Biochemical Studies on the Soya Bean, I
The Chemical Changes of the Protein during the Germination of the Soya Bean in Darkness

By

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As already mentioned by Palladin in his book[1], the chemical processes which take place in the constituents of seeds during germination differ according to the chemical nature of their reserved materials. One of the writers has already studied[2] this subject in the case of starchy seeds with rice germinated in darkness. The present study is concerned with soya bean seeds which are rich protein and also in fatty matter and are employed in the Orient as food material.

The investigations of various seedlings carried on by E. Schulze[3] from 1878 to 1907, which were concerned mostly with the isolation of amino acids and other nitrogenous matters in them have stimulated many chemists to turn their attention to the study of the structure of seed proteins and the chemical changes of proteins in the seeds during germination. T. G. Thompson's investigation of the Alaska pea[4], C. J. V. Pettibone and C. Kennedy's study of corn[5], and B. Sure and W. E. Tottingham's study of the pea[6] may be mentioned as important researches in the field of germination, especially that of the last-mentioned authors, who have emphasized that asparagine is accumulated in shoots of pea seedlings germinated in the dark.

1. B. E. Linvingston, "Palladin's Plant Physiology," (1917) 175.
2. Bull. of Chem. Japan, **5**. 65, 87, 109 (1930).
3. Landw. Jahrb., **7**, 411 (1878); **9**, 689 (1880); Zeit. Physiol. Chem., **12**, 405, (1888); **22**, 435 (1896); **24**, 19 (1898); Landw. Versuchsstat., (1906) 237.
4. Jour. Amer. Chem. Soc., **37**, 230 (1915).
5. Jour. Biol. Chem., **26**, 519 (1916).
6. Ibid., **26**, 535 (1916).

In the case of soya beans, P. Molliex[1] has studied the nitrogenous constituents of the seedlings, and W. Adolph has analysed the soya bean sprouts sold in a Chinese market, but their studies contain no information with regard to the chemical changes of the seed proteins during the germination. The writers, therefore, have undertaken the present research to find the chemical changes of the proteins, the germination of the seeds taking place by release of the stored substances.

Experiment

The beans used in this experiment are called "Hokaido" beans. They were harvested in Korea in 1928, and were kept in our laboratory.

To get fresh and healthy seedlings, avoiding putrefying action by moulds, the seeds were moistened with distilled water and kept in an incubator at 30°C for 4 days; the seedlings, from 2 to 7 cm. long, thus formed, were used in the experiment.

For comparison soya bean seeds were ground into powder and analysed.

In the analysis of seeds and seedlings, the content of moisture, the total reducing sugar, the soluble and insoluble polysaccharides, cellulose, pentosan, the total nitrogen, the total fat and ash were determined, and the results are shown in the following table I.

1. *Moisture.* About 10 gms. of the sample were dried at 105°C. in an air bath to a constant weight, the loss of weight being designated as the moisture content.

2. *Water extract.* About 10 gms. of the sample were treated with hot water and (a) total reducing sugars were estimated by determining the reducing power of the solution and calculated as glucose on an ash- and moisture-free basis; (b) soluble polysaccharides were determined by hydrolysing the extract with a 3 % sulphuric acid solution on a water bath for 3 hours; it was then neutralized with sodium carbonate and filtered, and the reducing sugars in this filtrate were estimated by means of Fehling's solution and calculated as d-glucose on an ash- and moisture-free basis. The difference between the content of the reducing sugars in the solution after and before the hydrolysis was designated the soluble polysaccharide content.

1. Ann. Chim. anal., **19**, 217 (1914).

3. *Insoluble polysaccharides.* The residue from the water extract was hydrolysed with a 3 % sulphuric acid solution on a direct flame for 3 hours, and the content of reducing sugars in the solution was estimated in the usual way.

4. *Pentosans.* These were determined by transforming them into furfural.

5. *Cellulose.* The amount of cellulose was determined by the method of Cross and Bevan.

6. *Total fat.* The samples were treated with petroleum ether and the extract was designated fat.

7. *Total nitrogen and protein.* The total nitrogen of the samples was estimated by Kjeldahl's method, and the protein content in the samples was calculated by multiplying the total nitrogen content by 6.25.

8. *Total ash.* The samples were ignited carefully at a low temperature and then heated again at a higher temperature to a constant weight.

Table I

	Seeds		Seedlings		Gain or loss
Dry wt. of 100 grains.	18.3 gms.	in %	17.8 gms.	in %	−0.5
Moisture	—	10.9	—	74.6	+47.96
Ash	—	4.9	—	4.3	−0.13
Fat	3.99	21.8	2.94	16.5	−1.05
Cellulose	0.83	4.5	1.31	7.4	+0.48
Pentosan	0.91	5.0	1.08	6.1	+0.17
Red. sugar	0.02	0.1	0.45	2.4	+0.41
Sol. polysacch.	0.78	4.3	0.39	2.2	−0.39
Insol. polysacch.	1.41	7.7	2.22	12.5	+0.81
Total N.	1.24	6.8	1.16	6.5	−0.08
Protein	7.75	42.6	7.25	40.6	−0.50

From the results shown in this table, loss in weight occurs during the germination of soya bean seeds as in the case of rice seeds, and in this the fat and nitrogenous matter are mostly concerned.

