THE CHEMICAL STUDIES ON THE DENATURATION OF PROTEINS
(Second report)

BY

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Since any manipulation in the production of food and industrial materials is always accompanied by the denaturation of proteins investigations into and study of these matters by experiments are very important as outlined in this treatise. This report is an extension of the first report by the author and K. YOSHIMURA entitled "Studies on the Denaturation of Proteins" (2). All the experiments described in the present report were conducted using the same method as before but with different samples; salmon being used as described in this paper: Eight salmon (four male and four female) of the same age were caught in the *Ishikari* river in the autumn of 1927 prepared and named as follows: The first two were preserved in an ice chamber and are called in this treatise "Original one", the second pair were frozen three times and called "Frozen one", the third pair were salted for six months and called "Salted one" and the last pair were smoked in the ordinary way and called "Smoked one". From each of these samples half male and female in content the muscle freed from bone and skin was mixed and used for analysis and in preparation of the proteins.

The references already stated in the first report have been omitted this time and here only new ones are given.

I. Short review of the experiments described in the first report

(A) The denaturated rice oryzenin:—

(1) The ash and sulphur content of boiled oryzenin is less than that of the original while that of the frozen one is just the opposite.

(2) In the oryzenin alkali solution, to obtain the maximum turbidity and surface tension of denaturated oryzenin a somewhat smaller quantity of acid is needed.

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(3) The specific rotatory power of boiled and frozen oryzenin alkali solution is higher than that of the original while the decomposition by illumination of ultraviolet ray is lower than in the original.

(4) The free amino acid content of denaturated oryzenin is greater than that of original oryzenin.

(5) The amide and the histidin nitrogen content of boiled and frozen oryzenin are superior to that of the original, while the lysin nitrogen content is just the opposite.

(B) The denaturated glycinin of soy-beans:—

(1) The phosphorus content of boiled and frozen- glycinin is less than that of the original while that of superheated-, gasoline- and benzin-glycinin is superior to that of the original-glycinin. On the contrary, the sulphur content of boiled- and superheated-glycinin is superior to that of the original while that of the others is less than that of the original.

(2) The content of free amino nitrogen of all denaturated protein is superior to that of the original and especially so in the case of superheated-, gasoline- and benzin-glycinin.

(3) In all denaturated glycinin, there is a predominance of amide, arginin and histidin nitrogen as compared with the case of original-glycinin. Among five kinds of denaturated glycinin, the frozen-glycinin is richest in arginin nitrogen content, while gasoline- and benzin-glycinin are rich in histidin nitrogen boiled or superheated cases show decreased arginin nitrogen and increased histidin nitrogen content.

(4) In glycinin alkali solution, to obtain the maximum turbidity of the boiled-glycinin a somewhat smaller quantity of acid is needed while the other denaturated glycinin showed no marked difference as compared with the original.

(5) The solubility of denaturated glycinin in NaOH and Na_2CO_3 solution decreased in the following order viz. Frozen- gl.→Boiled- gl.→Benzin- gl.→Gasoline- gl.→Superheated- gl. Almost all denaturated glycinin except Frozen-gl. showed their maximum solubility in 1/10–1/2.5 normality. The solubility of denaturated glycinin in acid solution is decreased in the following order viz. Frozen- gl.→Boiled- gl.→Benzin- gl.→Gasoline- gl.→Superheated→gl. The solubility of denaturated glycinin in salt solution is decreased in the following order viz. Frozen- gl.→Boiled- gl.→Benzin- gl.→Gasolin- gl.→Superheated- gl.

(C) The denaturated soy-bean:—

The heated soy-bean showed a decreased water and NaCl solution soluble nitrogen content and an increased NaOH soluble and insoluble

nitrogen content, in comparison with the original. This change observed in heating over 60°C and also in the case of heating over 110°C, showed that the insoluble nitrogen content was increased to a remarkable degree. In the case of frozen, water and NaCl solution soluble nitrogen content was decreased but increased in case of NaOH solution soluble nitrogen but not so in the case of insoluble nitrogen. In the case of boiling water and NaCl solution soluble nitrogen content was decreased and NaOH solution soluble and insoluble nitrogen increased. By the treatment with gasoline and benzin, the same changes were observed but not to a remarkable degree.

