SOME CHEMICAL CHANGES OF MUSCLE PROTEINS IN CANNING.*

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Received November 24th, 1933. Published February 28th, 1934.

Some changes take place in proteins and extractive matters of meats simply by heating in the case of canning. In this paper it is described on the study of the decomposition occurring in the muscle proteins.

The materials were prepared from muscles by removing substances soluble in water, alcohol and ether. The solutions of various pH values were added to the muscle proteins. The mixtures were sealed tightly in glass tubes and heated at 130–140°C. The purpose for adding the solutions of various pH values to the muscle proteins and heating them is to find out the changes of the muscle proteins in different hydrogen ion concentrations, and for using of glass-tubes is to learn the chemical changes free from the influence of any metallic can-materials.

(1) **Changes of the Hydrogen Ion Concentrations.** As the concentration of hydrogen ion of the contents of cans has great influences on etching the tin plated iron sheet of cans and on the chemical changes of the contents which occur during preservation, the experiments were carried out by the electric method for ascertaining the variation of pH value of muscle proteins by heating.

The proteins of whale, rabbit, hen, sea-bream, yellow-tail, carp, bonito, cuttle-fish and spiny-lobster were closen as experimental materials. Either a slightly acidic or alkaline solution or distilled water was added to the proteins before heating. The results of experiments always showed the tendency of pH to approach towards the neutral value. For example, when the muscle proteins of whale were heated at the pH values of 3.4, 6.2 (adding water) and 8.5, the pH changed to 4.5, 6.6 and 8.1 respectively. Thus, it is considered that these variations were probably due to the volatile basic and acidic substances, such as ammonia and hydrogen sulphide, as well as to the soluble amphoteric electrolytes, such as polypeptide and amino acid produced while heating.

(2) **Changes of the Form of Nitrogen.** When the muscle proteins of whale, rabbit, cow, hen and mackerel were heated in distilled water at 150°C. for one hour, about 20 per cent. of their protein-nitrogen changed to

* This paper was already published more precisely in Japanese in Journal of the Society of Agricultural Chemistry of Japan, **9** (1933), 835.

the forms of peptone, peptide, amino acid and ammonia. The formation of these soluble nitrogenous substances may cause a speedy putrefaction to the opened canned meat. The distribution of nitrogen of the muscle proteins after heating is shown in Table 1.

Table 1.

	In per cent. of dry matter					In per cent. of total N			
	Total-N	Protein-N	Amino-N	Ammonia-N	Residual-N	Protein-N	Amino-N	Ammonia-N	Residual-N
Whale	16.35	13.52	0.52	0.09	2.22	82.69	3.18	0.55	13.58
Rabbit	15.88	11.35	0.78	0.02	3.73	71.47	4.91	0.13	23.48
Cow	16.52	14.31	0.43	0.29	1.49	86.62	2.60	1.76	9.00
Hen	16.86	13.90	0.52	0.31	2.13	82.44	3.08	1.83	12.63
Mackerel	16.81	14.18	0.45	0.34	1.84	84 35	2.68	2.04	10.95

(3) **Changes in the Elementary Composition of Muscle Proteins.** In order to ascertain what changes take place in the elementary composition of muscle proteins through heating under pressure, and to find out the effects of pH values upon these changes, the muscle proteins of whale, rabbit, hen and sea-bream were heated at the initial pH values of 3.4, 6.2 and 8.5. The results of experiments showed the decrease of their nitrogen and sulphur contents and the amounts of the decrease were particularly marked when the substances were heated in the solutions having the greater values of pH. The results are given in Table 2.

Table 2.

		C	H	N	S	P
Whale	Original protein	51.60	6.91	16.48	0.74	0.085
	Protin heated at pH 3.4	51.88	6.88	16.28	0.74	0.076
	pH 6.2	51.93	6.87	15.97	0.73	0.086
	pH 8.5	52.54	6.85	15.57	0.70	0.071
Rabbit	Original protein	52.32	7.12	16.30	0.86	0.093
	Protein heated at pH 3.4	52.47	7 13	16.11	0.86	0.078
	pH 6.2	52.55	7.11	15.92	0.83	0.092
	pH 8.5	52.99	7.05	15.38	0.84	0.069

Table 2.—(*Concluded*)

