THE CHEMICAL STUDIES ON THE DENATURATION OF PROTEINS (Ist Report)

BY

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The denaturation of proteins is an irreversible change of their colloidal properties by the following treatment. For example, coagulation by heat or freezing and the action of acids and alkalies on proteins causing "*Protalbin Säure* and *Lysalbin Säure*" reported by PAAL (13). From the colloid chemical standpoint, FREDERICQ (6), HALLIBURTON (8), HEWLETT (10), NEUMEISTER (11), BRUNNER (1) and STARKE (16), reported that the coagulation by heat of protein occurs in weak acid medium, while if its acidity or alkalinity is increased, the coagulation is checked. HARNACK (9), BÜLOW (2), STARKE (16), PAULI (14) and ERB (5), reported that the coagulation by heat of protein occurs in the presence of salt while CHICK and MARTIN (3) reported that the coagulation by heat occurs without salts in any reaction medium, but in their presence the denaturation is concerned with the isoelectric point of the protein. OSBORNE (12) reported that when the protein solution was boiled many hours, the solution became alkaline by the formation of decomposition products and COHNHEIM (4), FODOR (7) and TARCHANOFF (17) reported their results concerning the temperature and the condition of the coagulation by heat but there is no report explaining the chemical properties which were changed by the denaturation. ROBERTSON (15) is the only man who investigated the chemical structure of the heat coagulated protein and he stated that the denaturated protein is the dehydrate product of the coagulation. Therefore the following investigations on the chemical changes of the denaturated proteins were made.

Part I. Denaturation of Rice Protein, Oryzenin

1. Materials

The denaturated oryzenin of rice was prepared by the following treat-

[Jour. Facul. Agric., Hokkaido Imp. Univ., Sapporo, Vol. XXV, Pt. 2, Dec., 1928.]

ment. 250 g. of defatted rice powder were mixed with 1500 ccm of 10% NaCl solution, shaken 30 minutes, settled for one night in an ice chamber, the upper liquid decanted and washed until the filtrate showed no chlorine reaction. The residue was mixed with 1500 ccm of 0.2% NaOH solution, shaken 30 minutes and after being set over night in an ice chamber, the upper liquid was decanted in a pulp filter and starch freed filtrate was obtained. Oryzenin in the filtrate was precipitated with acetic acid and washed several times with water. Then the precipitate was suspended in 70% alcohol and freed from 70% alcohol soluble protein. One part of oryzenin was boiled one hour and another part was frozen many times and these samples as well as the original were treated with the absolute alcohol and ether and dried in a H_2SO_4 desiccator.

2. Water, Ash and Sulphur content of denaturated oryzenins

The sulphur content was determined by DENIS-BENEDICT's method and the ash content by the ordinary way.

TABLE I

Samples	Water %	Ash (dry matter) %	Sulphur %	Sulphur (dry matter %)
Original oryzenin	4.529	0.6106	0.5509	0.5771
Boiled ,,	5.588	0.5158	0.4979	0.5244
Frozen ,,	5.339	0.6190	0.7299	0.7711

The ash and sulphur content of boiled oryzenin is less than that of the original, while that of frozen oryzenin is just the opposite.

3. Change of turbidity and surface tension of the alkali oryzenin solution in titration with HCl solution

A preliminary experiment on the iso-electric point estimation of the oryzenin solutions was made as follows. One tenth g of water free oryzenin was dissolved in 15 ccm of $\frac{1}{5}$ normal NaOH solution and diluted to 300 ccm with water. Ten ccm of the solution was titrated with $\frac{1}{50}$ normal HCl solution and the changes of surface tension and turbidity of the solution at 18°C were observed. Surface tension shown with dynes per 1 sq cm.

TABLE II. (Surface tension)

ccm HCl	5.20	5.25	5.30	5.35	5.40	5.45	5.50	5.55	5.60
Original oryz.	57.3	57.8	58.8	58.7	58.9	58.4	57.1	56.0	55.0
Boiled „	58.3	58.3	59.5	58.8	58.0	58.1	58.5	58.9	57.0
Frozen „	58.3	58.5	58.9	59.5	58.8	58.4	58.0	57.8	55.0

(Turbidity)

Original oryz.	+	+	++	+++	+++	+++	++	+	+
Boiled „	+	++	+++	+++	++	++	+	+	+
Frozen „	+	+	++	+++	+++	+++	++	+	+

According to the table, to obtain the maximum turbidity and surface tension of denaturated oryzenin a somewhat smaller quantity of acid is needed.

