results obtaind are shown in Fig. 16.

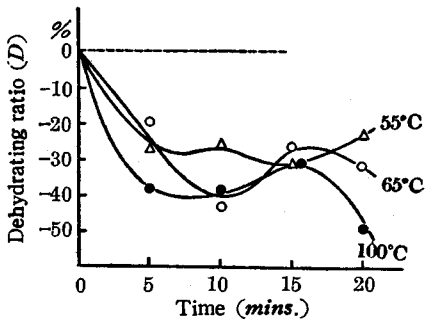

Fig. 16. Dehydrating curves of shoulder flesh of *Paralithodes camtschatica* in distilled water having various temperatures

As seen in Fig. 16, the dehydrating ratio, D, becomes maximum at 10 minutes. After 10 minutes' boiling, the coagulated protein first showed adsorption, and the dehydration occurred again with the increase of the boiling time owing to the increase of the dissolved amount of the soluble components.

(2) Relation between the heating-temperature and the ratio of the dehydration

As it clearly learned from the previous experiment that the ratio of the dehydration of the block (about 1 cm^3) of *Paralithodes camtschatica* flesh showed constant value as a result of heating for 10 minutes in dist. water of 50°~100°C, then it was observed in detail how the ratio of the dehydration of the blocks of the crab leg meat varies, when the blocks are heated for 10 minutes at various different temperatures. Further, in this experiment, in order to know the difference of the ratio of the dehydration in the various degrees of freshness of crab leg flesh, use was made of both fresh raw leg meat and unfresh meat which had been left for 24 hours at 15°C. The experimental method was the same as in the previous experiment.

The results obtained are shown in Fig. 17.

Curve I in Fig. 17 shows the ratio of the dehydration of fresh crab meat; curve II shows that of unfresh crab meat (left for 24 hours); curve III shows the ratio of the dehydration of the leg flesh of *Erimacrus isenbeckii* obtained by Kosakabe[8a]. As seen from curve I, in the fresh raw leg flesh, the dehydration occurred slowly until 40°C, then increased rapidly from 40°C to 65°C; the ratio of dehydration at 65°C showed about 32%, and the adsorption rathr than the dehydration occurred at from 65°~85°C; the phenomenon of dehydration predominated again at 85°~105°C.

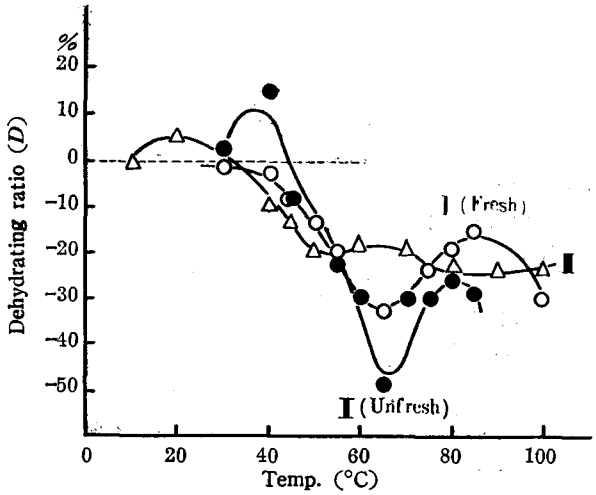

Fig. 17. Relation between the dehydrating ratio and the heating-temperature in fresh or unfresh meat of *Paralithodes camtschatica* (Heated in distilled water for 10 minutes)

If the remarkable phenomenon of the dehydration below 40°C or 85°C were considered according to the ideas of Shimidu[28], the dehydration of crab meat is interpreted as follows. The dehydration at 40°~65°C is considered to be due to the heat coagulation of myosin of muscular protein, and that at 85° is considered to correspond to the heat coagulating process of myogen. The adsorption at 65°~85°C is considered to be due to the prevention of passage of water from inner part of the meat to the outside because of the heat coagulation of the protein in the surface of the meat block. In the unfresh raw leg meat (left for 24 hours), as seen from curve II, the adsorption occurred until 40°C showing above 10 % of the ratio of the adsorption, and when the heationg temperature ascended over 40°C, the dehydration occurred rapidly as a result of a few degrees heating temperature. At 65°C, the ratio showed the maximum, about 48%. In comparison with the fresh leg flesh, the unfresh leg flesh is easily dehydrated: the dehydrating ratio decreased at 65°~80°C and adsorption occurred, and then the dehydration continued again at above 80°C. As seen in curve III obtained by Kosakabe as to *Erimacrus isenbeckii*, the maximum value of the ratio of dehydration was about 25 %, and the dehydration is not as easy as in the *Paralithodes camtschatica* flesh. This curve also shows two stages. The 1st stage of dehydration is observed from 20° to 50°C, and the 2nd stage from 70° to 80°C. In the flesh of *Erimacrus isenbeckii* also it is considered that the two kinds of heat coagulable proteins, myosin at lower temperature and myogen at higher temperature, are present. The coagulating temperature of the proteins in *Erimacrus isenbeckii* is apparently lower than that in *Paralithodes camtschatica.* The cause of this difference is considered to be the histological difference of the two kinds of crab. But the detailed explanation will be provided by studies in future.