		C	H	N	S	P
Hen	Original protein	52.53	7.18	16.76	0.85	0.093
	Protein heated at pH 6.2	52.88	7.14	16.33	0.84	0.094
Sea-bream	Original protein	52.79	7.08	16.42	0.95	0.095
	Protein heated at pH 6 2	52.92	7.03	16.07	0.93	0.093

(4) Generation of Ammonia and Hydrogen Sulphide. The results of experiments with four kinds of muscle proteins revealed the fact that both NH_3 and H_2S were generated in larger quantities when they were heated in the solutions having the greater values of pH. This fact agrees with the result of the elementary analysis. When the material of the tin is bad and iron dissolves in the liquid contained in the can, then sulphide of iron precipitates and causes the deterioration and blackening of the canned meats. The amounts of NH_3 and H_2S in mg. generated from 100 gr. of muscle proteins are shown in Table 3.

Table 3.

NH_3 (mg. in 100 gr. of proteins)

pH before heated	Whale	Rabbit	Hen	Sea-bream
3.4	0.15	0.21	—	—
6.2	0.58	1.76	1.36	1.88
8.5	28.44	36.89	—	—

H_2S (mg. in 100 gr. of proteins)

pH before heated	Whale	Rabbit	Hen	Sea-bream
3.4	0	0	—	—
6.2	9.37	1.14	0.87	15.62
8.5	27.72	12.89	—	—

(5) Changes of the Amounts of Arginine, Histidine, Lysine, Cystine, etc. In order to learn how the distributions of arginine, histidine, lysine, cystine and other nitrogen in the muscle proteins of whale, rabbit, hen and sea-bream change by heating, the quantitative analyses were carried out by the Van Slyke method before and after the heating. The proteins were heated at three different values of pH to ascertain the effects of solutions of various pH upon the proteins. The results are as follows (Table 4).

Table 4.

	Total-N	Ammonia-N	Melanine-N	Cystine-N	Arginine-N	Histidine-N	Lysine-N	Mono-amino-N	Non-amino-N
Whale									
In per cent. of protein									
Original protein	16.48	0.69	0.33	0.29	2.49	2.20	1.34	8.02	1.18
Protein heated at pH 3.4	16.28	0.59	0.45	0.16	2.59	2.28	1.16	7.79	1.23
pH 6.2	15.97	0.55	0.44	0.14	2.36	2.36	1.26	7.66	1.21
pH 8.5	15.57	0.45	0.43	0.10	2.49	1.96	1.34	7.45	1.35
In per cent. of total N									
Original protein	100	4.29	2.02	1.45	15.08	13.37	8.15	48.66	7.15
Protein heated at pH 3.4	100	3.62	2.79	1.04	15.94	14.03	6.48	47.84	7.67
pH 6.2	100	3.42	2.73	0.87	14.77	14.79	7.88	47.95	7.57
pH 8.5	100	2.91	2.73	0.64	15.97	12.59	8.62	47.85	8.64
Rabbit									
In per cent. of protein									
Original protein	16.30	0.74	0.36	0.19	2.49	1.93	1.26	8.17	1.15
Protein heated at pH 3.4	16.11	0.73	0.46	0.15	2.58	1.92	0.94	7.82	1.41
pH 6.2	15.92	0.50	0.38	0.13	2.34	2.17	1.15	7.89	1.34
pH 8.5	15.38	0.39	0.43	0.10	2.49	1.75	1.23	7.59	1.41
In per cent. of total N									
Original protein	100	4.56	2.17	0.18	15.28	11.85	7.74	50.15	7.06
Protein heated at pH 3.4	100	4.55	2.83	0.92	16.04	12.53	6.04	48.45	8.78
pH 6.2	100	3.81	2.36	0.79	14.41	13.60	6.92	49.61	8.43
pH 8.5	100	2.57	2.77	0 65	16.16	11.38	8.02	49.32	9.18

Table 4.—(*Concluded*)