4. The specific rotatory power of oryzenin alkali solution

One tenth g of each kind of oryzenin was dissolved in 25 ccm of $\frac{1}{5}$ normal NaOH solution and the specific rotatory power of solution was examined by a HAENSH-SCHMIDT half shadow polariscope. After portions of each solution had been illuminated 30,60 and 90 minutes respectively under a quartz lamp at a height of 1 foot, their rotatory power was determined in the same way.

TABLE III

Samples	α	$[\alpha]_D^{20}$	Illumination		
			30 m	60 m	90 m
Original oryzenin	−1.4	60.55	77.85	67.93	60.55
Boiled „	−2.0	86 50	88.66	69.20	60.55
Frozen „	−2,1	90.82	77.85	69.20	69.20

In the above table, 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 rays is lower than with the original.

5. The determination of free amino nitrogen

The content of free amino nitrogen of oryzenin was determined by SÖRENSEN's method as follows; 0.1 g of water free oryzenin was dissolved in 10 ccm of $\frac{1}{5}$ normal NaOH solution and after settling 3 days, 10 ccm of neutral formalin was added and titrated with $\frac{1}{20}$ normal HCl until the solution showed a light red colour.

TABLE IV

Samples	N/20 HCl ccm	Nitrogen g.	Nitrogen (dry matter %)
Original oryzenin	1.60	0.00112	1.120
Boiled „	1.80	0.00126	1.260
Frozen „	1.65	0.00115	1.155

The free amino nitrogen content of denaturated oryzenin is a little greater than that of original oryzenin

6. The separation and determination of amino acids

It is said that the amide, melanin and arginin nitrogen of denaturated casein is superior to that of the original and the same results were observed in frozen vegetable globulin (*Japanese tofu*). The author also examined amino acid nitrogen by VAN-SLYKE's method (18)(19) which will be described thoroughly in the next chapter.

TAELE V

	Original oryzenin		Boiled oryzenin		Frozen oryzenin	
	Dry matter %	Total N %	Dry matter %	Total N %	Dry matter %	Total N %
Total nitrogen	17.1095	100	16.2773	100	16.9585	100
Amide „	1.0223	5.9753	1.7048	10.4737	1.1249	6.6331

	Original oryzenin		Boiled oryzenin		Frozen oryzenin	
	Dry matter %	Total N %	Dry matter %	Total N %	Dry matter %	Total N %
Melanin nitrogen	0.3463	2 0240	0.3101	1.5056	0.2940	1.7337
Monoamino „	10.4713	61.2020	9.3763	57.6049	10.3645	61.1170
Diamino „	5.2694	30.7985	4.8857	30.0156	5.1750	30.5161
Arginin „	3.7302	21.8019	3.4450	21.1645	3.5356	20.8489
Cystin „	0.2262	1.3223	0.2160	1.3274	0.2852	1.6819
Histidin „	1.0978	6.4168	1.1118	6.8303	1.2186	7.1862
Lysin „	0.2151	1.2574	0.1128	0.6932	0.1355	0.7990

According to the above table, amide and histidin nitrogen content of boiled and frozen oryzenin is superior to that of the original, while lysin nitrogen content is just the opposite.

Part II. Denaturation of Soybean Protein, Glycinin

1. Materials

The water extracts of defatted soy-bean powder was filtered through a pulp filter and the clear filtrate was dialyzed in a bladder. The precipitate upon dialysis was washed with alcohol and ether and "glycinin A" was obtained.

50 g of glycinin A were dissolved in 2 liters of 0.2% NaOH solution and the solution was filtered through a pulp filter and divided into 3 parts. One of them was precipitated with diluted HCl, washed with water until no chlorine reaction was observed, then washed with alcohol and ether and dried in the ordinary way. Another part was precipitated with dilute HCl, boiled 2.5 hours in a boiling water bath, the precipitate was washed and dried in the same way as above. Another part was also precipitated with diluted HCl, and frozen many times, the precipitate collected, washed and dried in the ordinary way.

Glycinin A was suspended in water, treated 2.5 hours by superheated steam, washed with alcohol and ether and dried. Glycinin A was also mixed with gasoline for 24 hours, filtered and treated with superheated steam to remove the absorbed gasoline and dried. Glycinin A was also treated with pure benzin and treated in the manner above mentioned.