II. Water, ash, fat and nitrogen content of denaturated salmon muscle

The water, ash, fat and nitrogen content were determined by the ordinary way and the following results obtained.

TABLE I

Constits. / Samples	Water %	% in dry matter					
		Ash %	Ratio	Fat %	Ratio	Total N %	Ratio
Original one	64.787	3.606	100	6.674	100	10.661	100
Frozen one	66.307	3.828	106	7.485	112	10.534	99
Salted one	50.423	30.759	855	8.754	131	8.449	79
Smoked one	43.775	16.932	469	12.729	191	8.949	74

The ash and fat content of frozen, salted and smoked muscle is greater than that of the original, while their nitrogen content is just the opposite. The increase of ash in case of salted one is caused by addition of NaCl and the same inclination of ash content of smoked and frozen ones would be caused by translocation of mineral substances from body juice to muscle tissues by denaturation process. The increase of fat content in case of salted and smoked ones would be caused by the movement from fatty tissues to muscle by denaturation process. Therefore nitrogen percent of muscle would naturally be decreased.

III. Estimation of myosin, myogen and other soluble nitrogen

By the denaturation of muscle, its myosin, myogen and other soluble nitrogen content would be changed and it is probable an inter change between myosin, myogen and insoluble protein might take place by decreasing their solubility. Therefore we undertook the following experiment to determine the nitrogen distribution in denaturated salmon muscle.

Five g. of muscle from a definite part of the back was ground with quartz sand in a mortar and extracted with 100 ccm of 0.6 % NaCl, shaken with a shaking machine for 30 minutes. After 24 hrs., the mixture was centrifuged, filtered the upper liquid and 10 ccm of the clear filtrate was used to estimate total nitrogen (A). When the remaining filtrate was treated 3 minutes at 51° C in a water bath, myosin was precipitated and 10 ccm of its filtrate was used to estimate nitrogen (B). Further, its remaining filtrate was kept at 65° C for 3 minutes and myogen was precipitated and 10 ccm of its filtrate was used to estimate nitrogen (C). Therefore A=Soluble total nitrogen, A—B=Nitrogen of myosin form, B-C =N: trogen of myogen form and C=nitrogen of nonproteid, were produced.

TABLE II

Distr. of N	A		A—B		B—C		C	
Samples	Dry matter %	Total N %	Dry matter %	Total N %	Dry matter %	Total N %	Dry matt %	Total N %
Original one	7.764	40.018	4.290	25.430	2.043	12.110	1.430	8.476
Frozen one	7.870	45.874	4.164	24.843	2.135	12.743	1.238	7.392
Salted one	2.859	18.520	0.408	2.645	0.218	1.410	2.203	14.206
Smoked one	2.882	18.298	0.360	2.287	0.240	1.524	2.281	14.486

According to the table, the quantity of soluble nitrogen (0.6 % NaCl sol.) in case of salted and smoked ones is decreased markedly and corresponds to 40 % of original one. The quantity of myosin and myogen form nitrogen of salted and smoked is decreased also markedly and corresponds to only 10 % of original. On the contrary, the quantity of insoluble and nonproteid nitrogen is increased to twice that of original one but in case of frozen one, these changes are very small.

IV Preparation of myosin, myosinfibrine, myogen and muscle fiber

Salmon muscle which were freed from skin and bone were ground in a mortar, mixed with the same volume of 0.6 % NaCl solution, shaken vigorously and extracted for 24 hrs. in an ice chamber. This mixture was filtered with a linen cloth and filtered again through a paper pulp to obtain a clear liquid. The filtrate was mixed with same volume of saturated ammonium sulphate solution and after 10–15 hrs. myosin was precipitated. It is then filtered, the precipitate was dissolved in NaOH solution, filtered through paper pulp and reprecipitated with 1/10–1/5 normal HCl. The precipitate was washed 3 times with water, dialyzed for many days in a water current and after being freed from salt, the myosin was washed 3 times with 90 % alcohol and 3 times with absolute alcohol and ether using a centrifugal machine and dried in a H_2SO_4 desiccator.

For preparation of myosinfibrine, the above filtrate was warmed 51°C for 3–4 minutes precipitating myosinfibrine and treated the same as myosin.