	Total-N	Ammonia-N	Melanine-N	Cystine-N	Arginine-N	Histidine-N	Lysine-N	Monoamino-N	Nonamino-N
Hen									
In per cent. of protein Original protein	16.76	1.10	0.48	0.18	2.71	1.97	1.36	8.19	0.76
Protein heated at pH 6.2	16.33	0.89	0.63	0.14	2.55	2.04	1.15	7.97	0.97
In per cent. of total N Original protein	100	6.58	2.85	1.07	16.14	11.73	8.12	49.60	4.53
Protein heated at pH 6.2	100	5.42	3.88	0.85	15.61	12.51	7.04	48.78	5.95
Sea-bream									
In per cent. of protein Original protein	16.42	0.77	0.36	0.28	2.54	2.24	1.47	8.35	0.42
Protein heated at pH 6.2	16.07	0.45	0.48	0.12	2.46	2.36	1.37	8.20	0.51
In per cent. of total N Original protein	100	4.70	2.17	1.69	15.46	13.61	8.92	50.83	2.55
Protein heated at pH 6.2	100	3.43	2.99	0.77	14.68	14.71	7.93	50.42	3.17

It was found that the total nitrogen, ammonia and cystine nitrogen always decrease by heating, and the higher the value of pH the greater the rate of the decrease, while melanine nitrogen generally increases. The amounts of cystine, arginine, histidine and lysine contained in the proteins before and after heating were calculated in per cent. from the above results.

Table 5.

		Cystine	Arginine	Histidine	Lysine
Whale	Original protein	2.49	7.74	8.12	6.99
	Protein heated at pH 3.4	1.37	8.04	8.41	6.05
	pH 6.2	1.20	7.33	8.71	6.57
	pH 8.5	0.86	7.74	7.23	6.99
Rabbit	Original protein	1.63	7.74	7.11	6.57
	Protein heated at pH 3.4	1.29	8.02	7.09	4.90
	pH 6.2	1.11	7.27	8.01	6.00
	pH 8.5	0.86	7.74	6.46	6.41

Table 5.—(*Concluded*)

		Cystine	Arginine	Histidine	Lysine
Hen	Original protein	1.54	8.42	7.27	7.09
	Protein heated at pH 6.2	1.20	7.92	7.53	6.00
Sea-bream	Original protein	2.40	7.89	8 27	7.67
	Protein heated at pH 6.2	1.03	7.64	8.71	7.14

According to Table 5, the quantities of cystine, arginine, histidine and lysine contained in the proteins after heating showed a tendency of decrease, as compared with those contained in the original proteins. Such tendencies were remarkable on cystine at the higher pH before heating and on lysine at the lower pH.

(6) **Changes of Tyrosine and Tryptophane.** The quantities of tyrosine and tryptophane contained in the muscle proteins of whale, hen, rabbit, yellow-tail, carp, bonito, sea-bream, cuttle-fish and spinylobster were determined before and after heating. The amounts of those amino acids decreased by heating, but very little, as it is obvious in Table 6.

Table 6.

	Tyrosine		Tryptophane		pH	
Proteins	Before heating	After heating	Before heating	After heating	Before heating	After heating
Whale	5.11	4.97	1.51	1.32	6.2	6.6
Hen	4.81	4.78	1.58	1.59	6.2	6.6
Rabbit	5.49	5.27	1.68	1.57	6.2	6.6
Yellow-tail	4.97	4.69	1.70	1.62	6.51	6.54
Carp	4.71	4.53	1.50	1.47	5.02	5.92
Bonito	4.84	4.60	1.63	1.46	7.17	6.65
Sea-bream	5.00	4.74	1.63	1.56	6.37	6.51
Cuttle-fish	4.83	4.71	1.37	1.29	4.67	5.57
Spiny-lobster	5.05	4.81	1.46	1.34	3.98	5.29

(7) **Change of Proline.** The experiments were carried out by using each 40 gr. of gelatine to find out the difference between the quantities of

proline contained in the materials before and after heating. It was observed that there was almost no difference between them, and when the pure solution of proline was heated, there was no detectable decomposition. Therefore the amount of proline in muscle proteins may probably be the same.

