On the Differences in Physico-chemical Properties of Various Proteins in Plant Seeds, (Third Report).

On the differences in the physico-chemical properties of the different kinds of Soy-bean proteins

By

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In a former report, the differences in physico-chemical properties of four rice proteins were examined by one of the authors.[1] The references already listed in the former report have been omitted this time and here only a short review of the former reports is made. In the four kinds of rice proteins i. e. water soluble albumin, NaCl soluble globulin, NaOH soluble oryzenin and alcohol soluble prolamin in this order, the solubility decreases, the iso-electric points approach the alkali side, the specific rotatory power of their alkali solutions increases and also the HCl combining power increases.

These physical properties of these four different rice proteins have intimate relation to the following chemical properties. In the same order as above the contents of carbon and sulphur decrease, nitrogen increases and in their hydrolytic products, the contents of amide nitrogen and histidin-form nitrogen increase while arginin, and lysin-form nitrogen decrease. The authors undertook to learn whether or not such chemical differences of the four kinds of rice proteins may be observed in the case of soy bean proteins. They undertook also to learn about the characteristic properties of soy bean proteins. Experiments were carried out accordingly as decribed hereafter.

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I. Isolation and purification of the different proteins.

Samples for the research were collected from Manchuria and Hokkaido. The powdered soy bean was extracted with ether in a Soxhlets apparatus and again dried in the open air.

The defatted soy bean powder was mixed with 10 volumes of water and the mixture was shaken a long time by a shaking machine. After that it was placed over night in an ice chamber. The upper liquid was poured through a Buchner's funnel which contained filter pulp of 1–2 cm thickness. The filtrate did not become clear though the filtration was repeated many times. The filtrate was dialysed with bladder in a water current and after many days, freed from salt, the protein solution formed a cloudy precipitate. The precipitate was centrifuged in order to remove the liquid then washed 3 times with alcohol and ether in a centrifugal machine. The dried protein which was kept for many days in a H_2SO_4 desiccator, was powdered in an agate mortar and called "Glycinin A."

To the mother clear liquid from which the glycinin A had been separated, was added HCl until it changed to white turbid solution and precipitated with the addition of ammonium sulphate. The precipitate was dialysed until the H_2SO_4 and chlorine reaction was no more detectable and the precipitate was treated in the same manner as above. This protein was named "Legumelin."

The residue from which the water soluble protein was separated, by washing 3 times with water, was mixed with water and NaCl solution and made up to 10% solution of NaCl. The mixture was shaken for 30 minutes by a shaking machine. After that it was placed over night in an ice chamber. The upper liquid was decanted and the same treatment was repeated 3–4 times until the extracts had no protein reaction. The upper liquid was collected and filtered several times by a Buchner's funnel and then NaCl soluble protein was precipitated by addition of HCl and ammonium sulphate. The NaCl soluble protein was also dialysed and dried in the same manner as above. The protein was named "Glycinin B."

The residue was washed with water until no chlorine reaction was observed, NaOH solution was added to make up to 0.2% of NaOH. The mixture was shaken for 30 minutes by a shaking machine and placed over night in an ice chamber. The upper liquid was filtered several times and to the clear filtrate was added dilute HCl whereupon

Glutelin was precipitated. The purification of glutelin was carried out in the same manner as above.

II. Water and ash contents of samples.

Water and ash contents of the samples were estimated by the ordinary method and the following results obtained.

TABLE I.

Water and ash contents of the protein samples.

	Legumelin		Glycinin A		Glycinin B		Glutelin	
	Water	Ash	Water	Ash	Water	Ash	Water	Ash
Manchuria	9.258	0.9002	10.541	1.2670	5.831	0.7822	8.154	0.7715%
Hokkaido (Tokachi)	9.881	1.1695	9.059	1.2944	8.992	0.8690	8.341	0.6873%
Hokkaido	7.421	1.2228	10.063	1.0889	7.383	0.5926	8.226	0.7732%

The ash contents of protein decreases in the following order i. e. legumelin→glycinin A→glycinin B→glutelin.