Fig. 18 shows the comparison of curve I shown in Fig. 17 with the curves of other marine animals, Atka mackerel flesh[18] (curve II), *suketo*-cod flesh[11] (curve III), squid meat [23] (curve IV), sea-ear meat[15] (curve V) and sea-cucumber meat[14] (curve VI).

Curve IV is shown for squid meat heated for 30 minutes in water. All other curves represent values for samples heated for 10 minutes.

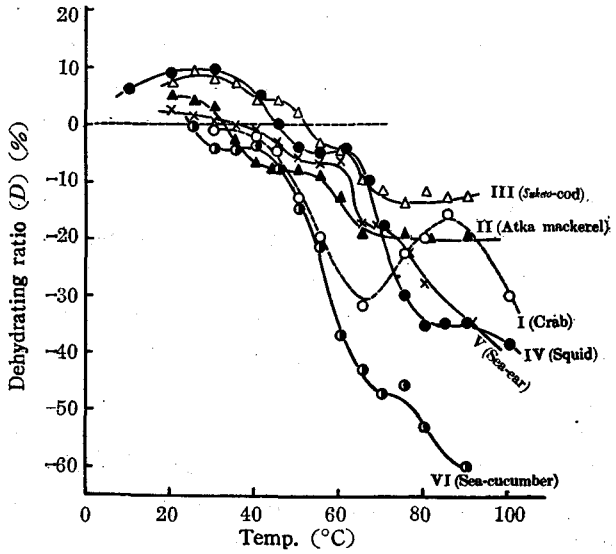

Fig. 18. Comparative data on the dehydration of *Paralithodes camtschatica* flesh with those of other marine animals

As seen in Fig. 18, the curves of the dehydration of fish flesh, such as Atka mackerel (II curve) and *suketo*-cod (III curve) are almost parallel; they show that the maximum values of the ratio of the dehydration (Atka mackerel (20%), *suketo*-cod (14 %)) are less than those of other marine animals. In the curves of dehydration of Atka mackerel and *suketo*-cod, the parts of the curves shown as below 30°C for the former, and as below 50°C for the latter respectively are predominantly the phonomenon of adsorption. Atka mackerel flesh has two kinds of heat coagulable proteins which are coagulated within the ranges of 30°~45°C and 55°~65°C; *suketo*-cod flesh has also two kinds of heat coagulable proteins which are coagulated in the ranges of 45°~55°C and 60°~75°C. In the curve of squid meat (curve IV), above 45°C the phenomenon of dehydration was shown, there being two kinds of heat coagulable proteins which are coagulated within the ranges of 45°~55°C and 60°~80°C; the maximum ratio of dehydration at 80°C was 36 %, which was remarkably larger than that of fish flesh. From the curve of sea-ear meat (curve V), the similar two kinds of heat coagulable proteins which are coagulated in the ranges of 40°~55°C and 60°~70°C are considered to be present, however, their presence is not so clearly shown as in the squid meat, and the increase of the dehydrating ratio continued gradually at temperatures above 70°C. In the curve of dehydration of sea-cucumber (curve VI), a larger ratio of dehydration was shown (about 48 % at 75°C), and the inclination of the curve was very steep; the adsorption of water was not to be observed at near 20°C, showing that the dehydration was gentle until 45°C. Above 45°C, remarkable dehydration was shown, and at near 70°C one step was shown on the curve which was undistinguishable from the curve seen in sea-ear flesh. Above 75°C, dehydration was continuously observed. The presence of two kinds of heat coagulable proteins which coagulate within the ranges of 25°~40°C and 45°~70°C was indistinctly observed.

As above stated, from the curves of dehydration, it is assumed that there are two kinds of heat coagulable proteins. Generally speaking, the fish flesh is less dehydratable

than that of squid and sea-cucumber; it is interesting that the dehydration from the flesh becomes larger with the lowering of the animals in the scale of life. It is also interesting that *Paralithodes camtschatica* shows the medium properties between fish and sea-cucumber, and that there was more remarkable variation of the dehydration and the adsorption than in the other animals.