(8) **Changes of the Forms of Cysteine, Cystine and Sulphur.** When the solutions of pure cystine and cysteine were heated under the different pH, a part of the one always changed into the other. That is, when the solution of any of the two acids was heated, it was found that the solution contained the both acids, but the sum of the two quantities always decreased. The results of the experiments are as follows:

Table 7.

pH, before heating	Sample	Used, mg.	Found, mg.		Sum
			Cysteine	Cystine	
1.7	Cysteine	37.5	32.0	3.8	35.8
4.8	Cystine	45.6	2.9	39.9	42.8
6.8	Cysteine	25.1	9.4	6.6	16.0
6.8	Cystine	22.6	2.7	17.1	19.8
8.3	Cysteine	25.8	5.1	5.3	10.4
8.6	Cystine	25.0	2.7	16.2	19.2

The muscle proteins of sea-bream were heated at pH 6.1 (a) and 9.2 (b), and the forms of sulphur were determined. The results obtained are as follows:

Table 8.

Mg. per cent. of protein	Total S	Cysteine	Cystine	H_2S S	Sulphide S	Sulphate S	Soluble-organic S
Before heating	690	660	190	—	—	—	—
After heating (a)	—	490	200	6.6	0.4	18	96
,, ,, (b)	—	440	110	20.9	4.6	30	103

In per cent. of total S	Total S	Cysteine S	Cystine S	H_2S	Sulphide S	Sulphite S	Soluble-organic S	Protein S
Before heating	100	26.0	7.2	—	—	—	—	—
After heating (a)	—	19.0	7.5	0.9	0.06	2.6	14.0	82.4
,, ,, (b)	—	17.5	4.3	2.9	0.7	4.3	15.0	77.1

Namely, when it was heated in alkaline solutions, the decompositions of cysteine and cystine were especially considerable, and hydrogen sulphide and other sulphides were formed in large quantities.

In conclusion the authors wish to express their thanks to Messrs. U. Endo, S. Nagatake, S. Shigematsu and K. Katai for their analytical works and also to "Hatutori-Hokokuwai" for a research grant which has defrayed a part of the cost of this investigation.

Summary.

(1) The muscle proteins of some hens, various fishes and certain animals were prepared as samples for the purpose of studying what changes take place in the muscle proteins at the time of canning meats under pressure and heating. The materials were sealed in glass-tubes and heated at 130–140°C. for one hour, and changes of materials were studied.

(2) The hydrogen ion concentration of all of the proteins showed a tendency to approach toward the neutral point.

(3) About 20 per cent. of the protein nitrogen was transformed by heating to peptone, peptide, and amino acid nitrogens. The formation of soluble nitrogen compounds, such as these, is considered to cause a speedy putrefaction of contents of an opened can.

(4) As the results of elementary analyses of carbon, hydrogen, nitrogen, sulphur and phosphorus, it was found that the quantities of nitrogen and sulphur contained in the proteins generally decreased by heating, and the changes were more marked the higher the values of pH. There was no appreciable change in phosphorus when the solution was neutral, but its amount decreased when the solution was acidic or alkaline, and hence the percentage of carbon increased.

(5) The quantities of NH_3 and H_2S generated by heating were greater in proportion to the values of pH of the solutions. This result explains the fact that, when the tin of cans are bad and the pH of the contents is high, the contents of the can deteriorate more readily and the colour changes to black.

(6) As the results of the analyses of the proteins by the Van Slyke method, the decrease of the total nitrogen, ammonia nitrogen and cystine nitrogen and the increase of melanine nitrogen were detected especially when the pH value increased. The quantities of cystine, arginine, histidine and lysine contained in the materials showed a tendency of decrease in all

three cases and the same tendencies were very remarkable on cystine at the higher value of pH and on lysine at the lower.

(7) Tryptophane by the May-Rose method and tyrosine by the Folin-Denis method were quantitatively analyzed, and the decreases of these substances by heating were very insignificant.

(8) The test for proline was made by the Fisher-Bechner method, but no decomposition by heating was observed.

(9) Cysteine and cystine were analyzed quantitatively by the Okuda-Katai method, and it was found that a part of any one of these acids always changed to the other acid by heating. But the sum of the two always decreased, and the decomposition of both of these acids was remarkable at the higher value of pH, and the decomposition of cysteine was greater than that of cystine.

(10) A part of the protein-sulphur changed by heating to hydrogen sulphide, other sulphides, sulphuric acid and soluble organic sulphur, and the quantities of these substances generated were greater at the higher pH of the solutions, as in the case of the decompositions of cysteine and cystine.

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