III. Some physico-chemical properties of different proteins.

(a) *Change of the surface tension and turbidity of the alkali protein solution in titration with HCl solution* :—The experiment was performed in the same manner as described in the former report, using 1/20 normal HCl solution for titration.

TABLE II.

Surface tension and turbidity of the protein samples.

HCl solution ccm	2.40	2.44	2.48	2.52	2.56	2.60	2.64	2.68	2.72
Manchuria Legumelin	53.51	52.48	52.70	53.46	56.00	56.30	54.22	54.15	55.50
Glycinin A	56.30	56.37	57.06	57.71	56.18	55.78	56.89	57.65	59.15
Glycinin B	52.15	53.85	53.38	51.72	51.41	52.23	52.89	53.83	54.91
Glutelin	54.78	54.93	55.46	55.46	54.37	53.96	53.87	53.37	54.15
Hokkaido (Tokachi) Legumelin	54.93	55.44	55.56	54.59	54.67	55.10	55.50	53.27	52.49
Glycinin A	52.87	53.22	53.51	53.72	53.59	52.74	52.93	54.30	54.39

TABLE II. (Continued).

HCl solution ccm	2.40	2.44	2.48	2.52	2.56	2.60	2.64	2.68	2.72
Hokkaiko (Tokachi) Glycinin B	50.00	51.20	51.73	50.00	49.89	50.85	51.62	—	—
Glutelin	53.60	52.23	53.48	53.23	54.46	54.21	54.09	54.78	54.79
Hokkaido Legumelin	56.09	55.20	54.86	53.02	53.41	56.11	53.51	53.27	55.74
Glycinin A	56.06	56.61	56.78	57.26	57.02	56.06	56.66	56.69	58.29
Glycinin B	55.42	55.98	56.83	53.76	53.58	55.74	56.06	56.69	—
Glutelin	54.07	53.34	51.64	52.55	52.10	53.93	52.83	53.62	54.22
Manchuria (Turbidity) Legumelin	±	+	++	+++	+++	+++	+++	+++	+++
Glycinin A	+	+	++	+++	+++	++	+	+	+
Glycinin B	+	+++	++	+	+	±	±	±	±
Glutelin	±	±	+	+++	+++	++	++	+	+
Hokkaido (Tokachi) Legumelin	±	+	+	+	+	++	+++	+	+
Glycinin A	±	+	++	+++	+++	++	+	+	+
Glycinin B	±	++	+++	++	+	±	±	±	±
Glutelin	±	±	±	+	+++	+++	++	+	+
Hokkaido Legumelin	±	±	±	±	+	+++	+++	+	±
Glycinin A	±	+	+	+++	++	+	±	+	+
Glycinin B	+	++	+++	++	+	+	±	±	±
Glutelin	±	±	+	+	+	+++	+++	++	+

In the above table, the maximum point of surface tension and turbidity shows a marked difference between the different kinds of soy bean proteins. Glycinin B solution reached its maximum point at the least addition of HCl, glycinin A solution needed more, glutelin solution needed still a little more and legumelin solution needed the greatest quantity of HCl. From these facts, we know that there is great difference in their iso-electric point comparing with the case of oryzenin of rice and that the iso-eleciric point of glutelin is more acidic than that of glycinin.

(b) *The specific rotatory power of diffeeent protein alkali solutions:*— Three tenths g. of water free protein were dissolved in 50 ccm of 1/10 normal NaOH solution and treated in the same manner as described in the former report.

TABLE III.

Specific rotatory power of the various protein alkali solutions.

Protein	Legumelin		Glycinin A		Glycinin B		Glutelin	
Time (hours)	24	48	24	48	24	48	24	48
Manchuria	-57.66	-54.78	-89.38	-76.40	-83.61	-74.96	-67.66	-86.50
Hokkaido (Tokachi)	—	—	-83.61	-72.08	-80.73	-77.85	-67.66	-80.73
Hokkaido	-51.90	-51.90	—	—	-77.85	-86.50	-67.66	-69.20

The rotatory power of the alkali solution of glycinin shows the highest value, that of glutelin next and that of legumelin shows lowest value. These results coincide with the difference of the iso-electric point of the proteins.