The difference of the curve of dehydration is perhaps due to the difference of quantity and quality of the muscular protein with the animals, but the authors have assumed that the cause is more largely the histological difference of animal tissue.

According to Tanikawa[14], the characteristic properties of sea-cucumber meat are observed to be (1) the amount of protein is small, (2) the amount of water content is large, and (3) the meat consists mainly from connective tissue forming a net work of collagen fibre.

Therefore, the animal muscle having such characteristics of histological properties contracts in the collagen fibre of the connective tissue with the increase of heating temperature (In sea-cucumber meat, the remarkable heat contraction occurs at 30°~65°C. According to Takahashi[24], the collagen fibre of the connective tissue in shark skin contracts at 37°~55°C), and the dehydration will occur at the same time; this phenomenon will be shown as a steep inclination of the curve. From those considerations, it is assumed that the histological difference of the meat largely affects the curve of the dehydration.

As seen in the previous report of experimental results, II, *Paralithodes camtschatica* has a constitution in which blocks of muscular fibre bundles are surrounded by a comparatively thick layer of gelatinous tissue; this is similar to sea-cucumber meat. In those meats, the phenomenon of dehydration by heat contraction of gelatinous tissue is considered to occur. On the contrary, muscular fibre of *Paralithodes camtschatica* itself apparently is more thick than that of fish flesh. Therefore, the heat coagulation of muscular protein can not be overlooked.

At this time, the chemical components in body fluid contained in *Paralithodes camtschatica* flesh, especially the presence of haemocyanin which is in the crab blood and is heat coagulable, must be considered. According to Kosakabe[8b], haemocyanin in the body fluid of the crab is coagulated at near 70°C. Therefore, at temperatures below 70°C the remaining haemocyanin which has not yot been coagulated will flow out during the heating, but above 70°C haemocyanin is heat-coagulated, and the ratio of the dehydration decreased; then the curve of dehydration will show the phenomenon of adsorption at 70°~85°C as shown in curve I of Fig. 18.

It can be said that the fact that *Paralithodes camtschatica* is situated histologically oetween the fish such as Atka mackerel or *suketo*-cod and sea-cucumbers, may be a cause of the result that the curve of dehydration of *Paralithodes camtschatica* (curve I) was situated between those of Atka mackerel (curve II) or *suketo*-cod (curve III) and

that of sea-cucumber (curve VI).

(3) Relation between the heating temperature and the amount of dissolved components

From the previous experimental results described in (2), it was seen that there are two stages on the dehydrating curve of *Paralithodes camtschatica* flesh according to the difference of the heat coagulabilities of the meat proteins.

The authors undertook to estimate the amount of the dissolved components from which the kinds of proteins or their heat coagulability can be indirectly known.

To 10 *g* of the crushed and homogenized leg flesh of *Paralithodes camtschatica* dist. water was added to make a volume of 100 *cc*, and the mixed solution was shaken for 30 minutes at a definite temperature. After standing for one hour, the upper clear liquor was centrifuged (3000 r.p.m. for 20 minutes). Aliquot volume of the centrifuged liquor was estimated for the determination of the total amounts of nitrogen and of free amino-acids nitrogen.

The results obtained are shown in Fig. 19.

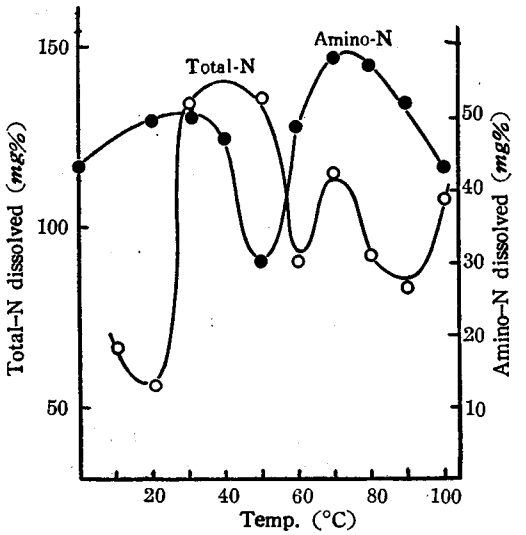

Fig. 19. Relation between the heating temperature and the amount of nitrogen dissolved in distilled water