Thus the following 6 different samples of glycinins were prepared,

namely, glycinin A, boiled-, frozen-, superheated-, gasoline-, and benzin-glycinin.

2. Water and ash content of denaturated proteins

TABLE VI

	Glycinin A	Boiled-gl.	Frozen-gl.	Super-heated-gl.	Gasoline-gl.	Benzin-gl.
Water %	10.633	12.680	9.731	8.732	9.031	9.222
Ash %	0.515	0.466	0.405	0.618	0.738	0.538
Ash in dry matter %	0.577	0.534	0.449	0.677	0.812	0.615
Ratio to orig.	100	93	78	118	141	107

3. Sulphur and phosphorus content of denaturated proteins

The sulphur content was determined dy DENIS-BENEDICT's and the phosphorus content by NEUMANN's method. The following results were obtained.

TABLE VII

	Glycinin A	Boiled-gl.	Frozen-gl.	Super-heated-gl.	Gasoline-gl.	Benzin-gl.
P_2O_5 in 1 g.	0.01097	0.00647	0.00739	0.01745	0.01661	0.01876
P_2 in dry matter %	0.23970	0.14150	0.16160	0.38120	0.36290	0.40990
Ratio	100	59	67	159	159	171
S_2 in dry matter %	0.58770	0.63020	0.61560	0.48650	0.50780	0.44070
Ratio	100	107	104	82	86	75

In the above table, the phosphorus content of boiled and frozen-glycinin is less than that of the original, while that of superheated-, gasoline- and benzin-glycinin is greater than in the original. On the contrary, the sulphur content of boiled and frozen-glycinin is superior to that of the original while that of the superheated-, gasoline- and benzin-glycinin are less than that of the original. Therefore the denaturation of the former would differ from that of the latter.

4. The determination of free amino nitrogen

The content of free amino nitrogen of denaturated proteins was determined by SÖRENSEN's method. 0.1 g of water free glycinin was dissolved in 20 ccm of $\frac{1}{10}$ normal NaOH solution to which was added 10 ccm of neutral formalin (50 ccm of formalin of purchase was titrated with $\frac{1}{5}$ normal NaOH solution using 1 ccm of 1% phenolphthalein as indicator until the solution was colored a slight pink). The solution was titrated with $\frac{1}{20}$ normal HCl solution until it became slightly pink in color. With 20 ccm of $\frac{1}{10}$ normal NaOH solution and 10 ccm of neutral formalin, a control experiment was carried out and necessary correction made.

TABLE VIII

	Glycinin A	Boiled-gl.	Frozen-gl.	Super-heated-gl.	Gasoline-gl.	Benzin-gl.
N/20 HCl ccm	2.460	3.100	2.600	3.200	3.180	3.150
Free amino N in dry matter %	1.722	2.170	1.820	2.240	2.226	2.221
Free amino N in total N %	10.219	13.346	11.303	14.575	14.138	14.348
Ratio	100	126	106	130	129	129

According to the table, the content of free amino nitrogen of all denaturated proteins is superior to that of the original and especially so in the case of superheated-, benzin- and gasoline-glycinin.

5. The separation and determination of amino acids

The determination of amino acids of denaturated proteins was undertaken according to VAN SLYKE's method (18). To a given quantity of the sample was added 20 times its weight of 20 % HCl; the mixture was boiled and hydrolysed for 16 hours in a sand bath under a reflux condenser. Next, almost all the HCl of the solution was driven off under a diminished pressure at below 40°C. The residue was neutralized and made slightly alkaline by the addition of 10% solution of calcium hydroxide. The ammonia nitrogen liberated was distilled into standard sulphuric acid under a diminished pressure at below 40°C. The remaining fluid was filtered, and separated from *melanin nitogen*. The *melanin nitogen* was determined by KJELDAHL'S method. The filtrate was acidified with HCl and evaporated under diminished pressure at below 40°C. To the concentrated filtrate were added

18 ccm of conc. HCl and 15 g of phosphotungstic acid and the diamino acids were filtered by suction and treated with a mixture of ether and amyl-alcohol according to VAN SLYKE's modified method (19). The diamino-acid nitrogen was determined separately. By using VAN SLYKE's micro-apparatus the free amino nitrogen was determined.