Next myogen was precipitated completely from the above filtrate adding powdered ammonium sulphate until the solution formed a precipitate. After standing 10–15 hrs. myogen was precipitated and separated, dialyzed, and dried same as above samples.

At last the residual musclefibers viz, sarkolemm was freed from NaCl solution soluble substances, washed with water until the liquid showed no chlorine reaction, pressed, freed from pieces of bone and skin and washed with alcohol and ether and dried as mentioned above.

V. Physico-chemical properties of muscle fibers (sarkolemm)

(A) Water, ash, sulphur and phosphorus content.

Water and ash content was estimated by ordinary method, sulphur by DENIS-BENEDICT's method and phosphorus by JVERSEN's method (1) and the following results obtained.

TABLE III

Const. / Samples	Water %	Ash %	Ratio	Sulphur %	Ratio	Phosphorus %	Ratio
Original one	17.971	0.682	100	0.947	100	0.146	100
Frozen one	17.418	0.460	67	0.891	94	0.104	71
Salted one	9.275	0.209	30	0.935	98	0.081	55
Smoked one	10.407	0.123	18	0.914	96	0.079	54

According to the above table, denaturated muscle fibers showed no difference in its sulphur content comparing with original one, while ash and phosphorus content were decreased always by change of its chemical structure from original one.

(B) Maximum point of surface tension and of the turbidity of the protein alkali solution in titration with HCl solution

Two tenths g. of each muscle fiber protein was dissolved in 50 ccm of 1/5 normal NaOH solution. After standing 24 hrs. at room temperature 5ccm of the protein solution was titrated with 1/5 normal HCl solution at 18°C. The maximum point of the surface tension and of the turbidity was estimated by NOUY's apparatus and DUBOSCQ's nephelometer respectively.

TABLE IV

Surface tension								
ccm of HCl / Samples	4.950	5.000	5.025	5.050	5.075	5.100	5.150	5.200
Original one	57.8	56.4	—	56.1	—	58.4	55.7	5.20
Frozen one	57.8	56.4	—	57.4	—	56.4	54.4	52.0
Salted one	58.4	57.4	56.0	56.4	—	54.4	52.3	52.0
Smoked one	57.2	56.4	—	57.0	57.4	57.8	55.0	53.2
Turbidity								
Original one	++	+++	—	+++	—	++++	++++	+++
Frozen one	++	+++	—	++++	—	++++	++	+++
Salted one	++	+++	++++	++++	—	+++	+	+
Smoked one	++	++	—	++++	+++	++++	+++	+

From the results of the above table, the different maximum point of precipitation and surface tension of frozen and salted musclefiber protein solution from original one indicates different iso-electric points.

(C) Determination of pH value which is produced by musclefiber in neutral salt solution

The frequently noticed development of acidity in neutral salt solution in the presence of the material from which no detectable quantity of acid can be extracted by pure water. In the experiment, Na_2SO_4 (KAHLBAUM's best) was recrystallized twice from pure distilled water of pH 6.9 and dried in a H_2SO_4 desiccator. The salt was dissolved in pure distilled water (pH 6.9) and 1/2, 1/5, 1/10 and 1/20 normal solutions were prepared. 0.5 g of water free protein was mixed with 10 ccm of salt solution, shaken and settled 24 hrs. For the estimation of pH value the mixture was treated with a potenttiometer of CAMBRIDGE system using platinum quinhydrone electrode and the following results were obtained.

TABLE V

Normality of HCl Samples (pH value)	1 norm.	1/2 norm.	1/5 norm.	1/10 norm.	1/20 norm.	Average
Original one	6.71	6.74	6.79	6.79	6.82	6.77
Frozen one	6.45	6.48	6.55	6.56	6.61	6.51
Salted one	7.05	6.97	6.94	7.01	7.00	6.99
Smoked one	6.66	6.64	6.62	6.57	6.52	6.60

The table shows that all denaturated protein solution developes greater acidity than the original except the salted one.

(D) Separation and determination of amino acids

Differences of the amino acids of different proteins both in quantity and kind were determined by VAN-SLYKE's advanced method which was described in the first report. The following results were obtained.