The proteins in both seeds and seedlings were isolated after the powderd samples had been treated with chloroform, by extracting with water at room temperature for 1 hour, the process being repeated 3

times, then with 10 % NaCl solution, and then 0.2 % and 7% NaOH solutions, and the nitrogen content in each extract was determined with the following results:

Table II

		in Seeds	for Total N	in Seedlings	for Total N	Gain or loss
N	Water extract	0.866 gms.	72.0 %	0.894 gms.	77.4 %	+0.028gms.
	10% NaCl	0.089	7.7	0.050	4.9	−0.039
	0.2% NaOH	0.058	5.0	0.034	3.3	−0.024
	7% NaOH	0.084	7.3	0.032	3.0	−0.052
	Sum	1.097	92.0	1.010	88.6	−0.087
N	Residue	0.14	12	0.15	12	+0.01

In the seedlings, compared with the seeds, the nitrogen in the water extract increased but was decreased in the other extracts, and the conversion of some proteins in the seeds into soluble nitrogenous compounds proceeded during the process of germination. The chemical processes taking place in the plant tissues were studied more closely by examining the chemical nature of the nitrogenous matter in the aqueous extract, and Wasteney's method[1] was employed to separate the hydrolytic products (proteose, peptone etc.) of proteins in the water extracts after albumin and globulin had been separated by coagulation, the solution being made up to pH 6 by adding a few drops of 10% acetic acid. The results are shown in the following table:

Table III

	in Seeds	for T.N.	in Seedlings	for T.N.	Diff.
Water extract-N	0.866 gms.	72 %	0.894 gms.	77 %	+0.028
Globulin-N	0.656	54.0	0.572	52.0	−0.084
Albumin-N	0.025	2.0	0.020	1.7	−0.005
Meta protein-N	0.020	1.6	0.004	0.4	−0.016
Proteose-N	0.051	4.0	0.112	9.8	+0.061
Peptone-N	0.057	4.5	0.060	5.2	+0.003
Sub. peptone-N	0.010	0.8	0.075	6.6	+0.065
Polypeptid-N	0	0	0.041	3.6	+0.041

1. Jour. Biol. Chem., **62**, 1 (1924).

When the seeds germinated in the dark, some of the proteins, especially globulin, were converted by partial hydrolysis into simpler forms such as proteose and peptone, and one part would evidently be decomposed further into urea and ammonia, showing an actual increase in the proteose, sub-peptone and polypeptide fractions. The nitrogen distribution in both seeds and seedlings will give clearer information as to the proteolytic changes of the proteins. These were studied with the whole grains and with the water extract, the latter being divided into precipitate and filtrate by making the solution PH 6, and both were examined separately.

Each sample was boiled with 20 % HCl for 20 hrs. and amide-N, humin-N, monoamido-N and diamino N were determined.

Table IV

	100 Grains of Seeds			100 Grains of Seedlings		
	whole grain	Water extract		whole grain	Water extract	
		soluble part	ppt. at pH 6		soluble part	ppt. at pH 6
Amide-N	135 mg.	18mg.	120mg.	185(+50)mg.	106(+88)mg.	103mg.
Humin-N	64	30	28	30(−34)	14(−16)	42
Mono-amino-N	798	62	775	770(−28)	200(+138)	585
Diamino-N	246	20	175	200(−41)	32(+12)	147
Sum	1243	130	1098	1185	352	877

The difference in the nitrogen distribution between seeds and seedlings is especially marked in the soluble part of the water extract (shown in brackets in the table); i. e. in the seedlings as may be seen in the table, the nitrogen of the amide- and mono-amino-forms was immensely increased owing to the presence of an acidic amino acid, such as glutaminic and aspartic acid, which probably forms the main constituent of the proteose and subpeptone fractions of the water extract, and the process of the conversion of the proteins in the seeds by the germination, as a whole, is very similar to the action of superheated water on proteins[1].

1. These Memoirs, **10**, 163, 241, 249 (1927); Jour. of Japan Chem. Soc., **51**, 729 (1930).

One unusual feature of the germination of seeds when compared with the chemical action of superheated water on proteins is the liberation of urea from the proteins. The content of urea and ammonia in both the seeds and the seedlings was estimated with 10 gms. of the samples by extraction with 250 c.c. boiling water and cooling to 40°C; and ammonia was fixed with $\frac{N}{14}$ H_2SO_4 solution which was generated from the solution by adding $Na_2CO_3^1$. The result was calculated for 100 grains of the seedlings and was found to be 5 mg. ammonia and 1.5 mg. of urea, but no trace was obtained from the seeds.

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1. O. Folin, Zeitschr. f. Physiol. Chem., **37**, 161 (1902).