Table IX shows the results of the experiments described and table X shows the percentage of each nitrogen to the total nitrogen of denaturated proteins.

TABLE IX

	Total-N.	Amide-N.	Melanin-N.	Monoamino-N.	Diamino-N.	Arginin-N.	Cystin-N.	Histidin-N.	Lysin-N.
Glycinin A	0.16855	0.01533	0.00259	0.10046	0.05015	0.02364	0.00073	0.01390	0.01186
Boiled-gl.	0.16261	0.01700	0.00282	0.09297	0.04981	0.02262	0.00127	0.01374	0.01218
Frozen-gl.	0.16102	0.01513	0.00256	0.09382	0.04950	0.02494	0.00093	0.01345	0.01016
Superheated-gl.	0.15368	0.01659	0.00304	0.08715	0.04691	0.01991	0.00105	0.01739	0.00856
Gasoline-gl.	0.15745	0.01603	0.00305	0.09050	0.04784	0.02225	0.00132	0.01359	0.01066
Benzin-gl.	0.15479	0.01618	0.00278	0.08755	0.04827	0.02284	0.00099	0.01619	0.01824

TABLE X

	Total-N.	Amide-N.	Melanin-N.	Monoamino-N.	Diamino-N.	Arginin-N.	Cystin-N.	Histidin-N.	Lysin-N.
Glycinin A	100.00	9.09836	1.53688	59.61071	29.75405	14.03081	0.43813	8.24810	7.03705
Boiled-gl.	100.00	10.45653	1.73209	57.17638	30.63500	15.91105	0.78171	8.45035	7.49188
Frozen-gl.	100.00	9.39932	1.59274	58.26586	30.74208	15.49205	0.57789	8.35669	6.31544
Superheated-gl.	100.00	10.79665	1.97849	56.69635	30.52851	12.95585	0.68441	11.31936	5.56878
Gasoline-gl.	100.00	10.18534	1.93755	57.49322	30.38389	14.13589	0.83781	8.63646	6.77374
Benzin-gl.	100.00	10.45694	1.80141	56.55520	31.18645	14.76069	0.64048	10.46347	5.32180

Thus it is seen that in all denaturated glycinin, there is a predominance of amide, arginin- and histidin-nitrogen as compared with 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 and boiled glycinin or superheated glycinin show decreased arginin nitrogen and increased histidin nitrogen content.

6. Change of turbidity of the alkali glycinin solution in titration with HCl solution

As a preliminary experiment on the iso-electric point estimation of the glycinin solutions, the following manipulation was used. One tenth g. of glycinin was dissolved in 15 ccm of 1/25 normal NaOH solution. After standing for 24 hours (15° C), 1 ccm of the protein solution was diluted with 135 ccm of pure water. Ten ccm of the mixture was titrated with 1/100 normal HCl solution. These figures show the turbidity, i. e. the "+" sign indicates degree of turbidity.

TABLE XI

HCl ccm	Glycinin A	Boiled-gl.	Frozen-gl.	Super-heated-gl.	Gasoline-gl.	Benzin-gl.
5.16	++	+++	+++	++	+++	+++
5.20	+++	+++	+++	+++	+++	+++
5.24	+++	+++	+++	+++	+++	+++
5.28	+++	+++	+++	+++	+++	+++
5.32	+++	+++	+++	+++	+++	+++
5.36	+++	+++	+++	+++	+++	+++

According to the table, to obtain the maximum turbidity of the boiled glycinin there is needed a somewhat smaller quantity of acid, while the denaturated glycinin showed no marked difference as compared with the original.

7. Solubility of denaturated glycinin

The solubility of denaturated glycinin in different solvents was examined. 0.05 g of glycinin was mixed with 20 ccm of solvent, shaken for 30 minutes and settled 24 hours. The mixture was then filtered, 15 ccm of filtrate was used for nitrogen estimation by KJELDAHL'S method and the quantity of dissolved protein was calculated.