TABLE VI

	Total N.	Ammonia N.	Melanin N.	Monoamino N	Diamino N.	Arginin N.	Cystin N.	Histidin N.	Lysin N.
Dry matter %									
Original one	16.872	0.9671	0.1750	9.9693	5.7615	2.7161	0.2633	1.6706	1.1115
Frozen one	16.758	1.0219	0.1941	10.2284	5.3136	2.2067	0.2579	1.4762	1.3728
Salted one	15.439	0.5857	0.1751	9.6562	5.0225	2.1579	0.2645	0.8808	1.2319
Smoked one	15.748	0.5934	0.1908	10.0456	4.5190	2.1098	0.2478	0.6612	1.9002
Total nitrogen %									
Original one	100.0	5.7317	1.0372	59.0847	34.1465	16.3671	1.5605	9.9011	6.5875
Frozen one	100.0	6.0980	1.1582	61.0357	31.7078	13.1680	1.5390	8.8089	8.1919
Salted one	100.0	3.4824	1.1241	62.5414	32.5298	13.9763	1.7131	5.7048	7.9788
Smoked one	100.0	3.7679	1.2115	63.7864	31.2341	13.3966	1.5735	4.1984	12.0557

According to above table, frozen, salted and smoked musclefiber proteins showed a decreae of ammonia nitrogen, of diamino nitrogen, especially of arginin and histidin nitrogen content and an increase of monoamino nitrogen content comparing with original.

(E) Determination of free amino nitrogen of musclefiber proteins

The free amino nitrogen contents of the following proteins were determined by SÖRENSEN's method which procedure has already been described in the first report. Two tenth g. of protein was dissolved in 40 ccm of 1/5 normal NaOH solution and the experiment was carried out after 48 hours.

TABLE VII

Samples	Original one	Frozen one	Salted one	Smoked one
N/10 ccm of HCl	0.84	1.01	1.00	0.96
% in dry matter	2.352	2.800	2.800	2.685
% in total nitrogen	13.9004	18.1312	17.0489	17.0489
Ratio	100.00	122.58	130.48	122.72

From the above results the free amino nitrogen content of all denaturated proteins showed a greater quantity than the original one.

VI. Physico-chemical properties of myosin and myogen

(A) Water, ash and nitrogen content

Water, ash and nitrogen content were determined by ordinary method and the following results obtained. The numbers are percentage of dry matter except water.

TABLE VIII

Samples / Constit.	Myosin				Myogen			
	Original	Frozen	Salted	Smoked	Original	Frozen	Salted	Smoked
Water	8.6775	6.3086	10.2535	7.4715	9.0878	7.0762	9.1365	9.6078
Ash	0.7665	0.7471	0.8914	0.9726	0.6600	0.1291	0.6604	0.7744
Nitrogen	15.7084	15.7794	15.6078	16.4153	16.0773	15.9836	15.9745	16.4307

	Water		Ash		Nitrogen	
	Original	Frozen	Original	Frozen	Original	Frozen
Myosinfibrine	9.4936	7.0717	0.8839	0.3228	16.2235	15.9821

(B) Maximum point of surface tension and the turbidity of the protein alkali solution in titration with HCl solution

Two tenth g of each sample was dissolved in 20 ccm of 1/25 normal NaOH solution. After standing 24 hours at room temperature, 4 ccm of the protein solution was diluted with 4 ccm of purest water and titrated with 1/125 normal HCl solution at 20°C. The maximum point of the surface tension and of the turbidity was estimated by NOUY's apparatus and DUBOSCQ's nephelometer respectively.

