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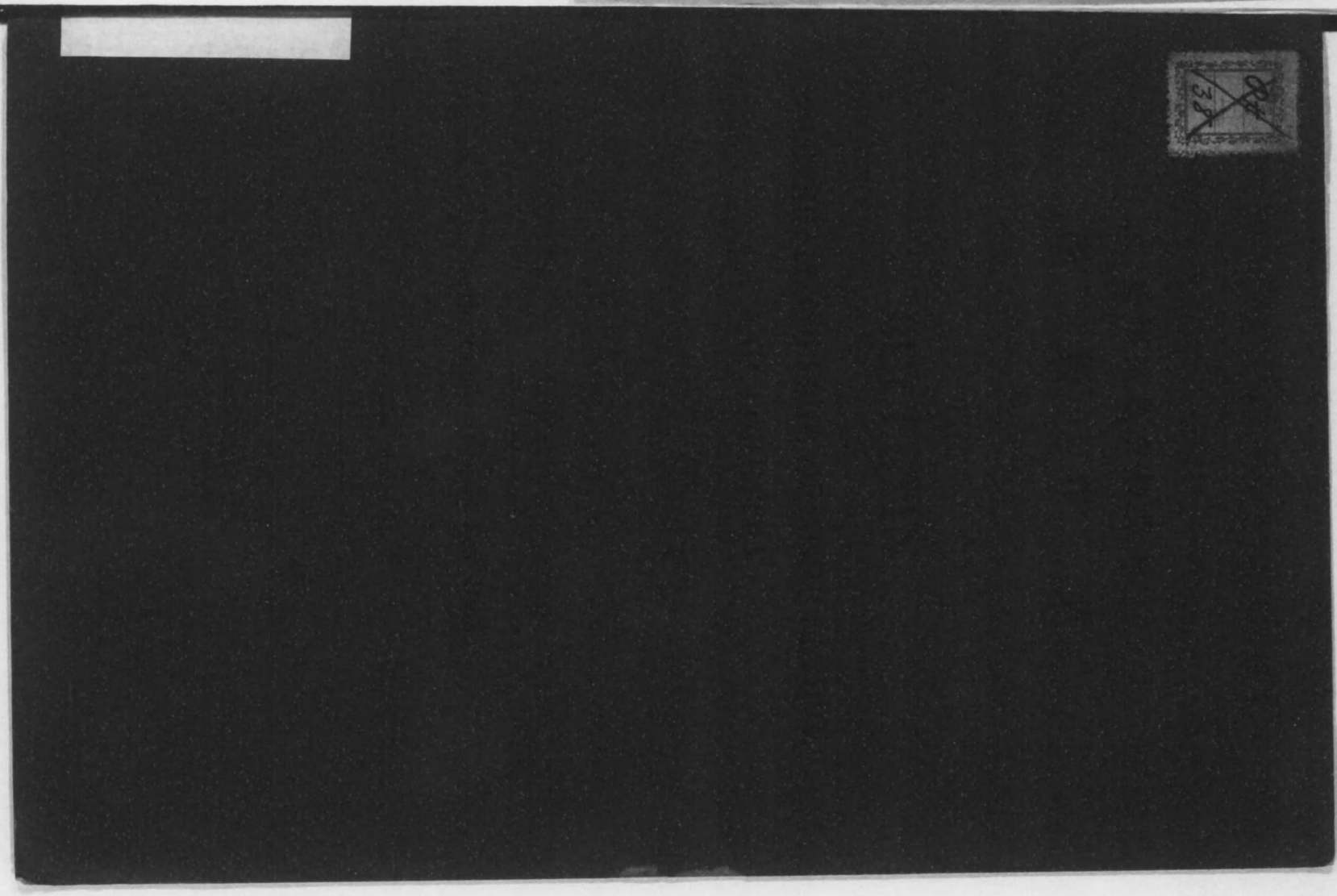

盛岡高等農林學校

學術報告

第七號

THE

BULLETIN

OF THE

IMPERIAL COLLEGE OF AGRICULTURE

AND FORESTRY

MORIOKA,

JAPAN

No. VII.

On the Composition of Soy Bean

By

Syunsuke Muramatsu

大正十三年十一月

MORIOKA, NOV., 1924.

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Chemical Studies on the Soy Bean Proteins.

By S. Muramatsu

Introduction

The soy bean is the most useful food material in this country and it is extensively used as by-food either cooked or in several other preparations. Owing to the richness in protein (ca. 40%) and fat (ca. 18%) soy bean is very suitable food material especially when it is taken with rice, as the rice is rather poor in protein and fat; therefor the people who take neither meat nor egg can find sufficient nourishment by taking soy bean cooked in various ways.

The soy bean is also most important raw material for technical purposes, as it contains much amount of fat; and it is separated with a hydraulic press or by extracting with benzene. The soy bean oil is used as a food material or to make soaps, and the oil cakes may be used as a food material or as a nitrogenous manure.

The soy bean flour is especially suited for the diabetics as it contains only slight traces of starch and comparatively small quantities of those carbohydrates which are available to the human beings. As Osborne and Mendel (1) say it is very suitable for animal food and we can understand why it is so when we see that it contains both vitamines soluble in water and fat. They say (2) that soy beans fed as the sole source of protein, or as a supplement to corn gluten, are suitable for the nutrition of rats.

They contain sufficient water-soluble vitamine to promote normal growth; for the diets containing soy bean flour, butter fat, starch and an artificial salt mixture have promoted growth just as well as similar rations in which natural protein-free milk was used instead of the soy bean flour.

Dr. T. B. Osborne and G. F. Campbell (3) isolated the proteins of soy bean and studied their composition and chemical properties and Dr. Harris (4) made some studies of the soy bean albumins. There have been many articles dealing with the compositions other than proteins

of the soy bean in this country, for instance, on the soy bean oil Kametaka (5), Nishiyama (6), Keimatsu (7), Tsuzimoto (8), and Okada (9); on the carbohydrates Yukawa (10); on creatinin Oshima and Ariizumi (11); on the nitrogenous compounds Yoshimura (12); but nothing about its proteins until recently Sato (13) published an article about the use of soy bean protein for technical purposes.

As I have made precise studies on the proteins of the soy bean I shall report the results. There are many difficulties on isolating soy bean proteins. The fat which is very rich in soy bean is emulsified when the water or salt extract is pressed out and it differs from hemp seeds or castor beans in that it is very difficult to remove it from protein. Dr. Osborne proposed to extract the oil with petroleum ether previously but I have isolated the proteins from its natural flour.

The soy bean used for the analysis was moderate sized yellow variety (variety name unknown), that was raised on the farm of the Agric. Exper. Station of Conn., U. S. A., which I could get through the courtesy of Dr. Jenkins, the director of the Agric. Exper. Station.

Experiment 1.

To see how the nitrogenous matters are dissolved out in its extract I made the following experiments:

25 g soy bean flour (moisture: 10.61%) was extracted thrice with distilled water and the extract made up to 1200cc. For the extraction I mixed with a small amount of water and the doughy mass was passed through the hand mill to make the extraction complete. Then some more water was put in and stirred thoroughly. The mixture was centrifuged (number of revolution was 3000 per minute) for twenty minutes and the aqueous solution was poured off. Some fat was separated on the surface in the form of scum. Water was added in the residue and after mixing thoroughly it was centrifuged again as before. The great deal of the soluble nitrogenous matters were dissolved out and the extracts were mixed and filtered through paper pulp in a Buchner funnel.

The residue from the aqueous extract was mixed thoroughly with 10% sodium chloride solution and it was centrifuged as before. The extractions were made thrice and the filtered solution was made up to 700cc.

The residue from the salt extract was washed thoroughly with water and then extracted with 0.2% sodium caustic solution as before and the extract was made up to 600cc.

The contents of nitrogen in the extracts above mentioned were as follows:

	In 100 parts of dry flour	In 100 parts of total nitrogen in the substance
Total nitrogen in the substance	6.94	100.00
Do. in its water extract	5.97	86.02
Do. in its salt extract after extracted with water	0.26	3.75
Do. in its alkali extract after extracted with water and salt solution	0.16	2.30
Nitrogen remained in the final residue	0.55	7.93

From this table we will see that great deal of the nitrogenous matters are extracted by water and some part of globulin remains in the residue and small amount of nitrogen which is insoluble in sodium chloride solution but soluble in alkali remains in the salt extract residue. Some author calls this kind of protein which is soluble in dilute alkali solution "Glutelin", but I am in doubt that this is a mixture of different substances which are soluble in alkali. I got this substance in dry state 1.10% of the dry flour, and I found that it contains only 11.72% nitrogen.

To see the different forms of nitrogen in its water solution I determined them as follows:

1. Total nitrogen. By Kjeldahl's method.
2. Globulin-nitrogen. Globulin was precipitated by acidifying with dilute acetic acid avoiding large excess of the acid, as the precipitated protein will come in solution again. The precipitate was centrifuged and separated, in which the nitrogen was determined by Kjeldahl's method.
3. Albumin-nitrogen. Albumin was coagulated by boiling in the acid solution, and the precipitate collected on a filter paper and its nitrogen was determined in the same way as before.
4. Proteose-nitrogen. Proteose was precipitated by copper sulphate in its neutral solution, and its nitrogen was determined in the same way as before.
5. Non-albuminoid nitrogen. By difference.

	In 100 parts of dry flour	In 100 parts of total nitrogen soluble in water
Total nitrogen	6.94	—
Total nitrogen in water solution	5.97	100.00
Globulin-nitrogen	5.03	84.25
Albumin-nitrogen	0.32	5.36
Proteose-nitrogen	0.26	4.36
Non-albuminoid nitrogen	0.36	6.03

Thus I found that a great deal of protein in the soy bean is extracted by water and even the globulin comes in the aqueous extract. Only small amount as 3.75% of the total nitrogen is extracted by 10% brine and another 2.30% extracted by alkali solution and still 7.93% remains in the final residue, which is very difficult to extract.

The reason why so much amount of soy bean globulin is dissolved out in the aqueous extract due to the presence of potassium phosphate and other salts.

Dr. Osborne (14) says about the solubility of edestin, the globulin of hempseeds, that it becomes soluble in water when it is combined with acid radicals.