TABLE XI

Normality of NaOH	1/5	1/10	1/20	1/50	1/100	1/150	1/200	1/500	1/1000
Glycinin A	100.0	100.0	100.00	100.00	85.28	75.28	68.68	46.71	17.03
Boiled-gl.	100.0	100.0	96.85	91.13	45.56	17.08	14.24	4.56	2.84
Frozen-gl.	100.0	100.0	100.00	100.00	—	—	74.78	21.57	5.75
Superheated-gl.	100.0	100.0	100.00	100.00	69.89	12.05	—	9.04	4.32
Gasoline-gl.	100.0	100.0	80.59	64.71	47.06	5.88	4.41	2.94	—
Benzin-gl.	100.0	100.0	100.00	98.73	86.76	32.95	14.95	—	—

Normality of Na_2CO_3	2	1/2.5	1/10	1/50	1/100
Glycinin A	86.38	95.73	97.72	100.00	93.36
Boiled-gl.	15.38	18.79	25.63	19.93	9.34
Frozen-gl.	91.97	97.72	97.72	99.63	97.72
Superheated-gl.	9.04	16.27	18.08	9.04	4.52
Gasoline-gl.	7.64	16.41	17.64	12.35	4.70
Benzin-gl.	8.97	17.95	26.92	14.96	8.93

According to the table, 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.

TABLE XIII

Normality of HCl	1/10	1/25	1/50	1/100	1/200	1/500
Glycinin A	95.73	100.00	99.66	98.92	81.57	19.23
Boiled-gl.	5.69	13.10	14.24	14.24	8.54	1.61
Frozen-gl.	37.43	86.28	83.31	61.55	50.04	8.62
Superheated-gl.	3.01	3.01	6.03	6.03	1.30	0.00
Gasoline-gl.	4.12	8.82	8.82	7.67	5.25	1.67
Benzin-gl.	2.99	5.98	6.58	8.97	7.78	5.98

Normality of oxalic acid	1	1/5	1/50	1/100	1/200	1/500
Glycinin A	20.88	49.46	52.21	21.98	5.49	2.21
Boiled-gl.	10.25	11.39	8.54	5.54	2.84	0.00
Frozen gl.	21.85	37.39	34.51	11.50	4.60	1.49
Superheated-gl.	10.48	12.05	8.82	7.21	3.01	0.00
Gasoline-gl.	7.06	8.82	8.82	2.94	2.07	0.00
Benzin-gl.	5.98	9.89	8.24	6.59	2.74	0.00

Normality of acetic acid	1	1/10	1/50	1/100	1/200
Glycinin A	93.35	87.52	87.52	4.67	2.91
Boiled-gl.	15.12	7.25	6.04	6.04	3.05
Frozen-gl.	78.17	25.04	9.16	9.16	3.02
Superheated-gl.	9.59	6.39	6.39	3.19	0.00
Gasoline-gl.	6.24	6.24	3.12	3.12	0.00
Benzin-gl.	9.53	6.35	6.35	5.08	0.00
Normality of citric acid	1	1/5	1/50	1/100	1/200
Glycinin A	100.00	98.02	23.34	14.01	5.83
Boiled-gl.	21.17	10.88	9.67	3.02	0.00
Frozen-gl.	100.00	100.00	18.17	10.99	6.11
Superheated-gl.	9.04	8.95	6.39	3.19	0.00
Gasoline-gl.	8.82	8.74	8.74	6.24	3.12
Benzin-gl.	13.17	12.71	11.43	3.17	0.00
Normality of tartaric acid	1/5	1/50	1/100	1/200	1/500
Glycinin A	100.00	49.59	18.17	14.58	2.91
Boiled-gl.	18.14	9.07	8.46	6.04	3.02
Frozen-gl.	100.00	24.43	13.43	6.10	3.05
Superheated-gl.	12.79	5.12	3.19	3.19	0.00
Gasoline-gl.	6.24	6.25	3.12	3.12	0.00
Benzin-gl.	12.79	6.35	6.35	3.17	0.00

From the above table, the solubility of denaturated glycinin in acid solution is decreased in the following viz. Frozen- gl.→Boiled- gl.→Benzin-gl.→Gasoline- gl.→Superheated- gl.