TABLE IX

Surface tension

HCl ccm	Myosin				Myosinfibrine		Myogen			
	Original	Frozen	Salted	Smoked	Original	Frozen	Original	Frozen	Salted	Smoked
3.000	—	—	—	—	—	—	—	—	—	—
3.100	—	—	—	—	—	—	—	—	—	—
3.200	—	—	—	—	—	—	—	—	—	—
3.250	—	—	—	—	—	—	62.9	—	—	—
3.300	—	—	—	—	—	—	62.5	63.2	—	—
3.325	—	—	—	—	—	—	61.8	0	—	—
3.350	—	—	—	—	—	—	62.1	62.5	—	—
3.400	—	—	—	—	64.3	—	61.2	61.5	—	—
3.425	—	—	—	—	0	—	60.8	0	—	—
3.450	—	—	—	—	63.2	—	60.5	62.1	—	—
3.475	—	—	—	—	62.9	—	60.1	0	—	—
3.500	63.2	—	—	—	63.2	—	60.8	61.5	—	—
3.550	62.9	—	—	—	63.9	—	—	60.8	—	—
3.575	0	—	—	—	62.9	—	—	60.1	—	—
3.600	0	—	—	—	61.8	—	—	60.1	—	—
3.650	61.2	—	—	—	—	—	—	0	—	—
3.700	0	—	—	—	—	—	—	59.5	—	—
3.750	61.8	—	—	—	—	—	—	0	—	—
3.800	62.6	—	—	—	—	—	—	59.1	—	—
3.850	60.5	—	—	—	—	—	—	0	—	—
3.900	59.8	63.6	—	—	—	—	—	58.5	—	—
3.950	—	63.2	—	—	—	—	—	—	—	—
4.000	—	62.5	—	—	—	—	—	—	—	—
4.025	—	62.2	—	—	—	—	—	—	—	—
4.050	—	61.8	—	—	—	—	—	—	—	—
4.075	—	60.8	—	—	—	—	—	—	—	—
4.100	—	62.5	—	—	—	—	—	—	—	—
4.150	—	63.0	59.8	—	—	—	—	—	—	—
4.200	—	61.8	0	—	—	—	—	—	63.2	—
4.250	—	61.9	60.5	—	—	61.9	—	—	0	—
4.300	—	61.5	61.2	—	—	62.2	—	—	0	—
4.325	—	0	0	—	—	61.2	—	—	0	—
4.350	—	0	0	—	—	60.5	—	—	0	—
4.375	—	0	0	—	—	62.2	—	—	0	—
4.400	—	60.8	61.9	58.8	—	61.5	—	—	62.2	—
4.425	—	—	0	0	—	61.2	—	—	0	—

HCl ccm	Myosin				Myosin-fibrin		Myogen			
	Original	Frozen	Salted	Smoked	Original	Frozen	Original	Frozen	Salted	Smoked
4.450	—	—	61.2	0	—	60.8	—	—	61.0	—
4.500	—	—	60.8	59.1	—	60.5	—	—	61.5	—
4.550	—	—	0	59.8	—	—	—	—	61.2	—
4.600	—	—	60.5	59.8	—	—	—	—	62.9	—
4.650	—	—	0	60.8	—	—	—	—	61.5	—
4.700	—	—	60.1	59.8	—	—	—	—	60.5	62.2
4.750	—	—	—	59.4	—	—	—	—	—	0
4.800	—	—	—	—	—	—	—	—	—	0
4.850	—	—	—	—	—	—	—	—	—	60.5
4.900	—	—	—	—	—	—	—	—	—	60.1
4.950	—	—	—	—	—	—	—	—	—	60.1
5.000	—	—	—	—	—	—	—	—	—	61.2
5.050	—	—	—	—	—	—	—	—	—	62.2
5.100	—	—	—	—	—	—	—	—	—	60.5
5.150	—	—	—	—	—	—	—	—	—	60.1
5.200	—	—	—	—	—	—	—	—	—	59.8
Turbidity										
3.000	—	—	—	—	—	—	—	—	—	—
3.100	—	—	—	—	—	—	—	—	—	—
3.200	—	—	—	—	—	—	—	—	—	—
3.250	—	—	—	—	—	—	+++	—	—	—
3.300	—	—	—	—	—	—	+++	++	—	—
3.325	—	—	—	—	—	—	+++	++	—	—
3.350	—	—	—	—	—	—	++++	++	—	—
3.400	—	—	—	—	+++	—	+++	+++	—	—
3.425	—	—	—	—	O	—	+++	O	—	—
3.450	—	—	—	—	+++	—	+++	++++	—	—
3.475	—	—	—	—	+++	—	++	O	—	—
3.500	+	—	—	—	+++	—	++	+++	—	—
3.550	+	—	—	—	++++	—	O	+++	—	—
3.575	+	—	—	—	+++	—	O	+++	—	—
3.600	+	—	—	—	—	—	++	+++	—	—
3.650	++	—	—	—	—	—	++	O	—	—
3.700	O	—	—	—	—	—	—	++	—	—
3.750	+++	—	—	—	—	—	—	++	—	—
3.800	++++	—	—	—	—	—	—	++	—	—
3.850	+++	—	—	—	—	—	—	—	—	—
3.900	+++	++	—	—	—	—	—	—	—	—