As the protein has very large molecular weight as 15000 or its

multiples as proposed by Krieger and Osborne, a very small amount of mineral acid radical can combine with much amount of protein and can make it soluble in water.

I also compared the amount of different proteins contained in the different varieties which grew in Morioka and its vicinity. The method of analysis was made as follows:

10g air-dried soy bean flour was taken and putted in some water. The whole mixture was macerated in a mortar and some more water added, and then it was centrifuged for twenty minutes. The residue was ground again in the mortar to make extraction complete and centrifuged again. The mixing and the separation was made successively five times in all. The separated aqueous extract was filtered through paper pulp and made up to 400cc.

The residue from the water extract was extracted thrice with 10% sodium chloride solution and washed then twice with distilled water, and the saline extract filtered through paper pulp and made up to 250cc.

The residue from the saline extract was extracted with 0.2% sodium caustic solution four times successively and washed with the same solution and after being filtered clear it was made up to 250cc.

The method of the determination of the nitrogen in the different forms was the same as in the former experiments.

1. Shiratama:

Moderate sized and yellow variety which was raised on the farm of Agric. Exper. Station of Iwate Pref.

	In 100 parts of dry matter	In 100 parts of total nitrogen
Total nitrogen	6.977	100.00
Do. in the aqueous extract	6.341	90.88
Do. in the saline extract	0.277	3.97
Do. in the alkali extract	0.114	1.64
Do. in the final residue	0.245	3.51

	In 100 parts of total nitrogen in the aqueous extract
Globulin-N	82.66
Albumin-N	4.68
Proteose-N	3.61
Non-albuminoid nitrogen	9.05

2. Awomame:
Moderate sized and green variety which was raised on the farm of the Agric. Exper. Station of Iwate pref.

	In 100 parts of dry matter	In 100 parts of total nitrogen
Total nitrogen	6.783	100.00
Do. in the aqueous extract	5.130	75.63
Do. in the saline extract	0.360	5.31
Do. in the alkali extract	0.164	2.42
Do. in the final residue	1.129	16.64

	In 100 parts of the total nitrogen in the aqueous extract
Globulin-N	82.69
Albumin-N	3.53
Proteose-N	1.29
Non-albuminoid nitrogen	12.49

3. Kurohiramame:
Large sized, flatted and black variety which was raised on the farm of Morioka Agric. College.

	In 100 parts of dry matter	In 100 parts of total nitrogen
Total nitrogen	6.931	100.00
Do. in the aqueous extract	5.578	80.48
Do. in the saline extract	0.324	4.67
Do. in the alkali extract	0.203	2.93
Do. in the final residue	0.826	11.92

	In 100 parts of total nitrogen in the aqueous extract
Globulin-N	86.81
Albumin-N	1.74
Proteose-N	2.91
Non-albuminoid nitrogen	5.54

4. Yagi:
Moderate sized and yellow variety which was raised on the farm of Morioka Agric. College.

	In 100 parts of dry matter	In 100 parts of total nitrogen
Total nitrogen	6.528	100.00
Do. in the aqueous extract	4.256	65.20
Do. in the saline extract	0.418	6.40
Do. in the alkali extract	0.228	3.49
Do. in the final residue	1.626	24.91

	In 100 parts of total nitrogen in the aqueous extract
Globulin-N	88.58
Albumin-N	3.58
Proteose-N	4.64
Non-albuminoid nitrogen	3.20

5. Nioiwase:
Large sized and yellow variety, which grew on the farm of Morioka Agric. College.

	In 100 parts of dry matter	In 100 parts of total nitrogen
Total nitrogen	6.918	100.00
Do. in the aqueous extract	5.470	79.07
Do. in the saline extract	0.417	6.03
Do. in the alkali extract	0.199	2.88
Do. in the final residue	0.832	12.02

	In 100 parts of total nitrogen in the aqueous extract
Globulin-N	86.64
Albumin-N	4.64
Proteose-N	2.32
Non-albuminoid nitrogen	6.40

Thus we will see that the amount of nitrogen which is extracted by water, saline solution and dilute alkali solution, and that which remains in the final residue, is different according to the different varieties, as we can make it clear from the following table.

	Shiratama	Awomame	Kurohiramame	Yagi	Nioiwase
Total nitrogen in 100 parts of dry matter	6.977	6.783	6.931	6.528	6.918
Do. in the aqueous extract	90.88	73.63	80.48	65.20	79.07
Do.in the saline extract	3.97	5.31	4.67	6.40	6.03
Do. in the alkali extract	1.64	2.42	2.93	3.49	2.88
Do. in the final residue	3.51	16.64	11.92	24.91	12.02

Shiratama, Kurohiramame and Nioiwase are rich in nitrorgen which is soluble in water and the amount of nitrogen which remains insoluble in the final residue is very rich in Yagi and next comes Awomame.

There are also some difference in the amount of nitrogen in the difierent forms of protein in the aqueous extract as we can see from the following table.

	Shiratama	Awomame	Kurohiramame	Yagi	Nioiwase
Globulin-nitrogen	82.66	82.69	89.81	88.58	86.64
Albumin-nitrogen	4.68	3.53	1.74	3.58	4.64
Proteose-nitrogen	3.61	1.29	2.91	4.64	2.32
Non-albuminoid nitrogen	9.05	12.49	5.54	3.20	6.40

Thus we will see that Kurohiramame and Yagi are very rich in globulin-nitrogen and Kurohiramame is very poor in albumin-nitrogen while non-albuminoid nitrogen is most scanty in Yagi.

Moreover I compared the amount of the different forms of protein in the unripened soy bean which is used as a food material in this country, and the variety Nioiwase is most suited for this purpose.

The soy bean was dried at 50°C and after being ground it was treated as the former experiments and got the following results:

	Moisture in the fresh state
Nioiwase:	
It was harvested on Sept. 18,1919	64.55%
Yagi:	
It was harvested on Oct. 2,1919	63.22%

Both varieties were full sized but were green and soft and just fitted for cooking.

Nioiwase:	In 100 parts of dry matter	In 100 parts of total nitrogen
Total nitrogen	6.825	100.00
Do. in the aqueous extract	3.871	56.72
Do. in the saline extract	0.377	5.52
Do. in the alkali extract	0.635	9.30
Do. in the final residue	1.942	28.46

	In 100 parts of total nitrogen in the aqueous extract
Globulin-N	84.84
Albumin-N	3.23
Proteose-N	2.45
Non-albuminoid nitrogen	9.43

Yagi:	In 100 parts of dry matter	In 100 parts of total nitrogen
Total nitrogen	5.772	100.00
Do. in the aqueous extract	3.949	68.41
Do. in the saline extract	0.328	5.68
Do. in the alkali extract	0.159	2.75
Do. in the final residue	1.336	23.15

	In 100 parts of total nitrogen in the aqueous extract
Globulin-N	82.27
Albumin-N	6.86
Proteose-N	2.03

Non-albuminoid nitrogen	8.84

There are also some difference in the amount of nitrogen which is extracted by water and the other different solutions, and that remains insoluble in the final residue in the unripened seeds of the different varieties.

	Yagi	Nioiwase
Total nitrogen in 100 parts of dry matter	5.772	6.825
Do. in the aqueous extract	68.42	56.72
Do. in the saline extract	5.68	5.52
Do. in the alkali extract	2.75	9.30
Do. in the final residue	23.15	28.46

Thus we can see that Yagi is far rich in nitrogen soluble in water and poor in nitrogen soluble in the dilute alkali solution.

We can see some difference in the amount of nitrogen in the different forms of protein in the aqueous extract of them from the following table.

	Yagi	Nioiwase
Globulin-nitrogen	82.27	84.84
Albumin-nitrogen	6.86	3.28
Proteose-nitrogen	2.03	2.45
Non-albuminoid nitrogen	8.84	9.43

There is not much difference in them but Nioiwase is rich in globulin-nitrogen while more albumin is contained in Yagi.

In the both varieties of Yagi and Nioiwase we can compare the same relation in nitrogen between the young and ripened seeds.

	Yagi		Nioiwase	
	Young	Ripened	Young	Ripened
Total nitrogen in the aqueous extract	68.42	65.20	56.72	79.07
Do. in the saline extract	5.63	6.40	5.62	6.03
Do. in the alkali extract	2.75	3.49	9.30	2.88
Do. in the final residue	23.15	24.91	28.46	12.02

Total nitrogen in 100 parts of dry matter	5.772	6.528	6.825	6.918
Do. in the aqueous extract	3.949	4.256	3.871	5.470
Globulin-N in the aqueous extract	82.27	88.58	84.84	86.64
Albumin-N „ „ „ „	6.86	3.58	3.28	4.64
Proteose-N „ „ „ „	2.03	4.64	2.45	2.32
Non-albuminoid nitrogen „	8.84	3.20	9.43	6.40

Thus we can see that there are some difference in the both varieties but globulin-N is generally rich in the ripened seeds and non-albuminoid nitrogen is scanty in them.

Experiment 2.

A. Preparation of protein from soy bean.

The air-dried soy bean (moisture:10.61%) were ground to fine powder and 6 kg of this meal was taken and put in 20 L of tap water and it was passed twice through the pulveriser to make the extraction complete.

As it was exceedingly difficult to press out the solution I mixed with some amount of clean filter paper and stirred thoroughly. Then the doughy mass was enclosed in several heavy cloths and then squeezed out with a hydraulic press; thus I got 15600cc. aqueous extract.

The residue was separated into two equal parts in weight. One half of it was extracted with 10 L 10% sodium chloride solution, thus getting salt extract and other half was extracted with 0.2% sodium caustic solution, thus getting the alkali extract.

1. Proteins from the aqueous extract.

When the aqueous extract was saturated with ammonium sulphate all of the proteins were salted out and the precipitate was collected on a large quick filter. As the precipitate on the filter paper contained a large amount of fluid it was pressed out between the several sheets of filter paper.

The precipitate (A) was dissolved putting in water and the greater part of it dissolved in water as the precipitate contained some ammonium

sulphate; but for the sake to dissolve all of the globulin I added some 10% sodinm chloride solution and filtered through paper pulp, thus getting clear opalrescent solution and the solution was putted in several sheets of parchment paper and dialysed in water current for one week.