(c) *The determination of the quantities of HCl. combined with proteins*:—Twenty mg of each protein were dissolved in 5 ccm of 1/20 normal HCl solution and treated with 25 ccm of 5% cane sugar solution in the same manner as described in the former report.

TABLE IV.

Combining power of proteins with HCl.

Proteins	Legumelin		Glycinin A		Glycinin B		Glutelin	
	HCl in free sta. (mg)	HCl in combined state. (mg)	HCl in free sta. (mg)	HCl comb. state. (mg)	HCl in free sta. (mg)	HCl in combined state. (mg)	HCl in free state (mg)	HCl in comb. state. (mg)
Manchuria	3.9402	5.1773	3.4334	5.6841	4.6167	4.5008	3.5652	5.5523
Hokkaido	3.8926	5.2249	3.4887	5.6288	5.8359	3.2816	4.1566	4.9609

In the case of glycinin, the quantity of HCl. combined is greater than in the case of legumelin and glutelin.

IV. Some chemical properties of different proteins.

(a) *The content of nitrogen, sulphur and phosphorus*:—The different

proteins were analysed by the ordinary methods and the nitrogen, sulphur and phosphorus contents estimated.

TABLE V.

Nitrogen, sulphur and phosphorus contents of the proteins.

	Manchuria			Hokkaido (Tokachi)			Hokkaido		
	N	S	P	N	S	P	N	S	P
Legumelin	15.0524	0.8674	0.3826	15.9275	0.9878	0.3213	15.6918	0.9027	0.2666
Glycinin A	16.5481	0.5703	0.3337	16.2784	0.5565	0.2603	16.4161	0.6540	0.3554
Glycinin B	16.2671	0.6525	0.1321	16.8012	0.7567	0.1458	16.4953	0.7479	0.1268
Glutelin	15.1980	0.5252	0.0399	15.4190	0.5942	0.0615	14.8832	0.5076	0.0469

The nitrogen content of glycinin is highest, next glutelin and that of legumelin is lowest while the content of sulphur is the opposite and the content of phosphorus is irregular.

(b) *The determination of free amino nitrogen*:—Three-tenths g. of each water free protein was dissolved in 50 ccm of 1/10 normal NaOH solution and 20 ccm of the solution was titrated with 1/20 normal HCl solution in the presence of 20 ccm of formalin.

TABLE VI.

Free amino nitrogen of the protein samples.

	Manchuria		Hokkaido (Tokachi)		Hokkaido	
	Dry % matter	Total N%	Day % matter	Total N%	Dry % matter	Total N%
Legumelin	1.6625	11.05	1.5167	9.52	1.7208	10.96
Glycinin A	1.8667	11.28	1.9250	11.82	2.0416	12.43
Glycinin B	2.0709	12.73	2.3042	13.71	2.1292	12.90
Glutelin	1.7208	11.32	1.8667	12.10	1.8391	12.35

According to the table, the content of free amino nitrogen of glycinin is highest, next that of glutelin and that of legumelin is lowest. These results are in keeping with the differences of the iso-electric point of these proteins.

(c) *The separation and determination of amino acids*:—Different amino form nitrogens of the proteins were determined by Van-Slyke's method in the same manner as described in the former report.

TABLE VII.

Kinds of nitrogen found in various protein samples.