As seen in Fig. 19, the total amounts of nitrogen and of free amino-acids nitrogen in the dissolved components were maximum at 40° and 70°C. Below and above those temperature, the amounts of the dissolved components decreased. Those temperatures (40° and 70°C) are considered to be those at which the heat coagulation of myosin and myogen in *Paralithodes camtschatica* flesh begin. So it can be considered that within the ranges of 20°~40°C and 60°~70°C where the proteins remain uncoagulated, the amounts of the dissolved components increase but above 40° or 70°C the amounts of the dissolved components decrease with the progress of inactivation of the coagulated proteins. The fact that the maximum amount of dissolved components was seen at 70°C was considered possibly to be caused by the presence of haemocyanin of blood in the body fluid in addition to the presence of myogen in the flesh. The fact that the amount of total-nitrogen of the dissolved componts at 40°C was larger than that at 70°C is perhaps due to the fact that at 40°C myosin only are coagulable; on the contrary, at 70°C myosin in addition to haemocyanin and myogen are together coagulable, so the amount of insoluble nitrogen was

superior in higher temperatures.

(4) The relation between the dehydration and the pH value of heating solution

In this experiment, the crab flesh was boiled in solutions of various pH values, and the change of the dehydrating ratio was estimated. The blocks (about 1 cm^3) of the raw crab flesh were taken from the leg as in the previous experiment.

The blocks were put in 50 *cc* of buffer solutions having various values of pH, such as 3.2, 4.8, 6.5 and 8.5 at 60°C (pH values were adjusted by means of N/10 HCl and N/10 NaOH solutions), and heated for a definite time (5~20 minutes); the ratio of the dehydration was estimated. The results obtained are shown in Fig. 20.

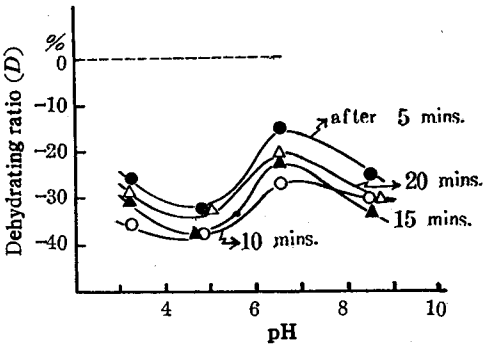

Fig. 20. Relation between the dehydration of *Paralithodes camtschatica* flesh and the pH value of heating solution

As seen in Fig. 20, the dehydrating ratio showed maximum at pH 4.8, and showed the decreases at pH 3.2 and 6.5. At pH 8.5 the dehydration increased again. The fact that the maximum dehydration was at pH 4.8, is interpreted to be due to the fact that the isoelectric reaction of *Paralithodes camtschatica* flesh is at pH 4.5~5.0, at which the flesh protein coagulates, the hydrating affinity decreases and dehydration occurs easily.

The increase of the dehydration on the alkali side is perhaps owing to the dissolving of the soluble component. On the contrary, the decrease of dehydration at pH 3.2 is perhaps because the acid swelling of the flesh protein acts antagonistically against dehydration.

2. Heat coagulation of flesh protein of *Paralithodes camtschatica*

In this experiment, the heat coagulation of water-soluble protein and of 0.5 Mol NaCl-soluble protein of *Paralithodes camtschatica* flesh was examined by the same method as described in Tanikawa's study[14].

The results obtained are shown in Fig. 21.

As seen in Fig. 21, in water-soluble protein solution, the amount of the proteins coagulated at 60°~75°C was remarkably increased, so the presence of water-soluble protein, *i. e.*, myogen was assumed. In this case, haemocyanin of blood in the body fluid is considerd to play a part in the coagulation. However haemocyanin is coagulated at near 70°C, not yet at 60°C, therefore the phenomenon of the coagulation of myogen is shown to predominate. On the other hand, in 0.5 Mol NaCl solution-soluble protein, it is assumed that there are two kinds of proteins, one is the protein coagulated at 30°~50°C and the other the protein whose coagulation begins at near 60°C. The former is considered to