The precipitate (B) which was dialyzed out was separated in a large mass and some part of it showed a coherent mass. To see how all globulin was separated or not I took a small portion of the liquid part and put into some distilled water, but there appeared no precipitate at all and found that all of the globulin was separated. The precipitate was collected on a filter paper.

The precipitate (B) was dissolved again in 10% sodium chloride solution and filtered. The solution showed a little milky appearance, so I centrifuged it and found that some fat separated and it was filtered nearly clear. Then the globulin solution was dialyzed again.

The precipitate (C) was separated in a big coherent mass. It was dissolved in 5% salt solution and precipitated by pouring into large bulk of water and the precipitate was washed with 50% alcohol until the filtrate showd no reaction of chlorine. The precipitate was transfered in 60% alcohol and kept overnight and it was treated in the same way with stronger alcohol (75% and 85%) and then kept in absolute alcohol for several days until the dried protein showed no horny appearance when it was dried. Finally it was filtered as soon as possible by suction pump and put in ether which was free from water and kept for several days. Then it was collected on filter paper and dried in vacuum desiccator giving 157g of preparation (C).

To the filtrate from the precipitate (A) I added some dilute acetic acid and found some white precipitate was formed but it gave no biuret reaction, so I discarded it.

As, in the filtrate from the precipitate (B) may be present some globulin combining with some base I took a small portion of it and passed some carbon dioxide in it and found that a bulky white precipitate was formed. So to separate the globulin the filtrate was acidified with dilute acetic acid and white precipitate was collected on a filter paper and dissolved in 10% sodium chloride solution and after filtering clear by

paper pulp, it was dialysed in water current. The precipitate was redissolved in 5% sodium chloride solution and precipitated by pouring into a large bulk of water which contained some alcohol in it, and washed with 50% alcohol until its washing showed no chlorine reaction and it was purified and dried as in the case of preparation (C). The preparation (D) weighed 43g.

The filtrate from the globulin (D) contain albumins and proteose. The albumins were separated by fractional coagulation. All of the filtrate were put in a large double walled kettle and heated by steam, stirring constantly. When the temperature was raised to 55°C the fluid showed a milky appearance and the coagulation was completed at 60°C, the liquid part getting quite clear. The precipitate was collected on a filter paper and washed with distilled water and then with 50%, 80%, 95%, and finally with absolute alcohol and ether as in the case of the globulin (C) and dried in vacuum desiccator giving 31g of preparation (P).

The filtrate of the preparation (P) was heated again in the same kettle and it was kept stirring constantly while the heating continued. The next coagulation begun at 68°C and completed at 72°C. So, the coagulum was collected on a filter paper and washed with alcohol and ether as before and it was dried giving 21g of preparation (Q).

The filtrate of the preparation (Q) was heated again in the same kettle and it was kept stirring constantly. The third coagulation begun at 85°C and was completed at 90°C. The coagulum was collected on a filter paper and washed with alcohol and ether as before and it was dried; which gave 14g of preparation (R). When the filtrate of the preparation (R) was heated again to higher temperature there occurod no coagulation at all.

The filtrate of the coagulated albumins was saturated with ammonium sulphate and the precipitate salted out was dissolved in water and after filtering clear it was dialysed out in water current. After all mineral matters were dialysed out, the proteose was precipitated by dialysing in absolute alcohol. The precipitate was washed with absolute alcohol and ether giving 20.27g of preparation (S).

Short plan for the separation of the proteins from the aqueous extract.

Water extract
saturated with am. sulphate

Filtrate	Precipitate(A)
Acidified with acetic acid. Precipitate showed no biuret reaction–discarded.	Dissolved in water and 10% NaCl solution, it was dialysed for one week.

Filtrate	Precipitate(B)
Acidified with dilute acetic acid and white precipitate was formed.	Dissolved in 10% NaCl solution and dialysed.

Filtrate	Precipitate(D)	Precipitate(C)
Albumins were separated by fractional coagulation. Coagulum at 60°C gave 31g(P). Coagulum at 72°C gave 21g(Q). Coagulum at 90°C gave 14g(R).	Dissolved in 10% NaCl solution and dyalised in water. The precipitate was redissolved in 5% NaCl solution and precipitated by pouring into large bulk of water which had some alcohol in it. The precipitate was washed with alcohol and ether, giving 43g(D).	Separated in a big coherent mass, which was dissolved in 5% NaCl solution and precipitated by pouring into large bulk of water and washed with alcohol and ether weighing 157g(C).

Filtrate
Proteose was salted out by am. sulphate and redissolved in water and dialysed out in alcohol, weighing 20.27g(S).

2. Proteins from the salt extract.

The salt extract was filtered through paper pulp and saturated with ammonium sulphate. The precipitate was dissolved in 10% sodium chloride solution and filtered through thick paper pulp. But as the solution contain much amount of fat in the form of emulsion the solution showed milky appearance. So the solution was centrifuged for twenty minutes and the fat separated as a scum on the surface. By repeating this process the greater part of fat was separated from the solution and when it was filtered through paper pulp I could get clear solution and it was dialysed in water current for one week. The globulin separatd in a coherent mass and it was dissolved in 5% sodium chloride solution and

the filtered liquid was poured into large bulk of distilled water and the precipitate was collected on a filter paper and purified as in the case of the globulin from water extract weighing 94g (which corresponds 188g from the whole flour.)

The filtrate from the globulin of the first dialysis was acidified with dilute acetic acid and the precipitate was collected on a filter paper and it was dissolved in 5% sodium chloride solution and dialysed out; but the yield was very poor and its filtrate contained very small amount of nitrogen. So, all parts were discarded.

The filtrate from the acetic acid precipitate was heated in a double walled kettle by steam to 60°C. The coagulum which was formed at this temperature was collected on a filter paper and washed with alcohol and ether weighing 5.5g (which corresponds 11g from the whole flour.)

The filtrate from the coagulum at 60°C was heated stronger finally to 90°C, but the coagulum formed was very little, so it was discarded with its filtrate as the filtrate contained very small amount of nitrogen in it.

Thus we see that greater part of albumin comes in the aqueous extract and some part goes to the salt extract. This is the reason that the flour was extracted just once with water and some part remained in the residue and it is that which was extracted by the salt solution.

Short plan to separate the proteins from the salt extract.

Proteins were salted out with ammonium sulphate and it was redissolved in 10% NaCl solution and filtered. The clear solution was dialysed and the globulin was separated in the form of a coherent mass.

Filtrate
Acidified with dilute acetic acid

Precipitate
Dissolved in 5% NaCl solution and it was filtered clear.
The solution was poured into a large bulk of water.
The precipitate was collected on a filter paper and washed with 50% alcohol and finally with absolute alcohol and ether giving 94g (which corresponds 188g from the whole flour.)

Filtrate
Coagulum at 60°C weighed 5.5g (which corresponds 11g from the whole flour.)
Coagulum at 90°C was very little; so it was discarded.

Precipitate
Dissolved in 5% NaCl solution and dialysed out, but very small amount of globulin separated out and its filtrate contained a little nitrogen; all were discarded.

3. Proteins from the alkali extract.

The alkali extract had milky appearance and I could not get clear solution even when I filtered it through paper pulp. The protein was precipitated with dilute acetic acid by making it slight acid in reaction.

The protein was collected on a filter paper and dissolved in 0.2% sodium caustic solution. This solution was filtered through paper pulp and precipitated by dilute acetic acid. It was washed with 50% alcohol and the protein was soaked in 90% alcohol for several days. Thus all of the fatty matters were dissolved out in alcohol. (The alcohol was tested by pouring into water and when the alcohol got rid of fat no turbidity appeared.)

The fat-free protein was redissolved in 0.2% sodium caustic solution and it was filtered clear. The protein was precipitated by acetic acid and collected on a filter paper and washed with 50% alcohol and then with absolute alcohol and ether as usual. The dried precipitate weighed 139g (which corresponds 278g from the whole flour.)

The filtrate from the acid precipitate was saturated with ammonium sulphate and the separated precipitate was dissolved in 5% sodium chloride

solution. The protein solution was dialysed and the globulin separated was very small in quantity. The albumin was separated by coagulation heating in double walled kettle to 72°C and washed thoroughly with alcohol and ether giving 2g (which corresponds 4g from the whole flour.)

The filtrate was heated to 90°C and the coagulum formed weighing 0.35g (which corresponds 0.7g from the whole flour.)

Short plan to separate the proteins from the alkali extract.

The extract was acidified slightly with acetic acid.

Filtrate
Saturated with ammonium sulphate.

Precipitate
Dissolved in 0.2% sodium caustic and precipitated by acetic acid.
To get rid of fat the precipitate was washed with alcohol and soaked in strong alcohol for several days and washed with a mixture of alcohol and ether.
The precipitate was dissolved in alkali solution again and filtered clear. It was precipitated by acetic acid and washed with alcohol and finally with ether. It was dried in vacuum desiccator weighing 139g, which corresponds 276g from the whole flour.

Filtrate
Discarded.

Precipitate
Dissolved in salt solution and dialysed out, but slight traces of globulin separated, so, it was discarded.

Filtrate
Saturated with ammonium sulphate.

Filtrate
Discarded.

Precipitate
Dissolved in water and dialysed, but no globulin separated. This was filtered clear and albumins were separated by coagulation.
Coagulum at 77°C weighed 2g which corresponds 4g from the whole flour.
Coagulum at 95°C weighed 0.35g which corresponds 0.7g from the whole flour.

B. Chemical properties of proteins isolated from soy bean.

Albumins:

Albumins separated in three fractions give all protein reactions, e. g. Millon's, Adamkiewicz, biuret and xanthoproteic reactions.

In these reactions P and Q showed nearly the same but R showed

some difference.

In Adamkiewicz reaction the colour produced by P and Q was rather light while it was strong in the case of albumin R, in Millon's reaction albumin P and Q showed strong and R rather light in colour. Other reactions of albumin R were nearly the same as albumin P and Q.

Globulin:

Globulin is the same with that which Dr. Osborne called "Glycinin".