Dry m%	Amide-N.	Melanin-N.	Diamino N.	Mono-amino N.	Arginin-N.	Cystin-N.	His-tidin-N.	Lysin-N.
Manchuria								
Legumelin	1.0702	0.2397	5.0465	8.6961	2.2797	0.1223	1.7168	0.9277
Glycinin A	1.6029	0.2703	5.1735	9.5014	2.7139	0.0819	1.1399	1.2378
Glycinin B	1.4045	0.3931	4.8969	9.5726	2.2472	0.0924	1.3006	1.2567
Glutelin	1.0994	0.2487	4.7043	9.1456	1.8052	0.0866	1.2645	1.5480
Hokkaido (Tokachi)								
Legumelin	1.3669	0.1927	5.2829	9.0850	2.8386	0.1132	1.4763	0.8548
Glycinin A	1.6521	0.3115	5.6215	8.6933	2.6263	0.0643	1.5835	1.3474
Glycinin B	1.4835	0.5422	4.8929	9.8827	2.3458	0.1219	1.1715	1.2537
Glutelin	1.3455	0.2563	4.8712	8.9460	2.3085	0.0573	1.2335	1.2719
Hokkaido								
Legumelin	1.0746	0.1962	5.1851	9.2360	2.2173	0.1393	1.8622	0.9663
Glycinin A	1.5494	0.3841	5.0814	9.4012	2.2298	0.0701	1.4252	1.3563
Glycinin B	1.8669	0.2557	5.1148	9.2579	2.9325	0.0810	0.8993	1.2020
Glutelin	1.2810	0.2742	4.5811	8.7469	1.8755	0.0670	1.2767	1.3619
(The following figures are percentage of nitrogen).								
Manchuria								
Legumelin	7.1098	1.5925	33.5260	57.7717	15.1445	0.8127	11.4056	6.1632
Glycinin A	9.6866	1.6333	31.2633	57.4168	16.3997	0.4955	6.8885	7.4796
Glycinin B	8.6340	2.4165	30.1031	58.8464	13.8144	0.5680	7.9956	7.7251
Glutelin	7.2342	1.6367	30.9531	60.1760	11.8778	0.5695	8.3202	10.1856
Hokkaido (Tokachi)								
Legumelin	8.5823	1.2101	33.1683	57.0393	17.8217	0.7106	9.2691	5.3669
Glycinin A	10.1493	1.9133	34.5333	53.4041	16.1333	0.3952	9.7278	8.2770
Glycinin B	8.8297	3.2272	29.1224	58.8207	13.9622	0.7260	6.9726	7.4616
Glutelin	8.7263	1.6620	31.5922	58.0195	14.9721	0.3715	7.9999	8.2487
Hokkaido								
Legumelin	6.8478	1.2500	33.0435	58.8587	14.1305	0.8880	11.8672	6.1578
Glycinin A	9.4385	2.3396	30.9538	57.2681	16.5829	0.4267	8.6820	8.2622
Glycinln B	11.3178	1.5501	31.0076	56.1245	12.7777	0.4909	5.4518	7.2872
Glutelin	8.6069	1.8425	30.7804	58.7702	12.6012	0.4503	8.5783	9.1506

In the table, glycinin B is rich in melanin form nitrogen, while poor in histidin nitrogen and on the contrary, legumelin is rich in histidin nitrogen. These results show considerable differences from the rice proteins.

CONCLUSION.

The most important results from the foregoing experiments may be summarized as follows:—

(1) The protein which is extracted with water from defatted soy bean, was dialysed and glycinin A precipitate obtained, then from the filtrate, legumelin was precipitated by addition of ammonium sulphate. From the residue, glycinin B was extracted by 10% NaCl solution and from this residue, glutelin was extracted by 0.2% NaOH solution.

(2) The ash and phosphorus contents of legumelin, glycinin and glutelin decrease in the above order, which the nitrogen content is just contrary.

(3) It seems to be that the iso-electric point of glycinin is of the highest pH value and that of legumelin is lowest. In the specific rotatory power of alkali protein solution, in the combined HCl quantity, glycinin showed the highest value amongst the proteins.

(4) The content of free amino nitrogen of glycinin is greatest, that of glutelin is next and that of legumelin is smallest. The melanin nitrogen of glycinin is superior far beyond that of other proteins. On the contrary, the histidin content of legumelin is superior and the lysin nitrogen of glutelin is superior to that of other proteins.

(5) Glycinin, which is the main protein of soy bean, has the highest pH value, greatest specific rotatory power, nitrogen, free amino and melanin nitrogen content. From these chemical properties, glycinin seems to be most polymerized amongst these proteins. So the chemical properties of soy bean glycinin are quite different from those of oryzenin of rice.

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REFERENCES.

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