TABLE XIV

Normality of NaCl	7.5	5	2.5	1	1/2	1/4	1/10
Glycinin A	2.74	5.49	24.73	32.97	10.99	8.24	5.49
Boiled-gl.	—	0.00	1.70	5.69	2.84	0.00	0.00
Frozen-gl.	2.87	2.87	9.77	28.76	6.91	2.87	0.00
Superheated-gl.	0.13	3.01	4.21	6.03	—	3.01	0.00
Gasoline-gl.	—	1.27	2.94	4.12	0.00	—	—
Benzin-gl.	—	1.29	1.79	5.98	1.29	0.00	—

Normality of $MgCl_2$		1/5	1/10	1/50	1/100
Glycinin A		8.24	33.84	29.13	3.83
Boiled-gl.		7.95	7.95	7.36	0.00
Frozen-gl.		7.43	17.25	13.96	2.87
Superheated-gl.		4.82	6.03	4.20	0.00
Gasoline-gl.		2.94	2.94	0.00	0.00
Benzin-gl.		4.17	8.97	4.16	0.00
Normality of Na_2SO_4	1	1/5	1/10	1/25	1/50
Glycinin A	5.49	26.92	32.97	46.71	6.04
Boiled-gl.	2.84	16.67	17.08	16.67	2.84
Frozen-gl.	5.75	13.96	25.88	25.88	2.87
Superheated-gl.	1.61	9.04	12.05	3.50	0.00
Gasoline-gl.	1.67	8.82	14.71	10.00	1.67
Benzin-gl.	2.99	14.53	15.84	14.52	2.89

From the above table, the solubility of denaturated glycinin in salt solution decreases in the following order viz. Frozen- gl.→Boiled- gl.→Benzin-gl.→Superheated- gl.→Gasoline- gl.

Part III. Solubility of Proteins in Denaturated Soybeans

1. Materials

The denaturated soybeans which were heated, boiled, frozen, soaked in gasoline were defatted is the ordinary way and powdered to make analytical sample. The following table shows denaturation process of soy-beans and water and crude fat content of powdered samples.

Table XV

Denaturation process of soy-beans	Water %	Crude fat (dry matter%)
1. Original soybeans, ground and powdered	8.701	21.126
2. Soybeans, kept 48 hrs. at 30°C and powdered	12.852	21.514
3. „ „ „ „ „ 60°C „ „	11.831	21.849
4. „ „ „ „ 110°C „ „	10.415	22.392
5. Soybeans, soaked in water, frozen, dried at 60°C and powdered	11.269	22.245
6. Soybeans, boiled 4 hrs. dried 60°C and powdered	10.509	22.397
7. Soybeans, soaked in gasoline 4 days then dried and powdered	11.701	21.031
8. Soybeans, roasted in ordinary way and powdered	8.701	21.425

2. Estimation of water, NaCl, alcohol, alkali soluble and insoluble proteins

Five g of defatted soy-bean powder was mixed with 50 ccm of water, shaken 30 minutes, settled one night, centrifuged and 25 ccm of upper liquid was used to estimate water soluble nitrogen. The first residue was washed 3 times with 50 ccm of water, and in order to make 10% NaCl solution there were added 25 ccm of 20% NaCl solution and water, taking in account the water already contained in the sample, then shaken 30 minutes, settled one night, centrifuged and 25 ccm of upper liquid was used to estimate NaCl soluble nitrogen. The second residue was washed 3 times with 50 ccm of 10% NaCl solution, next with 50 ccm water and in order to make 70% alcohol there were added 96% alcohol and water already contained in sample, shaken, settled, centrifuged and 25 ccm of upper liquid was used to estimate alcohol soluble nitrogen. The third residue was washed 3 times with 50 ccm of 70% alcohol and water, and in order to make 0.2% NaOH solution there were added 50 ccm 0.4% NaOH solution and water, calculating the water already contained in sample, shaken, settled, centrifuged and 50 ccm of upper liquid were used to estimate NaOH soluble nitrogen. At last the residue was washed 3 times with 0.2% NaOH solution and insoluble nitrogen was estimated.