1. It is soluble in sodium chloride and other salt solutions, and can be separated from its solutions by salting out with ammonium sulphate, or dialysed out in water current or separated by dilution.
2. When it is dialysed out some part of it separates as coherent mass and other parts as white flocculents.
3. When soy bean is extracted with water repeatedly, greater part of the globulin dissolves out as it combines with salts and become soluble in water.
4. Globulin dissolved in 10% sodium chloride solution does not coagulate even when it is heated to boiling; and it is not also precipitated by acetic acid, $CaCl_2$, $BaCl_2$ nor $MgCl_2$ solutions.
5. Glycinin is precipitated by tannic acid, picric acid and other salts of heavy metals.
6. Millon's, Adamkiewicz, biuret, xanthoproteic and other reactions occur in its solution.

Proteose:

Nearly all of the proteose is extracted by water and contained in its aqueous extract. It is readily soluble in distilled water and gives all protein reactions. But there are some differences from other proteins in its reaction, e.g. its Adamkiewicz reaction is very week and the xanthoproteic reaction occurs but no precipitate is separated.

C. Composition of the soy bean proteins.

Carbon and hydrogen were determined by the ordinary method of weighing the amount of CO_2 and H_2O which were produced by combustion of the proteins and the total nitrogen by Kjeldahl's method while the amino-nitrogen was determined by van Slyke's method.

Sulphur was determined by the method which was proposed by Osborne (15) giving very satisfactory result. (About ten grams of sodium peroxide were converted into hydrate in a nickel crucible by adding a little water and boiling over an alcohol lamp until the excess of water was expelled. From one to two grams of the protein was then stirred into the slightly cooled hydrate and oxidised by gradually raising the heat and adding small portions of sodium peroxide until the oxidation was complete. The fused mass was then dissolved in 400cc. of water, its solution strongly acidified with hydrochloric acid, boiled until the excess of peroxide was destroyed and chlorine expelled, filtered through pure paper, made neutral with ammonia and an excess of 4cc. of concentrated hydrochloric acid added. From the boiling solution sulphuric acid was precipitated by gradually adding a solution containing one gram of barium chloride. After standing overnight on a steam table, the barium sulphate was filtered out, washed, ignited and weighed.)

Oxygen by difference.

(A) Albumins:

	Albumins			
	P	Q	Average of P and Q	R
Carbon	53.05%	53.24%	53.15%	52.98%
Hydrogen	6.82 „	6.63 „	6.73 „	6.79 „
Nitrogen	16.32 „	16.23 „	16.27 „	15.14 „
Amino-nitrogen	1.13 „	1.08 „	1.10 „	0.95 „
Do. after hydrolysed	8.47 „	8.24 „	8.36 „	8.49 „
Sulphur	1.03 „	1.05 „	1.04 „	0.29 „
Oxygen	22.78 „	22.85 „	22.81 „	24.80 „

Thus we see that the albumin coagulated under 72°C is very alike in its composition and it is the same with that of legumelin from pea, lentil, vetch or cow pea and that which Osborne isolated from soy bean.

	Legumelin from pea, lentil, vetch, cow pea	Albumin from soy bean		
		By Osborne	Average of PandQ	R
Carbon	53.35%	53.06%	53.15%	52.98%

Hydrogen	6.97%	6.97%	6.73%	6.79%
Nitrogen	16.28 „	16.14 „	16.27 „	15.14 „
Sulphur	1.07 „	1.17 „	1.04 „	0.29 „
Oxygen	22.33 „	22.66 „	22.81 „	24.80 „

Albumin R is quite different from legumelin in its composition containing 1.13% less nitrogen and 0.75% less sulphur, while it shows all protein reactions and it has the properties of albumin. In the ordinary way which is used to separate albumin it is mixed with legumelin.

There is no description about such an albumin as this, so I propose to call this "Soylegumelin".

(B) Globulins:

	Globulins	
	C	D
Carbon	51.87%	51.80%
Hydrogen	9.81 „	6.94 „
Total nitrogen	17.17 „	19.45 „
Amino-nitrogen	1.08 „	1.32 „
Do. after hydrolysed	8.41 „	8.72 „
Sulphur	0.78 „	0.42 „
Oxygen	23.57 „	24.39 „

Dr. Osborne found that the globulin of the soy bean contain 0.50% less nitrogen and 0.30% more sulphur than another legumin of pea, vetch, lentil etc. and called it "glycinin", as we can see from the following table.

	Legumin from pea, lentil, vetch etc.	Glycinin by Osborne	Globulin C
Carbon	51.72%	52.12%	51.87%
Hydrogen	6.95 „	6.93 „	6.81 „
Nitrogen	18.04 „	17.53 „	17.17 „
Sulphur	0.41 „	0.79 „	0.78 „
Oxygen	22.88 „	22.63 „	23.37 „

Thus we see that globulin C is the same to glycinin which Osborne has separated.

The globulin D which was precipitated by dilute acetic acid is different from others in its composition. Dr. Osborne says that soy bean may contain a small proportion of phaseolin which is more soluble in dilute salt solution than glycinin. I found that the globulin D is very alike to phaseolin in its composition and reactions.

	Phaseolin from cow pea, kidney bean etc.	Phaseolin from soy bean by Osborne	Globulin D
Carbon	52.47%	51.94%	51.80%
Hydrogen	6.92 „	6.88 „	6.94 „
Nitrogen	16.54 „	16.51 „	16.45 „
Sulphur	0.54 „	0.60 „	0.42 „
Oxygen	23.53 „	24.07 „	24.39 „

Thus we see that globulin D has the same composition with phaseolin.

(C) Proteose:

The composition of proteose is alike to that which Osborne separated from the other leguminous seeds.

	Proteose from pea, lentil, horse bean etc.	Proteose from soy bean	
		By Osborne	S
Carbon	50.05%	48.75%	48.69%
Hydrogen	6.74 „	6.28 „	6.30 „
Nitrogen	16.96 „	16.14 „	16.60 „
Sulphur	1.71 „	28.82 „	28.41 „
Oxygen	24.54 „		

Conclusion.

1. When soy bean flour is extracted with distilled water 86.02% of the total nitrogen comes in the solution; 3.75% in the salt extract from the residue extracted with water; and 2.30% in the dilute alkali extract from the residue extracted with water and salt solution.

7.93% of the total nitrogon remained in the final residue.

2. The protein extracted by dilute aikali solution which belong to "Glutelin" reaches 1.10% of the dry flour and it contains only 11.72% of nitrogen; it seems reasonable to conclude that it contains some impurities other than protein.
3. In the aqueous extract 84.25% of the total nitrogen consists of globulin, 5.36% albumin and 5.03% proteose and other 5.39% non-albuminoid nitrogen.
4. Great deal of the globulin in the soy bean comes in the aqueous extract combining with mineral matters contained in it.
5. When the protein was isolated in large scale the yield from 6 kg air-dry flour was as follows:

Clobulin:	From aqueous extract	200g
	From salt extract	188g
	Total	388g
Albumin:	From aqueous extract	66g
	From salt extract	11g
	Total	77g
Proteose:	From aqueous extract	20.27g

7. 78.5% of the globulin consists of "Glycinin and 21.5% of "Phaseolin".
8. 78.79% of the albumin consists of "Legumelin" but it contains 21.21% of a special albumin" Soylegumelin" which has the composition as

Carbon 52.98% Hydrogen 6.79% Nitrogen 15.14%
Sulphur 0.29% Oxygen 24.80%

9. Proteose is the same which presents in the other leguminous seeds.

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On the Starch of Soy Bean.

By S. Muramatsu

There are many investigations about the carbohydrates of soy bean. Street and Bailey (The Journ. of Indust. & Eng. Chem. Vol. 7, No. 10 853, 1915) found that in the Hollybrock variety the nitrogen-free extract exists in the following forms:

Total sugar	4.51%
Starch	0.50 ,,
Dextrin	3.14 ,,
Pentosan	4.94 ,,
Galactan (less 0.24 due to raffinose)	4.86 ,,
Cellulose	3.29 ,,
Waxes and colour principles &	8.60 ,,

Yukawa (Journ. of the Tokyo Chem. Society Vol. 38, No. 4, 1917) found that the carbohydrates of soy bean consist of the following substances:

	In 100 parts of dry matter	In 100 Parts of carbohydrates
Total carbohydrates	21.69	100.00
Cane sugar	5.90	37.20
Stachyose	3.52	16.22
Araban	3.30	17.52
Galactan	4.62	21.30
Crude cellulose	3.83	17.76

On the existence of starch in soy bean was not yet definitly proved. Winton (Microscopy of vegetable food P. 248) says that the starch is entirely absent in soy bean. Some European investigators (Meissl and Boecker) report from 3 to 5% starch, while the others have not identified starch in the soy bean. Hartz (Zeit. Allg. Oesterr. Apoth. Vers., 23, 40, 1885) found that when the beans do not ripen throughly or when they are allowed to ripen after the stems are cut, starch may be present, certain varieties being more likely to contain it than others, whereas if the beans are thoroughly ripened they are practically free from starch.

Street and Bailey found the equivalent of 0.5% of starch in the original material. Their method of determination was as follows: 4 g of the soy bean meal are extracted with ether and boiling alcohol and were washed with cold water first by decantation and then on the filter paper until the washings amounted to about 300cc. This residue was digested with freshly prepared malt extract in the usual manner for two ninety minutes periods. The solution was then hydrolysed and its reducing power determined.

Inouye (The bulletin of the Agr. Coll. Tokyo Univ. Vol. 2, P.210,1895) says that he has confirmed the absence of starch in the Japanese soy bean.

Yukawa says that he could not detect the starch in the hot water extract of soy bean meal which was previously extracted with ether and alcohol respectively; but when the residue of ether and alcohol extract was washed with cold water and then hydrolysed with malt diastase he found the equivalent of 0.05% of starch, thus concluding that he could not prove the presence of starch in soy bean and the several varieties of soy bean which he examined do not contain the starch practically.

Thus no body in his recent work could recognise the presence of the starch in the soy bean though they could determine its content only by the analytical method.

When I was analysing green soy beans I found that they contain much amount of starch which I could isolate. It is rather hard to prove the presence of starch by iodine solution putting on the surface of the section of soy bean as it is very rich in fat and protein. But by keeping the section longer in the iodine solution we can observe its blue reaction.