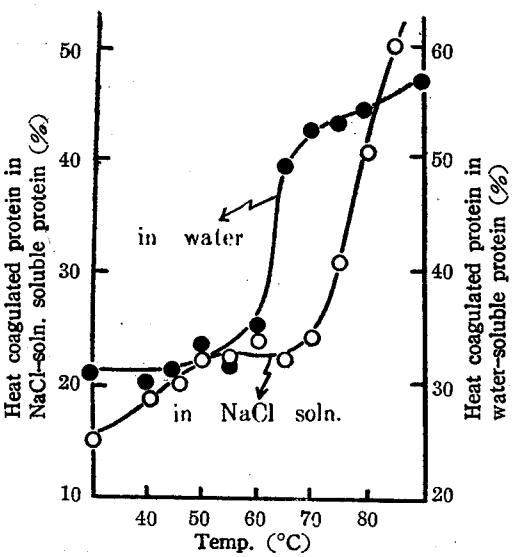

Fig. 21. Heat coagulating curves of flesh proteins of *Paralithodes camtschatica*

correspond to the range of coagulating temperature of the myosin, and the latter is considered to correspond to that of the myogen (at 70°C the presence of haemocyanin takes a part in the coagulation). Those results agreed with the results of Shimidu[25] which were obtained from the 0.1 Mol NaCl solution-soluble proteins in Formosan loach (Kamuruchi, *Ophicephalus argus CONTER*) and in clam meat (*Meretrix meretrix (LINNE)*).

IX. HYDRATION OF *PARALITHODES CAMTSCHATICA* FLESH

From the results obtained in the previous experiment, VIII, the presence of heat coagulable proteins, such as myosin and myogen has been strikingly observed and the dehydrating curve of *Paralithodes camtschatica* resembles that of fish flesh. It was also observed that *Paralithodes camtschatica* flesh has the characteristic of easily dehydration, resembling sea-cucumber meat or squid meat.

In the present experiment, in order to ascertain the hydrating affinity of *Paralithodes camtschatica* flesh, the authors have determined the water content—relative vapour pressure curve of the crab meat.

(1) Experimental method

Three kinds of samles, (1) fresh raw leg meat of *Paralithodes camtschatica*, (2) unfresh raw leg meat (left for 3 days at 5°C), and (3) boiled fresh leg meat (heated for 20 minutes at 100°C), were used for the comparison with each other.

The vapour tension method[26] was used by indirect procedure which estimates the equilibrium pressure by an oil manometer between the vapour pressure (P) of water contained in sample and the vapour pressure (P_o) of pure water (dist. water) maintained at the same temperature as that of the sample. As sample, about 2 g samples of crushed meat of *Paralithodes camtschatica* as above stated, were accurately weighed. Estimations were made at temperature of 20°±1°C. The results obtained are shown as the relative vapour pressure (P/P_o) to the amount of water content, "g", gram of water per gram of the dried matter.

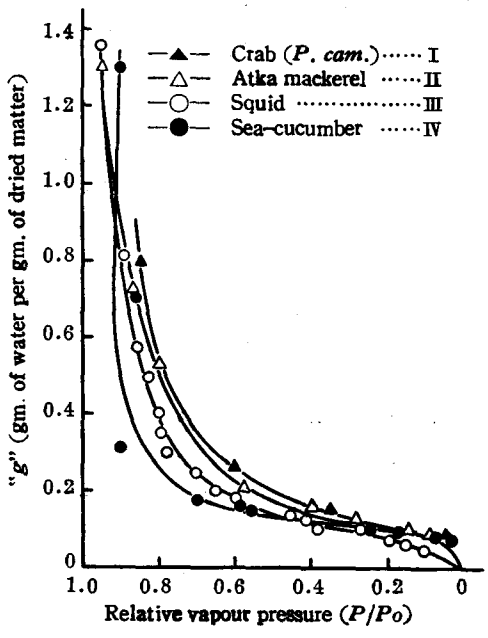

Fig. 22. Comparison of "*g-p/p₀*" curve of *Paralithodes camtschatica* flesh with those of other marine animals

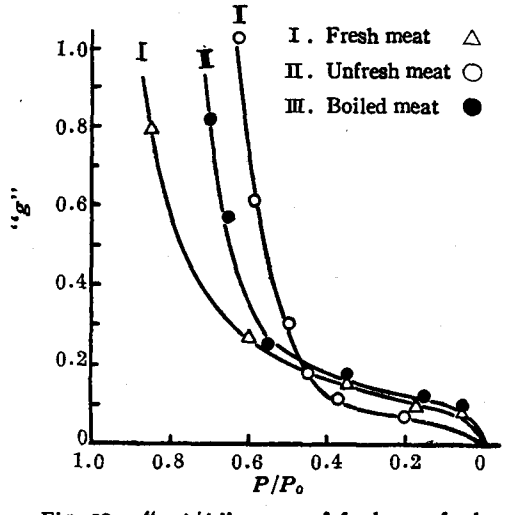

Fig. 23. "*g-p/p₀*" curves of fresh or unfresh meat and of boiled meat of *Paralithodes camtschatica*

(2) Experimental results

Results obtained are shown as curve I in Fig. 22. In order to compare the meat of *Paralithodes camtschatica* with those of other marine animals (Atka mackerel[13], sea-cucumber[14] and squid meat[26]), the results obtained are shown together in Fig. 22. Curve II is shown as "*g*"-P/P_o curve of Atka mackerel meat (20°C), curve III is squid meat (20°C) and curve IV is that of sea-cucumber meat.