TABLE XVI

		Total N.	Water sol. N.	NaCl sol. N.	Alcohol sol. N.	NaOH sol. N.	Insol. N.
Original	(Dry matter %)	5.904	3.730	1.238	0.021	0.626	0.446
	(Total N. %)	100.000	63.170	20 975	0.351	10.609	7.560
Heated 30°C	(Dry matter %)	5.725	3 348	1.187	0.022	0.755	0.506
	(Total N. %)	100.000	58 480	20.733	0.391	13.203	8.951
Heated 60°C	(Dry matter %)	6.080	2.344	1.071	0.015	1.162	1.154
	(Total N. %)	100.000	38.564	17.708	0.249	19.120	18.981
Heated 110°C	(Dry matter %)	6.788	0.289	0.027	0.022	0.375	4.952
	(Total N. %)	100.000	4.264	0.409	0.327	5.532	72.950
Frozen	(Dry matter %)	6.466	1.701	0.767	0.017	2.989	0.712
	(Total N. %)	100.000	26.311	11 866	0.266	46.222	11.022
Boiled	(Dry matter %)	6.225	0.508	0.109	0.020	2.766	2.876
	(Total N. %)	100.000	8.072	1.737	0.326	43.900	45.646
Gasoline	(Dry matter %)	6.536	3.332	0.984	0.014	1.127	0.711
	(Total N. %)	100.000	52.272	15.090	0.227	17.272	10.909
Roasted	(Dry matter %)	5.931	0.423	0.112	0.013	3.137	2.272
	(Total N. %)	100.000	7.142	1.897	0.223	52.901	38.303

According to the table, the heated soy-bean shows a decrease in water and NaCl solution soluble nitrogen and an increase in NaOH soluble and insoluble nitrogen content as compared with the original. These changes were observed in heating over 60°C and in the case of heating over 110°C, insoluble nitrogen content was increased to a remarkable extent. In the case of the frozen sample, water and NaCl solution soluble nitrogen content was decreased NaOH solution soluble nitrogen was increased but insoluble nitrogen remained about the same. In the case of boiling, water and NaCl solution soluble nitrogen content was decreased and NaOH solution soluble and insoluble nitrogen were increased. By the treatment with gasoline and roasted soybean, the same changes were observed but not remarkably.

SUMMARY

Form the above experimental results, we would summarize the following differences in the physico-chemical properties between the denaturated rice oryzenin and the denaturated soy-bean glycinin and both of the original ones.

(A) The denaturated 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.

(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 rays is lower than in the original.

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

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

(B) The denaturated glycinin

(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 on the contrary that of the others is less than that of

the original.

(2). The content of free amino nitrogen of all denaturated proteins 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 ones 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.→Gasoline- gl.→Superheated- gl.

(C). The denaturated soybean.

The heated soy bean showed a decreased water and NaCl sol. soluble nitrogen content and an increased NaOH soluble and insoluble nitrogen content in comparison with the original. The changes 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 sol. soluble nitrogen content was decreased but increased in case of NaOH sol. soluble nitrogen. In the case of boiling, water and NaCl sol. soluble nitrogen content was decreased and NaOH soluble and insoluble nitrogen increased. By the treatment with gasoline and benzin, the same changes were observed but not to a remarkable degree.

REFERENCE

(1) BRUNNER :—Dissertation, Bern (1894).
(2) BÜLOW :—Pflügers Arch. f. d. Ges. Physiol. 58, 207 (1889).
(3) CHICK u. MARTIN :—Journ. of Physiol. 40, 404 (1910), 43, 1 (1911), 45, 61 (1912).
(4) COHNHEIM :—Zeits. f. Physiol. Chem. 33, 455 (1901).
(5) ERB :—Zeits. f. Biol. 41, 309 (1901).
(6) FREDERICQ :—Zentralbl. f. Physiol. 3, 23, 601 (1890).
(7) FODOR :—Kolloid Zeitschr. 27, 29, 30 (1922).
(8) HALLIBURTON :—Journ. of Physiol. 5, 155 (1885).
(9) HARNACK :—Ber. d. deutsch. Chem. Ges. 22, II, 3046 (1889).
(10) HEWLETT :—Journ. of Physiol. 13, 493 (1892).
(11) NEUMEISTER :—Zeits. f. Biol. 24, 272 (1888).
(12) OSBORNE :—Ergebnisse d. Physiologie 10, 47 (1910).
(13) PAAL :—Ber. d. deutsch. Chem. Ges. 35, II, 2195 (1902).
(14) PAULI :—Pflügers Arch. f. d. Ges. Physiol. 78, 315 (1899).
(15) ROBERTSON :—physik. Chem. d. Eiweisskörper (1914).
(16) STARKE :—Sizungsber. d. Ges. f. Morphol. u. Physiol. (1897).
(17) TARCHANOFF :—Pflüger Arch. f. d. Ges. Physiol. 33, 303 (1886).
(18) VAN SLYKE :—Journ. Biol. Chem. 10, 15-85 (1911-1912).
(19) VAN TLYKE :—Journ. Biol. Chem. 22, 281 (1915).