As we will see from the accompanying figures the starch is present in younger seeds much more than in the ripened and it differs with time elasped since it is harvested.

Fig. 1. Section of a green seed stained with iodine solution.

Fig. 2. Section of a ripened seed stained with iodine solution.

Fig. 3. Section of an imperfectly ripened seed stained with iodine solution.

Fig. 4. Do.

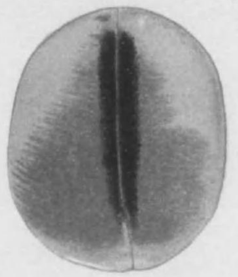

Fig. 1.

Fig. 2.

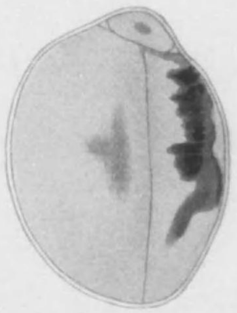

Fig. 3.

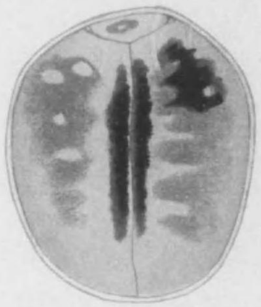

Fig. 4.

Fig. 5. Spermoderm of a green seed stained with iodine solution, showing that its pallisade cells contain starch granules.

Fig. 6. Spermoderm of a ripened seed stained with iodine solution.
ps — Pallisade cell.
ed — Endsperm.

Fig. 7. Section of a cotyledon of a green seed stained with iodine solution, showing the starch granules in it.

Fig. 8. Do. of a ripened seed.

Fig. 9. Section of a cotyledon of a green seed, showing the mode of agglegation of starch granules in the cell.

Fig. 10. Starch granules, ×1000.

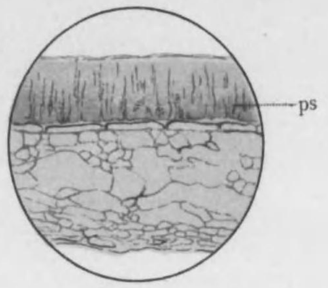

Fig. 5.

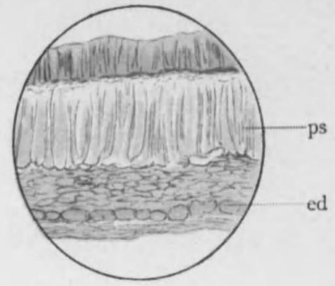

Fig. 6.

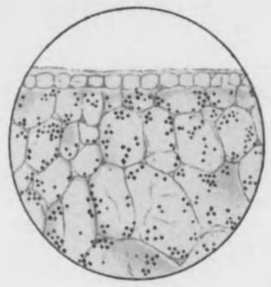

Fig. 7.

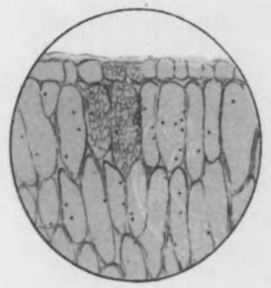

Fig. 8.

Fig. 9.

Fig. 10

In the green seeds, the pallisade cells of the spermoderm contain chlorophyll and there are contained the starch granules; but another parts of the spermoderm have no starch. In the ripened seeds the pallisade cells of spermoderm contain neither chlorophyll nor starch at all.

The endosperm consists of a single layer of moderately thick-walled cells which contain aleuron grains but no starch.

The cotyledons contain starch granules while epidermis has not. The cells of the cotyledon at the center of the seed where two cotyledons come in contact are rather rich in it as we can see from the figure.

The starch grains in the cell form an aggiegation, the severl grains forming a group.

In some seeds which are ripened imperfectly may have some spots where starch is very rich and if its section is stained with iodine solution that part will show dark blue in colour.

Experiment 1.

The method of determination of starch in soy bean was as follows:

Soy bean flour was thoroughly ground and it was extracted with ether and alcohol respectively and then with 5% sodium chloride solution in order to remove great deal of protein which is soluble in the brine. To extract with brine the residue from ether and alcohol extract was macerated in mortar with brine and it was centrifuged for twenty minutes. The residue was ground again in mortar to make the extraction complete and centrifuged again. The mixing and separation was made repeatedly and finally twice with distilled water. The starch in the residue was gelatinised by putting it in the water bath for ten minutes and then 3cc. of freshly prepared malt extract was added after the mixture was cooled. The whole mixture was kept for one hour at 60°C, then the temperature was raised to boiling point. The mixture was made up to 80cc. and filtered through dry quick filter paper and the aliquot part was acidified with sulphuric acid and the nitrogenous matter was precipitated with phosphotungustic acid and its filtrate neutralised with caustic soda and then made up to a definite volume. The content of sugar in the solution was determined by volumetric method. It was culculated as

maltose and deduced to starch by multiplying with 0.9468.

The malt extract was prepared by extracting the ground malt with thrice its weight of water and keeping 6 hours stirring several times and then filtered clear. In the 3cc. of the malt extract contained 0.17g of maltose and it was subtracted from the sugar contained in the inverted solution.

Name of variety	Amount of starch found in 100 parts of dry matter
Shiratama	0.48
Yagi	0.79
Nioiwase	0.43
Awomame	0.41
Kurohiramame	0.73
Yagi(unripened)	2.19
Nioiwase(unripened)	2.77

The unripened seeds of Nioiwase and Yagi were dried at 50-60°C before they were ground; so, there may be some change in the contents of the starch.

Thus we will see that the amount of starch contained in soy bean differs with its varieties and it is especially rich in the green seeds.

Experiment 2.

The starch was isolated from the soy bean in the following manner:

The soy bean flour was ground in the mortar mixing with some water in it and strained through a cotton cloth. The strained liquid was allowed to stand for several hours, when a layer of crude starch settles at the bottom of the vessel. The liquid with the impurities in suspension was decanted off and rejected. The time wanted for the settlement of the starch wanted rather long as the starch particles are very small in size. The deposit was then rubbed through a fine cloth, with a slight flow of water, and allowed to deposit a layer of starch as before. This was repeated as long as the starch was separated from other impurities. The whole of the starch was then collected on a filter paper and dried in desiccator. While it was hard to get the starch from the ripened

seeds, it was readily separated from the unripened seeds as they contain much amount of the starch in them.

Unripened seeds	Starch gain from 100 parts of dry matter
Nioiwase(cutted on Sept. 18 and it cotained 64.55% moisture)	2.60
Yagi(cutted on Oct. 2 and it contained 63.22% moisture)	4.76
Ripened seeds	
Nioiwase(cutted on Oct. 2 and it contained 29.69% moisture)	0.010
Yagi(cutted on Oct. 16 and it contained 32.19% moisture)	0.019
Shiratama(passed one year since it was harvested)	0.006
Awomame(do.)	00003

Experiment 3.

The properties of the soy bean starch: The microscopical appearance of the starch is irregularly oval showing no concentric ring. Its size is 2-5μ long and 2-4μ in weidth. As it is so small in size it shows Brownian movement under the microscope but no special character with the polarised light. It is coloured blue with iodine solution, It commences gelatinisation at 60°C.

To see how it is acted by diastase, 0.2g starch was put in test tube with 10cc. water and 2cc. malt extract. It was kept at 60°C in water bath for one hour and then it was boiled and the maltose formed was determined by the volumetric method and then it was deduced to starch.

	Saccharified in percent
Ungelatinised starch	64.67
Gelatinised starch	67.85

When the ungelatinised starch was digested at 37°C instead of 60°C, the other condition being the same, it was saccharified 35.47%.

Conclusion.

1. Soy bean contains the starch in it.
2. The amount of the starch contained in soy bean differs with its varieties and the unripened seeds contain much more starch than in the ripened ones. The amount of the starch which the auther could get was as follows:

	Unripened seeds	Ripened seeds
Yagi	4.78%	0.019%
Nioiwase	2.60 ,,	0.010 ,,
Shiratama	——	0.006 ,,
Awomame	——	0.003 ,,

3. The ripened seeds contain 0.34-0.77% starch.
4. The form of the soy bean starch is irregularly oval.
5. Its gelatinisation commences at 60°C.

On the natural Soap occuring in Soy Bean.

By S. Muramatsu

Introduction.

It is well known fact that when the soy bean is washed with water the washing froths extensively. The filtrate from the Tofu preparation is also very foamy and it is often used for the cleaning purpose of the cloth. Now, the reason why they are so foamy was not known until the present time and people thought that they are colloidal solution of protein and contain some mucilaginous matter which was dissolved out from soy bean.

While I was studying about the composition of soy bean I found that when soy bean flour previously extracted with ether it contains a substance which is soluble in alcohol and acetone. When some acid was added there formed a precipitate which is soluble in alcohol.

From its behaviour I suggested the presence of an organic acid which melting point is so high as 189°C (Journ. of Tokyo Chem. Society, Vol.41, p.311,1919)

The free fatty acids which are contained in the soy bean would not come in the alcohol extract as it is to be dissolved in the ether used for the extraction of fat. When the residue of the ether extract is extracted with hot alcohol of 80%, sugar will come in its solution. But when I added hydrochloric acid to this solution I found that there was produced a bulky precipitate of an organic acid which alkali solution being very foamy when it is shaken. When the precipitate was dissolved in sodium caustic solution there was formed a gelatinous mass when it was kept cold overnight. These facts led me to suggest that the precipitate is an organic acid which is contained in soy bean in the form of a soap.

I can not find the literature about such a natural soap occuring in the plant kingdom, though there are some soap found in the alimental canal of the animal, which is formed by the digestion of the neutral fat. I feel it very strange that such a natural soap of the vegetable kingdom have not been known until the present time while its solution had been

used for cleening purpose since an early age in this country. Saponin, a kind of glucoside, has a similar property with soap in some respects. Its water solution is very foamy and it is often used for some technical purpose at the early age before the artificial soap became so popular as the present time.