HCl ccm	Myosin				Myosinfibrine		Myogen			
	Original	Frozen	Salted	Smoked	Original	Frozen	Orignial	Frozen	Salted	Smoked
3.950	+	++	—	—	—	—	—	—	—	—
4.000	—	++	—	—	—	—	—	—	—	—
4.025	—	+++	—	—	—	—	—	—	—	—
4.050	—	+++	—	—	—	—	—	—	—	—
4.075	—	+++	—	—	—	—	—	—	—	—
4.100	—	+++	—	—	—	—	—	—	—	—
4.150	—	++++	—	—	—	—	—	—	—	—
4.200	—	+++	++	—	—	—	—	—	++	—
4.250	—	+++	O	—	—	++	—	—	O	—
4.300	—	++	+++	—	—	++	—	—	O	—
4.325	—	O	O	—	—	++	—	—	O	—
4.350	—	—	O	—	—	+++	—	—	O	—
4.375	—	—	O	—	—	++++	—	—	O	—
4.400	—	—	++++	++	—	+++	—	—	+++	—
4.425	—	—	+++	O	—	+++	—	—	O	—
4.450	—	—	+++	O	—	+++	—	—	+++	—
4.500	—	—	+++	+++	—	++	—	—	+++	—
4.550	—	—	O	+++	—	—	—	—	+++	—
4.600	—	—	++	+++	—	—	—	—	++++	—
4.650	—	—	O	++++	—	—	—	—	+++	—
4.700	—	—	++	+++	—	—	—	—	+++	++
4.750	—	—	O	+++	—	—	—	—	O	O
4.800	—	—	O	+++	—	—	—	—	++	+++
4.850	—	—	O	++	—	—	—	—	—	+++
4.900	—	—	O	++	—	—	—	—	—	+++
4.950	—	—	O	++	—	—	—	—	—	+++
5.000	—	—	+	+	—	—	—	—	—	+++
5.050	—	—	—	—	—	—	—	—	—	++++
5.100	—	—	—	—	—	—	—	—	—	+++
5.150	—	—	—	—	—	—	—	—	—	+++
5.200	—	—	—	—	—	—	—	—	—	++

From the above results the attainment of the maximum point of the surface tension and turbidity of the denaturated myosin and myogen alkali solution always requires a much larger quantity of acid than does that of the original.

(C) The specific rotatory power of myosin and myogen

The rotatory power of an organic compound is due to the presence

of asymmetric carbon atoms in its molecules and it is increased by the complexity of the molecule, by saturation in its structure, and by the special atomic groups. Therefore it is highly important in this investigation to examine the rotatory power of protein alkali solution. One tenth g of each kind of protein was dissolved in 15 ccm of 1/10 normal NaOH solution. After the indicated time, the solution was examined by means of a HAENSCH-SCHMIDT's half shadow polariscope.

TABLE X

	Samples	Myosin				Myosin-fibrine		Myogen			
		Original	Frozen	Salted	Smoked	Original	Frozen	Original	Frozen	Salted	Smoked
After 7 hrs.	Reading	-1.10	-1.44	-1.50	-.157	-1.22	-1.29	-1.43	-1.54	-1.54	-1.77
	Specific rotatory power	-57.09	-74.73	-77.85	-81.48	-63.32	-66.95	-74.21	-78.36	-79.92	-91.86
After 24 hrs.	Reading	-1.59	-1.60	-1.67	-1.78	-1.55	-1.51	-1.76	-1.67	-1.65	-2.38
	Specific rotatory power	-82.00	-83.04	-86.67	-92.38	-80.45	-78.37	-91.34	-86.67	-85.63	-123.52
After 48 hrs.	Reading	-1.31	-1.49	-1.62	-1.63	-1.27	-1.40	-1.60	-1.61	-1.54	-2.21
	Specific rotatory power	-67.98	-77.33	-84.07	-87.19	-65 91	-72.66	-83.06	-83.56	-79.92	-114.69

In the above table, the specific rotatory power of the denaturated myosin and myogen is always greater than that of the original one. The smoked one showed greatest value, next follow the salted one and the frozen one but still greater than the original one.