Fig. 23 is shown as "g-P/P_o" curve of three kinds of samples of *Paralithodes camtschatcia* which were as above described in order to compare with one another. Curve I in Fig. 23 is the "g-P/P_o" curve of the fresh leg meat, curve II that of unfresh leg meat, and curve III that of boiled meat of the fresh leg meat.

As seen in curve I in Fig. 22, when the meat of *Paralithodes camtschatica* has about 0.3~0.4 *g* of water content, "*g*", (0.3~0.4 of "*g*" corresponds to about 23~29% of the water content for the sample containing water). the value of P/P_o decreases rapidly and shows as an S-type curve which is characteristic for the vapour pressure curve. On the other hand, in the case of sea-cucumber meat, curve IV, when the value of "*g*" is 0.34 *g*, the value of P/P_o is 0.84~0.88.

Comparing the curve "g-P/P_o" of the sea-cucumber with that of *Paralithodes camtschatica*, when the value of "*g*" is the same, the value of P/P_o of the former is larger than that of the latter, and the curve of sea-cucumber meat is situated generally

farthest to the left of curve I of *Paralithodes camtschatica*. On the contrary the curve of *Paralithodes camtschatica* resembles curve II of Atka mackerel. Curve III of squid meat is situated between curve IV of sea-cucumber and curve II of Atka mackerel (or curve I of *Paralithodes camtschatica*).

The water content "*g*"—relative vapour pressure P/P_o" curve shown in Fig. 22 is regarded as a kind of dehydrating isothermal curve. The phenomenon of the mutual slipping of the curves is supposed to be caused by the difference of free energy of water in the sample, that is to say, to the difference of binding energy of water existing in the sample.

According to Lewis and Randall[27], 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 given by the following equation:

$$\Delta\bar{F}=F_o-\bar{F}=-RT\ ln\ P/P_o\equiv -RT\ ln\ a \cdots\cdots\cdots\cdots(2)$$

Here, F_o 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, R is gas constant, and "*a*" is water-activity of water in the sample.

As seen in equation (2), when two kinds of sample are estimated at the same temperature in the two curves of "g-P/P_o", the value of $\Delta\bar{F}$ decreases in the sample showing a small value of "P/P_o" at the same amount of water-content "*g*", and the water in the sample takes on the properties of pure water, that is to say, it comes to have the properties of free water having weak binding strength. Therefore, in Fig. 22 the values of P/P_o, that is the water-activity, "*a*", of water in the sample, corresponding to the same amount of water-content, "*g*", rank with those of crab, Atka mackerel, squid and sea-cucumber meat in order of larger values.

So the values of $\Delta\bar{F}$ of those marine animal meats are assumed to become contrarily smaller in the same order, and the hydrating affinity also shows decrease in that order. In this case, curve IV of sea-cucumber meat was obtained at 18°C, but with the rising of the estimating temperature, the curve slipped as a whole to the left (the value of P/P_o increases in comparison with the same amount of water-content "*g*"), so even if the estimation was made at 20°C as in other samples, the order as above described will be unchanged.

According to Higashi[28] *et al.* or Akiba[29], water having the value of P/P_o which is below 0.7, may be defined as a molecular theoretical bound water from the point of view of protein chemistry (the value of P/P_o of the bound water defined from the colloidal point of view is 0.8~0.9, and bound water estimated by cobaltous chloride method by Hatschek[30] or Ōyagi[31] is in this range of values of P/P_o); therefore the amount of molecular theoretical bound water may be considered to become progressively smaller in the meat of crab, Atka mackerel, squid and sea-cucumber in that order.

That is to say, sea-cucumber being rich in the gelatinous tissue and having property

of watery meat can be said to contain water having weak hydrating affinity and free state. On the other hand, *Paralithodes camtschatica* or Atka mackerel meat can be said to have water which is restrained from the freedom, added to the hydrating affinity of bound water of the meat protein itself. Squid meat can be said to have water of which the property is between the water of sea-cucumber and that of crab meat.