The pods of Saikachi (Gleditschia japonica Miq.) and the exocarp of soap-berry (Sapindus saponaria, L.) are populary used for the cleaning purpose of cloth, and the oil cake of the camelion seeds are often used for the cleaning of the human hair, Kumakiri (Journ. of Agric. Society, vol. 164, p.246, 1916) analysed the several kinds of plants which contain saponin getting the following result:

	Saponin In 100 parts of air-dry substance
Oil cake of camelion seeds	13.66
Seeds of Sazanka(Thea sasanqua Nois)	19.75
Exocarps of Mukuroji(Sapindus Mukurosi Gaertn.)	66.70
Onidokoro(Dioscorea Tokoro Makino)	15.95

Seki in this laboratory analysed the another kind of seeds and found:

Pods of Saikachi(Gleditschia japonica Miq.)	5.49
Seeds of Egonoki(Styrax japonica S. et Z.)	11.34

Saponin can be dissolved out by methyl and ethyl alcohol but it is not precipitated by acidifying its water solution with some mineral acid, while there is produced sugar and the precipitate of sapogenin by boiling with an acid. Saponin is also, poisonous and when it is added to blood it will dissolve the red corpuscles. In these respects saponin is quite different from the soap.

Not only the soap exists in the soy bean it is also contained in another kinds of seeds.

	In 100 parts of dry substance organic acid in the form of soap
Soy bean	1.52
Oil cake of soy bean	1.35
Azuki(Phaseolus Mungo L. var. subtrolobata)	1.06
Horse bean	1.03

Rice bran	1.11
Sesame(black variety)	0.44
Rape seed	Trace
Oil cake of rape seeds	Trace

Thus we will see that the soap is mostly contained in soy bean. While some seeds, which are rich in oil as soy bean and others, contain much amount of soap in them the another oil seeds as rape (sample contained 41.64% fat) contains only trace of it. So, we can not say that the seeds which are rich in fat always contain much amount of soap in it. Rice bran is also rich in the soap and it is the reason why it has been used for the cleaning purpose in this country.

1. The separation of the organic acids which are in the form of the soap.

The pulverised soy bean flour was passed through a seive of 0.5 mm. meshes and the oil in it was extracted with ether. The extracted residue was put in a flask and added about ten times its weight of 80% alcohol. It was heated on a water bath under reflux condenser for two hours. The alcoholic extract was decanted off and the same volume of alcohol was added in again and heated for thirty minutes. The same process was repeated again after the alcoholic solution was decanted off. Finally the whole alcoholic extract was filtered through Buchner's funnel and was kept overnight. The alcoholic extract was filtered clear again through a quick filter paper and alcohol in it was distilled off. After the greater part of alcohol was driven away it was cooled. Then it was acidified with hydrochloric acid, thus forming a bulky precipitate of an organic acid.

To see what kind of base would form the soap with the organic acid I determined the bases in the filtrate of the acid and got the following result:

	In 100 parts of dry matter
Organic acid	1.465
Potassium	0.361
Sodium	0.216

Thus we will see that the organic acid which forms the soap combines principally with potassium. The quantity of potassium and sodium which were dissolved out in the alcoholic extract in the form of the soap amount to about 40% of the whole metals which are contained in the soy bean, as we can see from the following table:

	Total amount	Found in the alcoholic extract	Ratio
Potassiun	1.758%	0.666%	37.9%
Sodium	0.569 „	0.228 „	40.0 „

At the same time we can say that the soap is a principal form of these metals in soy bean.

To determine the amount of the organic acid which forms the soap it is necessary to purify the acid by repeating the precipitation and resolution until we can get a colourless filtrate.

The amount of the organic acid do not differs much with the different varieties as we will see from the following result:

	Organic acid(in 100 parts of dry matter)
Shiratama	1.44
Yagi	1.72
Kariha	1.65
Awomame	1.86
Average	1.67
Yagi(unripened)	1.52

The organic acid does not dissolve in the absolute alcohol and acetone but when these solvents is diluted to about 80% it is readily soluble. When these solutions was evaporated at the ordinary temperature there was produced a cristalline mass. The crystal melts at 160°C and it darkens at its melting point.

When the precipitate of the organic acid would be washed with water on a filter paper, some part of it will dissolve. Thus we know that the organic acid is partly soluble in water or it may contain some acid soluble in water. To see how much of the acid will dissolve in water I added some water to the precipitate and it was collected on a filter paper after it was boiled. The amount of the acid dissolved in

water reached to 17.99% of the whole acid.

From these results I thought that the acid may be an oxyacid or it may contain some oxyacid, as the properties of the acid is quite different from that which we can get from the oil by saponifying it.

To separate the oxyacid from the other fatty acid I put the alkali solution in a separating funnel and then added petroleum ether and hydrochloric acid. The fatty acid which is precipitated by hydrochloric acid will dissolve in petroleum ether while the oxyacid will not.

The precipitated oxyacid suspends on the surface of the layer of the acid solution. The extraction of the fatty acid from the precipitate of the oxyacid was repeated for several times, but when there are much amount of oxyacid the extraction of the whole fatty acid is troublesome. So, the precipitate was dissolved in alkali again and repeated the same process as before by precipitatig with hydrochloric acid and then extracting with petroleum ether.

Petroleum ether was evaporated off from its solution of the fatty acid and the acid was got as a iight yeilow in colour and a radiant crystaliine mass when it was cooled. It is a mixture of the liquid and solid fatty acids melting at 26°C.

The amount of the fatty acid and oxyacid was as follows:

Oxyacid	57.78%
Fatty acid	42.22 „

Thus we will see that the greater part of the mixed acid consists of the oxyacid.

The fatty acid which was separated by extracting with petroleum ether contains a small amount of the neutral fat which has escaped the extraction by ether from the original flour; but the neutral fat may be taken off by extracting the alkali solution with ether.

The neutralisation value of the purified fatty acid is 177.1, its mean molecular weight being 316.9

To separate the liquid acid from the solid acid in their mixture I took the lead salt method. The mixed acid was dissolved in a weak alkali solution and the solution was neutralised with acetic acid and then precipitated with neutral lead acetate solution. The supernatant part

was decanted off and finaliy the precipitate was collected on a filter paper. Then the precipitate was pressed between the dry filter papers to take off the moisture as much as possible. The precipitate was dried in a desiccator until it could be done as a powder. The lead salt was extracted with ether under reflux condenser and the supernatant part was decanted off after it was cooled in a current of water for two hours. We can separate the lead salt of the liquid acid from the salt of the solid acid by repeating the same process. So, the ether solution was joined together and ether in it was evaporated. The residue was dissolved in potassium caustic solution and after that the fatty acid was precipitated by hydrochloric acid. As the lead salt of the solid fatty acid is not soluble in ether, the residue of the ether extract consists of the salt of the solid fatty acid. It was dissolved in potssium caustic solution and then precipitated with hydrochioric acid. The gain of the both fatty acids was as follows:

	In 100 parts of the whole fatty acid	In 100 parts of the whole organic acid
Liquid acid	69.41	29.31
Solid acid	10.98	4.63

When the liquid acid was dissolved in ether and the solvent was evaporated off it formed a soft crystalline mass melting at 38°C. when the solid acid was dissolved in acetone and the solvent was evaporated it formed a white lustrous crystal melting at 55°C.

The acid which is insoluble in petroleum ether was dissolved in alkali solution and ether in it was driven off by heating on water bath. The acid was then precipitated by acidifying the solution and collected on a filter paper. The precipitate was dried in a vacuum desiccator and extracted with ether again under reflux condenser to get rid of the acid which is soluble in ether. The amount of the acid which was dissolved out in ether was 11.79% of the whole organic acid and it is in the oiiy state at the ordinary temperature. The greater part of the acid is soiubie in petroleum ether. Thus we will see that some part of the acid which is soluble in petroleum ether will remain mixed in the oxyacid after it is extracted with petroleum ether in the separating funnei. So I separated

the fatty acid from the oxyacid by the following manner:

The refined mixed acid was collected on a filter paper and the moisture was taken off by pressing between filter paper. After it was dried in a desiccator it was extracted with ether under reflux condenser. The extraction was repeated several times until the whole ether soluble acid was separated. Thus it was separated into two parts with the following results:

Acid, soluble in ether	64.63%
Acid, insoluble in ether	35.37 „

The acid which is soluble in ether is principally composed of liquid and solid fatty acids. So, it was separated into two parts, one soluble in petroleum ether and the other insoluble in it.

Acid, soluble in petroleum ether	94.65%
Acid, insoluble in petroleum ether	6.35 „

Thus we came to the conclusion that the whole organic acid consists of 61.17% (64.63×0.9465) fatty acid and 38.83% oxyacid.

2. The isolation of the oxyacid.

The acid which is insoluble in ether contains some ether in it. So, it was driven out by evaporating on a water bath and the acid was dissolved in potassium caustic solution. The oxyacid was precipitated by hydrochloric acid and it was collected on a filter paper. The moisture of the precipitate was taken off by pressing the precipitate between several sheets of dry filter paper and by keeping in a desiccator. When it was dried the precipitate was extracted with ether again under reflux condenser. Then it was dissolved in alcohol or acetone of 80%. The oxyacid crystallised out when the solvent was driven away by evaporating at the ordinary temperature. The oxyacid which was got as crystal amounts to 36.93% of the whole organic acid which had composed the soap in soy bean. The mother liquor still contains the other acid which is not so easily crystallisable.

If some amount of alkali metal will remain in the acid as an impurity it would be very difficult to get the acid in the crystalline form, as the metal will combine with the acid and form the soap. The molecular

weight of the oxyacid being so large, even a trace of the alkali metal will form the soap with much of the oxyacid. To get rid of the alkali metal I precipitated the oxyacid by hydrochloric acid and the supernatant liquid was decanted off and again added some water in it with a little amount of hydrochloric acid and again the supernatant lipuid was decanted off. This process was repeated for several times and then the precipitate was collected on a filter paper and pressed between filter paper and dried in the desiccator.

3. The properties of the oxyacid.

To get the crystal of the oxyacid it is better to recrystallise from 70-80% of acetone. Alcohol of the same concentration may be used instead of acetone but acetone is preferable to alcohol to get the fine crystal. The oxyacid will crystallise out when its solution will be kept at the ordinary temperature. The acid of a purified crystal melts at 224°C in a capiliary tube.