(D) Separation and determination of amino acids

Differences of the amino acids of different proteins both in quantity and kind were determined by VAN-SLYKE's advanced method (4) which was described in the first report. The following results were obtained.

TABLE XI

	Samples	Ammonia N.	Melanin N.	Monoamino N.	Diamino N.	Arginin N.	Cystin N.	Histidin N.	Lysin N.
	(Amino acids % in dry matter)								
Myosin	Original one	0.7115	0.1109	10.8039	5.0321	2.0698	0.0788	1.9784	0.9551
	Frozen one	0.5764	0.0811	10.2465	4.8754	2.0896	0.1121	1.4552	1.2185
	Salted one	0.9219	0.1041	8.9404	5.6414	2.4170	0.1304	2.0952	0.9988
	Smoked one	0.7660	0.1095	10.2504	5.2894	2.3346	0.1102	1.5944	1.2500
Myosin-fibrine	Original one	0.6340	0.0893	10.0926	5.4076	1.8647	0.1192	1.9023	1.5213
	Frozen one	0.4450	0.1090	10.2521	5.1760	2.0340	0.1969	1.6371	1.3080
Myogen	Original one	0.7054	0.0928	9.8959	5.3832	1.8565	0.1617	1.8005	1.5942
	Frozen one	0.5489	0.0726	10.6397	4.7224	1.7441	0.1937	1.5024	1.2822
	Salted one	0.5648	0.1299	9.8930	5.3868	2.0799	0.1198	2.2649	1.2189
	Smoked one	0.5975	0.0934	10.5139	5.2259	2.0911	0.1029	1.6911	1.3405
	(% in total nitrogen)								
Myosin	Original one	4.5294	0.7060	62.4118	32.3528	13.1764	0.5016	12.5945	6.0802
	Frozen one	3.6286	0.5140	64.9359	30.8972	13.2458	0.7104	9.2222	7.7221
	Salted one	5.9067	0.6670	57.2816	36.1447	15.4858	0.8355	13.4241	6.3994
	Smoked one	4.6664	0.6671	62.4442	32.3528	14.2221	0.6713	9.7129	7.6148
Myosin-fibrine	Original one	3.9079	0.5504	62.2098	33.3319	11.4901	0.7354	11.7256	9.3771
	Frozen one	2.7844	0.6820	64.1474	32.3862	12.7267	1.2320	10.2432	8.1842
Myogen	Original one	4.3876	0.5772	61.5520	32.4832	11.5473	1.0058	11.1990	9.9158
	Frozen one	3.4341	0.4542	66.5664	29.5453	10.9118	1.2119	9.3996	8.0220
	Salted one	3.5356	0.8132	61.9300	33.7212	13.0201	0.7499	14.1782	7.6303
	Smoked one	3.6363	0.5684	63.9894	31.8057	12.7268	0.6263	10.2923	8.1585

According to the table, denaturated myosin and myogen showed a decrease of ammonium and diamino nitrogen, especially of histidin nitrogen content and an increase of monoamino nitrogen content comparing with original one. But the change of arginin nitrogen of denaturated one is quite different from that of musclefiber and it would be caused by transformation of myosin and myogen in musclefiber.

(E) Determination of free amino nitrogen of myosin and myogen

The free amino nitrogen content of the following proteins were determined by SÖRENSEN's method which procedure has already been described in the first report.

TABLE XII

Samples / Constituents	Myosin				Myogen			
	Original	Frozen	Salted	Smoked	Original	Frozen	Salted	Smoked
% in dry matter	2.0904	3.3620	3.5097	4.2666	3.4650	4.8364	3.2558	3.8555
% in total nitrogen	13.3075	21.3063	22.4868	25 9916	21.5521	30.2585	20.2560	23.4652
Ratio	100	160	169	195	100	140	94	181

	% in dry matter		% in total nitrogen		Ratio	
	Original	Frozen	Original	Frozen	Original	Frozen
Myosinfibrine	2.6451	3.6157	16.3041	22.6234	100	139

From the above results the free amino nitrogen content of all denaturated proteins showed a greater quantity than the original one.