From the results in this experiment, *Paralithodes camtschatica* flesh has the same amount of bound water as other fish flesh, and the hydrating affinity of the water is also the same as that of fish flesh.

As seen in Fig. 23, in which the curves of "g-P/P_o" of fresh flesh of *Paralithodes camtschatica*, unfresh flesh and boiled flesh are shown in their mutual relation, curve I of fresh flesh crossed with curve II of unfresh flesh at 0.45 value of P/P_o, (at 0.18 value of "g") and in higher range above 0.45 value of P/P_o, the value of P/P_o of the unfresh flesh corresponding to the same amount of water-content "g" shows smaller than that of fresh flesh. This may be due to the formation of volatile basic substances with the falling of the freshness of the crab flesh (the amount of volatile basic nitrogen in the sample was about 25 *mg%*), so the true vapour pressure can not be considered to have been estimated. But it is supposed that the hydrating affinity of the meat protein becomes the most important factor by introduction of water into the range of the molecular theoretical bound water, when the value of water-content "g" is below 0.2, at which the value of P/P_o decreases rapidly.

Therefore, the fact that below 0.45 of P/P_o, the value of P/P_o corresponding to the same amount of water-content "g" of unfresh flesh is larger than that of fresh sample, means the increase of water-activity of water in unfresh flesh, and the value of $\Delta\bar{F}$ in equation (2) becomes small, so the flesh is considered abundantly to contain water which possesses much freedom. That is to say, resultant from the falling of the freshness of the flesh, the hydrating affinty of the flesh becomes weak, and decrease of the amount of molecular theoretical bound water is considered to occur.

On the other hand, in the case of curve III which is for the boiled flesh of the crab, and which slipped as whole to the right side of the curve of fresh flesh (slipped to the small side of the values of P/P_o), as the flesh protein becomes denatured coagulated protein by heating, it is considered that the flesh is caused to contain water as a result of a different mechanism from the raw flesh. But, as for the hydrating affinity, the value of P/P_o of the boiled flesh becomes small and the value of $\Delta\bar{F}$ become large at the same water content "g", therefore the boiled flesh protein is considered to contain water more certainly than raw flesh.

DISCUSSION AND SUMMARY

Canned crab is one of the important exports of Japan. Fundamental studies on the

processing of canned *Erimacrus isenbeckii*, a commercially feasible production of crabs, were carried out in the past[32]. Here, as fundamental studies on the processing of canned *Paralithodes camtschatica*, another kind of commercial production of crabs, the properties of the crab flesh have been studied and reported from the chemical and physico-chemical points of view.

The results obtained are summarized with addition of some supplementary interpretation as follows.

(1) It is remarkable that *Paralithodes camtschatica* flesh has larger amount of water-content and less of crude fat than that of fish flesh, but thre is no difference of the protein-content between the two sorts of flesh. The change in the chemical components before and after the peeling of the crab was observable in the result that the hard crab is more rich in the amounts of non-protein nitrogen and ash than the soft shell crab. As for the difference of the chemical components by parts of the crab body, the leg flesh is more rich in pure protein and crude fat contents and less so in non-protein nitrogen than the shoulder flesh.

(2) From the histological ovservation of leg flesh of *Paralithodes camtschatica*, in the flesh, the blocks of muscular fibre bundles are, respectively, surrounded by a thick layer of gelatinous tissue. This is different from the fish flesh, in which the muscular fibre bundles are distributed closely and uniformly throughout the gelatinous tissue. Therefore, when the crab flesh is soaked in water, and the blocks are deformed, the blocks show the characteristic of becoming easily separated.

(3) Upon examination of the solubility of *Paralithodes camtschatica* flsh protein, the following results were obtained.

(i) The greater part of the total amount of nitrogen was extractive nitrogen in water-soluble state. The amount of dissolved nitrogen of *Paralithodes camtschatica* flesh is larger than that of fish flesh. One of the causes of the fact may be the histological characteristics of *Paralithodes camtschatica* flesh as described above. (ii) As to the solubility of the nitrogenous compounds in the flesh by NaCl solution, the maximum solubility was shown at 0.7 Mol NaCl solution. This solubility is larger than that by dist. water. The difference of the solubility is considerd to show the presence of NaCl solution-soluble protein. The solubility of the protein of the boiled flesh decreases during processing of the heat coagulation of the protein. (iii) Although the amounts of the dissolved nitrogen are different according to kind of acidic or alkali concentrations, the amounts are largely affected by the hydrogen ion concentration. By HCl or H_2SO_4 solutions the flesh is acid coagulated, and the amount of the dissolved nitrogen decreases. On the contrary, by an alkali solution, such as NaOH solution, a remarkable amount of the nitrogen is dissolved.