Crystals of hispidic acid
×450

It is insoiuble in ether, benzene and chloroform but sparingly soluble in water, absolute alcohol and acetone while it is readily soiubie in alcohol and acetone of 80%. It is also soluble in amyl alcohol, aniline and glacial acetic acid. Aikali and magnesium salts are readily soluble in water while its calcium and barium salts are sparingly soluble in it. No substance which has the reducing power is formed by boiling with a

mineral acid. No nitrogen is contained in it, and no quantative amount of ash remains when it is ignited.

The result of the quantitative elementary analysis was as foliows:

	Percentage	Ratio of the number of atoms
Carbon	56.03	2
Hydrogen	8.86	4
Oxygen	35.11	1

Thus we get the empirical formula C_2H_4O its minimum molecular weight being 44.

Its neutralisation value is 73 and the molecular weight culculated from this number is 768.

For the determination of the molecular weight it is better to use its silver sait. The salt was prepared by adding silver nitrate solution into its 70% acetone solution. This was done in a coloured beaker and it was ignited in a porcelain crucible after dried in a coloured desiccator. By igniting a known weight of the silver salt I found 12.79% silver in it and the molecular weight cuiculated from this number is 751.

	Molecular weight
From the neutralisation value	768
From the silver salt	751
From the formula($n(C_2H_4O)$ where $n=17$)	748

So, the molecular formula for the oxyacid will be $C_{34}H_{68}O_{17}$ and I will propose to call this acid as "Hispidic acid".

Hispidic acid has so many as fifteen atoms of oxygen besides the two atoms which are combined with carbon, thus composing carboxyl group. Six in fifteen oxygen atoms are combined with hydrogen atom to form hydroxyi group, as we can see from the following result.

The acetyl compound was prepared by adding acetic anhydride to the powdered hispidic acid and it was boiled under reflux condenser for two hours to complete the acetylation. After the acetylation came to end it was boiled again by adding enough water for thirty minutes to change acetic anhydride to acetic acid. The acetylated acid separated on the wall, and when the solution was cooled it was got as a brittle mass and

it was collected on a weighed filter paper.

Increase in weight	33.46%
Increase in weight culculated as six acetyl group combined for one molecule of the acid	33.68 „

The acetyl derivative was dissolved in absolute alcohol and neutrailised with alcoholic potash. The solution was boiled after adding a known quantity of a standard alkali, thus hydrolysing the acetyl compound and the excess of the potash was neutralised with a standard acid thus getting the acetyl value 340.6. The culculated acetyl value assuming that the acetyl compound contains six acetyl group is 337.2. So, the both numbers come very closely.

The result of the elementary quantitative analysis of the acetyl compound was as follows:

	By experiment	Culculated as $C_{46}H_{80}O_{23}$
Carbon	55.2	55.8
Hydrogen	8.0	8.1
Oxygen	36.8	36.1

Thus the acetyl compound of hispidic acid has the molecular formula $C_{34}H_{62}O_9(CH_3CO_2)_6$ COOH.

To assure how we can introduse more than six acetyl group for one molecule of the acid I added one third weight of anhydrous sodium acetate to the mixture to accerelate the acetylation. But I could not get acetylated more than six acetyl group for one molecule of hispidic acid. Thus we can say that hispidic acid is in the form of $C_{34}H_{62}O_9(OH)_6$ COOH.

4. Hispidic acid as a soap ingredient.

Hispidic acid has a slight astringent taste and when its alkali solution be shaken it will froth heavily, and we can use it for cleaning purpose. Its dilute water solution also froths. The alkali salts of the other acids which occur in soy bean accompanying with hispidic acid froth as their water solution are so viscous as the ordinary soap solution. Both the alkali salts of hispidic acid and other fatty acid will serve as a soap when the water extract of soy bean is used for a washing purpose.

5. Conclusion.

1. The auther found out that soy bean, Azuki and the seeds of several other plants contain soap.
2. The content of soap in soy bean reaches 1.67% of the dry substance as the organic acid.
3. The bases which combine with the organic acid forming the soap are potassium and sodium, and the amount of the both metals reach about 40% of the total metals contained in the original substance. So, we can say that the soap is a principal form of the alkali metals in soy bean.
4. There are not much difference in the content of the soap between the varieties of soy bean, and the unripened seeds contain nearly the same amount of it.
5. 61.17% of the whole acid consists of the fatty acid and the other being oxyacid.
6. The neutraiisation value of the mixed fatty acid is 177, the mean molecular weight being 316.9. It was separated into two parts, namely, 29.31% of the liquid acid and 4.63% of the solid acid, their melting points being 30°C and 55°C respectively.
7. The oxyacid is the principal acid of the soap and I could get 36.93% of the whole acid in the crystalline form.
8. The oxyacid forms a white crystal melting at 224°C. Its molecular formula being $C_{34}H_{68}O_{17}$, I named it "Hispidic acid".
9. Hispidic acid is soluble in water having an astringent taste, and the solution will froth when it is shaken. Its alkali salts being more soluble in water may be used for the cleaning purpose. The alkali salts of the other fatty acids which are accompanied with the oxyacid will serve as a soap when the water extract of soy bean is used for the cleaning purpose.

On the Preparation of Tofu.

By S. Muramatsu

Introduction.

Tofu is an important food material which is made from soy bean. It is used from ancient time in this country as it is very rich in fat and protein; at the same time it is very delicious and readily digestible. It is said that the method of its preparation was devised in China so early as Kan (23) and its method of preparation was brought to this country at the age of Toyotomi (1585) from Korea.

At the early time most Budhist did not take meat in their diet and the protein source in their daily food was supplied from vegetable protein especially from soy bean and its preparations. So, Tofu is an important food material in our daily life evon at the recent time from that early age. It is now prepared every day and sold in the form of a flat cube.

Raw materials.

Tofu is prepared from soy bean and a protein precipitant; the selection of the raw materials want much attension as the quality and the amount of production depend much upon the nature of the raw materiasl which are used for its preparation.

1. Soy bean.

Any kind of soy bean may be used as a principal material for the preparation of Tofu, but we want to use such a kind of it as to produce much amount of Tofu from a certain amount of soy bean. Such a soy bean is rather poor in the content of crude fibre and in generally small in size.

The composition of soy bean is quite different according to their varieties and the water soluble parts as sugar and other carbohydrates come in the filtrate which is to be discarded, while the great deal of the protein and fat are contained in Tofu. The coarce fibery part which is insoluble in hot water is separated as residue. The average composition

of soy bean raised in this country is as follows after Nagaoka (Chemical tables for daily use P. 68)

Moisture	10.0%
Crude protein	33.4 „
Crude fat	17.6 „
Crude fibre	4.8 „
Nitrogen-free extract	29.2 „
Ash	5.0 „

I analysed the severl kinds of soy bean which were grown on the farm of our college and its vicinity and got the following result:

In 100 parts of air-dry matter

	Shiratama	Yagi	Kohachigatsu	Nioiwase	Awomame	Kurohiramame	Riusan	Average
Moisture	10.50	13.70	13.81	13.02	12.01	12.11	10.86	12.29
Dry matter	89.50	86.30	86.19	86.98	87.99	87.89	89.14	87.71
In 100 parts of dry matter								
Crude protein	43.61	40.80	46.09	43.24	42.39	43.32	42.81	43.18
Crude fat	17.36	20.97	18.84	18.98	17.90	18.21	18.82	18.72
Lecithine	0.44	0.54	0.59	0.91	0.33	0.91	0.75	0.64
Crude fibre	7.12	7.14	5.77	7.82	5.90	6.69	4.88	6.47
N-free extract	26.40	26.31	24.65	25.54	28.84	26.74	29.33	26.83
Starch	0.48	0.79	0.64	0.43	0.41	0.73	0.56	0.58
Sugar (as sucrose)	5.20	5.37	5.67	5.37	5.33	5.97	5.73	5.53
Pentosan	4.23	2.66	3.73	2.55	8.85	2.35	3.21	3.94
Ash	5.51	4.75	4.65	4.42	4.97	5.04	4.16	4.79

The ash was mixed thoroughly and analysed with the following result:

In 100 parts of the crude ash

CO_2	12.67	C	1.82
Sand	1.35		

In 100 parts of pure ash

SiO_2	0.19	SO_3	6.32
P_2O_5	19.50	Cl	0.19
CaO	5.42	MgO	10.36

K_2O	47.20	Na_2O	7.52
MnO	0.88	Fe_2O_3	1.47

Thus we will see that Kohachigatsu is the richest in protein and then comes Shiratama. As the ash ingredients kali is the principal and then comes phosphoric acid, magnesia and lime.

The great deal of soy bean protein is extracted by water when it is ground and mixed with water showing the following result:

	Shiratama	Yagi	Kohachigatsu	Nioiwase	Awomame	Kurohiramame	Riusan
Total nitrogen	6.977	6.528	7.374	6.918	6.783	6.931	6.850
Nitrogen in the water extract	6.341	4.256	6.402	5.470	5.130	5.578	6.396
	90.88%	65.19%	86.82%	79.06%	75.63%	80.47%	93.37%
In 100 parts of nitrogen in the water extract							
Clobulin-N	82.66	88.58	82.67	86.64	82.69	89.81	83.53
Albumin-N	4.68	3.58	10.34	4.64	3.54	1.74	7.73
Proteose-N	3.61	4.64	2.45	2.32	1.29	2.91	3.22
Non-alb.-N	9.05	3.20	3.55	6.40	12.49	5.54	5.52

Thus we can see that so much as 93.37% of the total nitrogen comes in its water extract in the case of Riusan.

Furthermore, the ground mixture was boiled for twenty minutes and separated from its residue as in the case of the preparation of Tofu and the nitrogen found in the liquid part was as follows:

	Shiratama	Yagi	Kohachigatsu	Nioiwase	Awomame	Kurohiramame	Riusan
Total nitrogen	6.977	6.528	7.374	6.918	6.783	6.931	6.850
Nitrgen in the hot water extract	5.370	3.949	5.924	5.253	4.963	3.049	5.736
	76.96%	60.49%	80.33%	75.93%	73.16%	43.99%	83.73%

From this table we will see that there are some difference in the amount of nitrogen which comes in the hot water extract with the variety of soy bean. Riusan and Kohachigatsu which are small in size contain much amount of nitrogen which comes in the water extract, while Yagi and Awomame are rather poor in the same nitrogen.