(F) The composition of acetyl proteins

For the purpose of studying the constitutional differences of the three kinds of denaturated proteins from original one, the author acetylated the proteins and determined acetyl group and nitrogen content.

According to TROENSEGAARD's method (3), proteins were acetylated as follows:—1 g of protein was kept over night with 20 ccm of glacial acetic acid. The solution was heated in a boiling water bath with a reflux condenser. 3 ccm of acetyl chloride were added through the condenser and

the bath kept boiling. After 3 hrs. 3 ccm of reagent were added as formerly, 3 ccm more of the reagent were added twice at intervals of 1 hour. Thus after the addition of 12 ccm of acetyl chloride, the boiling was stopped and a solution of acetyl protein was obtained. 10–15 ccm of acetic acid anhydride were added to the solution and the mixed solution was evaporated to about 20 ccm under a diminished pressure. Again 10 ccm of acetic acid anhydride were added. To the mixture were added 4 g of newly fused and pulverized sodium acetate accompanied by a good shaking. The mixture was heated at 132°–135°C for 5 minutes in an oil bath. After its cooling, 30 ccm of dry chloroform were added and the whole was stood over night. The chloroform solution freed from insoluble matter by filtration was then poured into 150 ccm of dry ether. By this treatment, the acetyl protein produced was precipitated; the precipitate was washed twice with ether and dried over sulphuric acid under diminished pressure.

The nitrogen content of the compound was estimated by KJELDAHL's method and the acetyl group by WENZEL's method. (5)

TABLE XIII

Samples	Myosin				Myosinfibrine		Myogen			
	Original	Frozen	Salted	Smoked	Original	Frozen	Original	Frozen	Salted	Smoked
% of acetyl group	47.179	55.287	51.115	31.450	38.725	33.394	63.754	35.033	30.057	32.770
% of nitrogen	3.903	6.284	6.936	6.436	4.009	8.093	6.275	4.905	10.269	8.118
Acetyl/N.	12.038	8.799	7.366	4.887	9.659	4 126	10.160	7.142	3.927	4.036
Ratio	100	73	61	40	100	43	100	70	29	40

According to the above table, myosin and myogen of salmon muscle decreased their acetyl group contents by the above denaturation process, while their nitrogen contents were increased. The ratio of acetyl group to nitrogen was far less of denaturated protein than in case of original one.

VI Conclusion

Form the results of the above experiments we can summarize the following differences in physico-chemical properties between the denaturated protein and the original muscle protein of salmon.

(1) In frozen, salted and smoked procedure, the mineral substances of body juice seemed to translocate to muscle tissue and fat also showed the same tendency; therefore the ash and fat contents of denaturated muscle are greater than that of the original one.

(2) In the above denaturation process of muscle, its content of NaCl soluble protein viz. myosin and myogen, is decreased very markedly and especially in case of salted and smoked its content corresponding only to 10 % of the original one. On the contrary, the content of insoluble- and nonproteid in the denaturated muscle is increased very markedly, especially in case of smoked one.

(3) The musclefiber viz. sarkolemm which is isolated from denaturated muscle of salmon, contained always less mineral substances and phosphorus. The acid developing power of musclefiber in neutral salt solution, showed somewhat increasing tendency in case of denaturated one. Among the amino acids of denaturated musclefiber, ammonia and diamino nitrogen especially arginin and histidin are decreased while monoamino acid nitrogen and free amino nitrogen are increased.

(4) Myosin and myogen which are isolated from frozen, salted and smoked muscle of salmon, showed that in alkali solution need always a greater quantity of HCl attain to the maximum point of surface tension and turbidity than that of original. Both denaturated proteins in alkali solution always showed greater specific rotatory power than that of the original one. The quantity of monoamino and free amino nitrogen of denaturated one is greater than that of original while diamino and histidin content are less and the ratio of acetyl group to nitrogen of denaturated acetyl proteins especially in case of smoked one is always less than that of original one.

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