(4) The minimum amount of dissolved nitrogen from *Paralithodes camtschatica* was shown at near pH 4.5. The maximum amount of coagulated protein was shown at pH

5.0 in the water-soluble protein and at 4.5 and 5.1 in 0.5 Mol NaCl solution soluble protein. From those results the isoelectric point of the proteins of *Paralithodes camtschatica* was concluded to face within the range of pH 4.5～5.1.

(5) As 0.5 Mol NaCl solution-soluble protein was considered to have two components on the basis of the isoelectric reaction, the presence of two components was confirmed by electrophoresis.

(6) The water-extractive solution from *Paralithodes camtschatia* flesh did not show streaming birefringence, on the contrary, the extractive solution obtained with Weber's solution (0.6 Mol KCl) showed clearly streaming birefringence. As to the streaming birefringence, the *Paralithodes camtschatica* flesh resembles fish flesh, and does not show the characteristic of squid meat from which the dissolution of myosins (including actomyosin) into water extract is observed. Therefore, myosins in *Paralithodes camtschatica* flesh protein were considered to dissolve out into salt solution, but not to disolve out into water-soluble fraction.

(7) *Paralithodes camtschatica* flesh shows the phenomenon of swelling in various salt solutions and in acid or alkali solutions.

(i) The flesh showed two peaks in each swelling curve at about 0.2 Mol and 0.8～1.0 Mol in the monovalent salts solutions, such as NaCl, KCl, NH_4Cl or in alkali Na-salts solutions, such as Na_3PO_4, $Na_2B_4O_7$ and Na_2CO_3. The fact that in the swelling curve of *Paralithodes camtschatica* flesh, a peak is shown at lower concentration at near 0.2 Mol, may be due to the holding affinity of water of the galatinous tissue surrounding the blocks of muscular fibre bundles. The swelling phenomenon at higher concentration, at near 0.8～1.0 Mol, may be due to the hydrating affinity of muscular fibre protein itself. (ii) The minimum swelling of *Paralithodes camtschatica* flesh was shown at pH 5, which is within the isoelectric range. (iii) In the acidic side below pH 4 and alkali side above pH 9, remarkable acid swelling or alkali swelling was observed.

(8) According to results obtained in examining heat coagulation of *Paralithodes camtschatica* flesh, when the crab flesh was cooked, the dehydrating phenomena accompanied by heat coagulation were observed.

(i) In the dehydrating curves, there were observed two stages at the ranges of 40°～65°C and 80°～85°C. From those results the presence of two kinds of heat coagulable proteins was assumed; the coagulating temperature of the former corresponds to that of the myosin, and that of the latter corresponds to that of the myogen. (ii) *Paralithodes camtschatica* flesh is dehydrated more easily than that of fish. As one descends in the scale of animal life, viz., fish, mollusca, crustacea and echinoderma, he finds that the amount of gelatinous tissue becomes aboundant in the flesh and more easy to dehydrate. (iii) The larger amounts of protein-nitrogen and amino-nitrogen were dissolved at 40° and 70°C, and the maximum ratio of dehydration was shown at pH 4.8, which is the isoelectric point of *Paralithodes*

camtschatica flesh. (iv) In the water-extract of *Paralithodes camtschatica* flesh, there was a protein heat-coagulated at 60°~75°C, and in 0.5 Mol NaCl extracted solution, there were two kinds of protein heat-coagulated at 30°~50°C and at near 60°C. (v) The heat coagulated protein in the water-extract is considered to correspond to myogen and to haemocyanin; in 0.5 Mol NaCl-extracted solution the proteins coagulated at lower temperature are considered to correspond to the myosin and that at higher temperature is considered to correspond mainly to the myogen.

Those results obtained agree with the results obtained from the contraction of muscular fibre of *Paralithodes camtschatica* flesh. The deteiled description will be presented in the next paper.

(9) The hydrating affinity of *Paralithodes camtschatica* flesh was examined by the estimation of the curve of water content, "g",—relative vapour pressure, P/P_o. According to the results obtained, the hydrating affinity of the fresh crab flesh resembles that of fish flesh. With the falling of the freshness of the crab flesh, the hydrating affinity weakens and the amount of water in free state increases. The hydrating affinity of boiled crab flesh is larger than that of raw flesh. But, the hydrating mechanism of the boiled crab flesh which was denatured by the heating is considered to be different from that of raw flesh.

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