The protein in the hot water extract was precipitated by adding 1cc. of $CaCl_2$ solution (15%) for 50cc. and the precipitate was collected on a filter paper and the nitrogen in it was determined after it was weighed.

From 100 parts of dry soy bean flour

	Shiratama	Yagi	Kohachi-gatsu	Nioi-wase	Awomame	Kurohira-mame	Riusan
Precipitate (Tofu)	39.61	37.91	42.22	38.60	33.21	22.12	41.76
Nitrogen in the precipitate	4.32	3.10	4.86	4.24	2.15	2.65	4.95
Residue	28.68	30.25	28.05	28.41	39.36	44.85	30.72
Nitrogen in the residue	1.048	1.192	1.086	1.180	1.855	2.439	1.204

Thus we will see that there are much difference in the amount of the substance which may be used as Tofu, and the small variety such as Kohachigatsu and Riusan are most fitted for Tofu making and then comes Shiratama.

2. Nigari

Nigari is a water solution of several salts such as $MgCl_2$, $CaCl_2$, $MgSO_4$, Na_2SO_4, NaCl &, which come in the table salt as its impurities. When the table salt would be kept in moist air these impurities form a brine absorbing the moisture from air and is separated as a yellow solution. Nigari is also got in a solid state by evaporating the mother liquor of table salt. In the preparation of Tofu the solid Nigari is principally used and its solution is made by dissolving 8 Kwan (ca. 30 kg) in 35 Sho (63 L) water having the following composition:

Sp. gr.	1.143		
Dry matter	24.00%	Ash	14.94%
SiO_2	0.01 „	SO_3	4.53 „
Cl	2.81 „	CaO	2.11 „
MgO	4.19 „	K_2O	1.23 „
Na_2O	3.43 „		

The alkali earth metals in the brine serve as the precipitants of protein in the water extract thus forming Tofu.

The method of the preparation of Tofu.

Inouye (The Bull. of the Agric. Coll. Tokyo Univ. Vol. 2, 209, 1895) have studied on the preparation and chemical composition of Tofu. The method of preparation do not change from at that time. The soy bean is washed and steeped in water for severl hours to get absorb water in the grain and the time wanted for the steeping differs according to the temperature of water used. At the summer time it wants from 10 to 12 hours while at the winter time it wants to steep from 20 to 24 hours. When soy bean is steeped in water it absorbs water and soften by swelling. It increases 130% in its volume and about 100% in weight when it is steeped enough.

The steeped soy bean is ground in a stone mill adding some water in it continuously. The pulpy mixture is collected in a receiver and then put in an iron kettle which have some boiling water in it. When the mixture begins to boil the liquid will froth exceedingly on the surface. So, to avoid its overflow the foam must be stopped by stirring with a little quantity of oil added. The boiling is continued about ten minutes to dissolve the soluble matter in the ground mixture. The mixture is then put into a cotton cloth and the residue is separated from the liquid part pressing enough to squeeze out the liquid as much as possible. The milky liquid thus obtained contains much amount of protein and fat having a delicious taste like as a cow milk. So, it is popularly used as a drink calling "Bean milk".

The properly made milky liquid has the following composition:

Sp. gr.	1.013
Water	94.50%
Dry matter	5.50 „
Crude protein	2.79 „
Crude fat	1.24 „
Crude fibre	0.02 „
Nitrogen-free extract	1.13 „
Ash	0.32 „

When the liquid is separated it is kept in a wooden vessel and it is still so hot as from 70°C to 80°C. To precipitate the nitrogenous matter the brine is instantly added using a small quantity at a time and the

liquid is to be stirred slowly. About 300cc. of the precipitant was used for 24L liquid.

When the precipitate is settled the great deal of the supernatant liquid is skimmed off and the residual part is poured in a wooden four sided vat containing a cotten cloth in it. The vat has many holes on its sides and the liquid part comes out when it is pressed, gently putting some weights on the lid which comes doon by and by according to the contraction of the mixture. The precipitate consolidates to a soft and white mass, thus forming Tofu. The data for the preparation of Tofu was as follows:

Soy bean used	3225cc.=2328g
In which dry matter	2004.6g
Time wanted for steeping	18 hours
Steeped soy bean	7335cc.=4623g
In which dry matter	1969.9g
Time wanted for grinding	40 minutes
Water added	20 L.
Time wanted for heating	46 minutes
Oil used	14 cc.
Time wanted for pressing out the liquid	13 minutes
Amount of the liquid expressed out	24 L.
In which dry matter	1320 g
Amount of the residue	2844 g
In which dry matter	464 g
Nigari used	305 cc.
Time wanted for the precipitation	15 minutes
Time wanted for pressing	65 minutes
Tofu gain	7038 g
In which bry matter	1080 g
Filtrate separated	14600 cc.
In which dry matter	292 g

The composition of Tofu thus prepared was as follows:

In 100 parts of the fresh substance

Water	84.65
Dry matter	15.35
In 100 parts of dry matter	
Organic matter	96.24
Crude protein	29.93
Crude fat	27.64
Crude fibre	0.81
Nitrogen-free extract	34.10
Ash	3.76

Thus we will see that Tofu is very rich in protein, fat and carbohydrates. It has very delicious taste and is easily digestible. So, it is suited as our daily food material.

The filtrate was analysed with the following result:

Sp. gr.	1.0105
Water	98.00
Dry matter	2.00
Crude protein	0.52
Crude fat	0.01
Nitrogen-free extract	1.05
Sugar (as sucrose)	0.57
Ash	0.42
Total nitrogen	0.083
Albuminoid nitrogen	0.009
Non-albuminoid nitrogen	0.074

Thus we will see that the filtrate has yet much of the useful substances while the greater part of the nitrogenous matter is in non-albuminoid form. The carbohydrates consists principally of cane sugar and stachyose. So, it is used as a drink for the cattle or it may be used for cleaning purpose as it is viscous in nature.

The residue consists principally of the insoluble matter and has the following composition:

In 100 parts of the fresh substance

Water	82.59

Dry matter	17.41
In 100 parts of dry matter	
Organic substance	96.14
Crude protein	26.31
Crude fat	11.35
Crude fibre	20.18
Nitrogen-free extract	38.30
Ash	3.86

As it is rich in protein and other foodstufis it may be used as a food material or most commonly it is used as a rich fodder.

An improvement for the preparation of Tofu.

There are some improvements for getting the milky liquid which contain the principal constituents of Tofu but nothing about the precipitant. As Nigari has a bitter taste it will hurt the taste of Tofu if we would use it as the precipitant. The protein of soy bean can be precipitated by a calcium salt or acetic acid as well as magnesium salt. In the case of acetic acid when it would be used too much some part of the protein will dissolve after it is precipitated, while it is not the case by calcium or magnesium salt. Even in the case of calcium and magnesium salts if the solution would be used too much the precipitate will become compact and Tofu will be too hard and brittle. So the proper amount of the precipitant must be used to get Tofu of good quality.

The content of ash will change according to the precipitant used as we can see from the following table:

Precipitant	Ash in 100 parts of dry Tofu
Nigari	5.32
Magnesium chloride	5.57
Calcium chloride	5.92
Acetic acid	2.46

In the case of acetic acid the acid content of Tofu is especially small in amount, for the bases in the lipuid will form the soluble salts with the acid.

Lime is an important ash ingredient of the food material, but the rice and barley which are the principal food material in our daily life contain more magnesia than lime while it is wanted more than magnesia in our diet.

The amount of lime and magnesia in 100 parts of Tofu prepared by the different precipitants was as follows:

Precipitant	Lime	Magnesia
Nigari	0.61	2.19
Magnesium chloride	0.43	0.95
Calcium chloride	0.41	0.26
Acetic acid	3.21	0.22

Thus we will see that Tofu precipitated by calcium chloride solution contain further more lime than the others and that which were precipitated by Nigari and magnesium chloride solution were rich in magnesia. Tofu prepared by acetic acid was poor both in lime and magnesia.

The amount of Tofu produced from the same amount of the milky liquid differs with the precipitants used and that which produced by acetic acid was greatest and then comes magnesium and calcium chlorides, Niari being the least.

The amount of Tofu produced from 200cc. of the milky liquid and the proper amount of the precipitants was as follows:

	Nigari	$MgCl_2$	$CaCl_2$	Acetic acid
Weight of Tofu(g)	49.00	51.00	50.50	51.00
Dry matter(g)	7.99	8.29	8.17	8.54
Volume of the filtrate(cc)	143	144	141	134
Total N in the filtrate(g)	0.087	0.092	0.086	0.057

Thus we will see that the nitrogen contained in the filtrate is least in the case of acetic acid.

In the case of the another precipitants we can get more Tofu by adding more precipitant but if so the products would be too hard and inferior in its quality. So it wants to use proper amount of them. In the case of calcium chloride I would propose to use its 10% solution mixed with the same volume of 10% sodium chloride, to make the prepared Tofu tender and delicious in its taste.

Conclusion.

1. As a material for Tofu making it is better to use a small kind of soy bean such as Kohachigatsu, as we can get much amount of Tofu from a certain amount of the beans. If we would use such a variety we can get a white Tofu of the good quality.
2. Tofu is rich in protein and fat than the other vegetable food materials.
3. The nitrogen compound in Tofu is the protein combined principally with alkali earth metals, and the nitrogen in the filtrate is composed of non-albuminoid form.
4. Residue of Tofu preparation contain much amount of protein, fat and nitrogen-free extract; so, it is fitted as feeding material.
5. It is better to use calcium chloride solution as its precipitant, as we can get the product which is rich in calcium content.
6. If we would use calcium chloride as its precipitant we can get the Tofu so rich in calcium as five times more than that which made from Nigari.
7. If we would use calcium chloride the product is better in its quality than that which made by Nigari.
8. We can get more Tofu using a dilute acetic acid instead of the other precipitants.

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